Flow Cytometric Analysis of Peripheral Blood Lymphocytes in Patients with Vitiligo

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Vitiligo is an acquired cutaneous disorder characterized by progressive and selective destruction of melanocytes. Although, the exact etiology of vitiligo is still obscure, autoimmunity is strongly implicated in its pathogenesis. The aim of this study was to evaluate the immunological alterations in the peripheral lymphocytes in patients with vitiligo and their possible immunomodulation by narrowband ultraviolet B phototherapy. The study comprised of 44 patients with vitiligo (23 untreated, 21 treated) and 20 normal control subjects who were studied for peripheral blood lymphocytes imbalance using flow cytometry. The percentages of total T-lymphocytes, B-lymphocytes, helper T cells, cytotoxic T cells, activated T-lymphocytes, and natural killer cells were evaluated with the use of CD3, CD19, CD4, CD8, CD25, HLA-DR and CD56 monoclonal antibodies, respectively. A statistically significant lower difference in the median values of CD4+ cells (helper T lymphocytes) and CD4+/CD8+ ratio was found between untreated and both of treated and control groups. Natural killer cells (CD3-CD56+) were significantly higher in untreated than the other two groups. On the other hand, activated T-helper cells (CD4+CD25+) were significantly higher in both untreated and treated groups than the control subjects. In conclusion, the reported immunological alterations supported the role of cellular autoimmune mechanisms in the pathogenesis of the disease. Narrowband ultraviolet B phototherapy may enhance cellular immunity in vitiligo patients.

Vitiligo is an acquired cutaneous disorder characterized by progressive and selective destruction of melanocytes leading to a variable number of circumscribed white cutaneous macules, frequently affecting the exposed body areas (Taieb & Picardo, 2007). The disease is estimated to affect 1-2 % of the world's population, regardless of age, sex, and race (Sandoval-Cruz *et al.,* 2011). Although, vitiligo seems to be a mild disease, it is cosmetically a disfiguring disorder, its impact on the patient's psychology should not be underestimated (Ongenae *et al.,* 2006).

The exact etiology of vitiligo is still obscure; however, autoimmunity is strongly evidenced in its pathogenesis by the coexistence of other autoimmune disorders such as autoimmune thyroid disease, pernicious anemia and alopecia areata (Alkahateeb *et al.,* 2003; Ahmed *et al.,* 2007). Moreover, repigmentation in vitiligo patients treated with immunosuppressive agents indirectly supports the idea that an autoimmune-mediated process is involved in the pathogenesis of the disease (Boone *et al.,* 2007; Hartmann *et al.,* 2008). Humoral (Gilhar *et al.,* 1995) and cellular (Van den Boorn *et al.,* 2009) immunities have been implicated in the pathogenesis of vitiligo. The role of humoral immunity is supported by the presence of melanocyte-specific auto-antibodies in the sera of vitiligo patients (Cui *et al.,* 1995; Farrokhi *et al.,* 2005). On the other hand, cellular immunity is suggested by the infiltration of perilesional skin areas with helper and cytotoxic T lymphocytes. Furthermore, the circulating as well as skin infiltrating cytotoxic T lymphocytes against melanocyte-specific antigens, including Melan-A (MART-1), melanosomal matrix protein gp100 and tyrosinase express high levels of a skin homing receptor, cutaneous lymphocyte-associated antigen (CLA), its frequency correlates with the extension and

severity of the disease (Ogg *et al.,* 1998; Lang *et al.,* 2001; Palermo *et al.,* 2001).

Various management options are available for vitiligo with acceptable results in most patients. Different types of phototherapy have been used in the treatment of vitiligo yielding reasonable therapeutic responses. The most classic treatment regimen was topical or oral psoralen and ultraviolet A (PUVA) therapy until the introduction of narrowband ultraviolet B (NB-UVB) in 1997 which represents the current effective and safe therapeutic option in patients with vitiligo (Westerhof & Krobotova, 1997).

The aim of this study was to evaluate the immunological alterations in the peripheral lymphocytes in patients with vitiligo and the impact of narrowband UVB phototherapy as an immunomodulator.

Patients and Methods

Study Design

This study was conducted at the department of Dermatology & Venerelogy in collaboration with the Medical Microbiology & Immunology department, Sohag university hospital, during the period from October to December 2011.

The study included patients with vitiligo recruited from the outpatient's clinic of the of Dermatology department. Written informed consents were obtained from all enrollees. Twenty healthy volunteers from the hospital staff were also included as control.

Study Exclusion Criteria

Patients with age less than 15-years, clinically and laboratory diagnosed with autoimmune diseases such as Hashimoto thyroditis, pernicious anaemia, type 1 diabetes and alopecia areata and patients receiving immunosuppressive therapy.

The eligible patients (n= 44) were allocated into two groups; untreated patients (n = 23) and treated patients with NB UVB phototherapy (n = 21).

Definitions

Vitiligo was classified into segmental and non-segmental types according to Koga classification (Koga & Tango, 1988).

Non - segmental (type A); is more common, has a potential lifelong evolution and is associated with Koebner phenomenon and frequently with autoimmune diseases, such as thyroid disorders, juvenile diabetes mellitus, and pernicious anemia.

Segmental (type B); is rarer and has a dermatomal distribution; after rapid onset and evolution it usually exhibits a stable course.

Stable vitiligo; was defined by the absence of new lesions, non progression of existing lesions, and absence of Koebner phenomenon during the last one year (Parsad & Gupta, 2008).

Vitiligo Area Scoring Index (VASI); is a quantitative parametric score to measure the severity of the disease indexes and treatment evaluation criteria. It is calculated using a formula that includes contributions from all body regions with a possible range of (0–100) (Hamzavi *et al.,* 2004).

VASI=

Σ(All body sites) (hands units) X (depigmentation)

Fitzpatrick phototyping scale; is a numerical classification for the color of skin to evaluate the response of different types of skin to UV light. It measures several components, genetic disposition, reaction to sun exposure and tanning habits (e.g. skin type III: darker white skin that tans after an initial burn; type IV: light brown skin, typical Mediterranean Caucasian skin, burns minimally and tans easily (Fitzpatrick, 1975).

Narrowband UVB Phototherapy

Treated patients received at least 40 sessions of narrowband ultraviolet B (NB UVB) phototherapy; twice per week on non-consecutive days. The dose regimen was determined by Fitzpatrick skin phototypes. Patients with skin phototype III were started on 180mJ/cm^2, while skin phototype IV patients received 150mJ/cm^2. The dose was increased at each session by 20% of the previous dose until minimal erythema of the vitiligo lesions was achieved. Further increments, if needed, were 10% of the last given dose. If symptomatic erythema or blistering developed, NB-UVB was withheld for one week and the dose was decreased by 20% of the last given one.

Flow Cytometric Analysis of Peripheral Lymphocytes

Two ml of peripheral venous blood samples were withdrawn from all the participants using vacutainer tubes containing potassium ethylene diamine tetraacetate (EDTA) anticoagulant. Samples were

stained and analyzed in the same day by flow cytometry according to manufacturer's instructions. One hundred µl of anti-coagulated blood stained with 10 µl of either combination of conjugated monoclonal antibodies (Beckman Coulter, France); namely anti-HLA-DR, anti-CD4 labeled with fluorescein isothiocyanate (FITC), anti-CD8, anti-CD25, anti-CD56 labeled phycoerythrin (PE), anti-CD3 or anti-CD19 conjugated to electron coupled dye (ECD). Tubes were vortexed then incubated for 10 min at room temperature (RT) in the dark. One ml of lysing solution (Beckman Coulter, France) was added to the mixture while vortexing followed by incubation for 15 min at RT in the dark. Finally, analysis of the cells was done by EPICS XL flow cytometry using SYSTEM II version 3.0 software (Beckman Coulter, USA) with a standard 4-color filter configuration. Lymphocytes were gated via their forward and side scatter properties. T-cells were identified based on their expression of CD3+CD4+ (helper T-lymphocytes) or CD3+CD8+ (cytotoxic T-lymphocytes). B-lymphocytes were identified by expression of CD3-CD19+ and natural killer by CD3-CD56+. Gates were analyzed for number and percentage of cells.

Statistical Analysis

Values are presented as percentages or range and median. Chi square test was used for categorical data and non parametric test was used for quantitative data. Variables with uneven distribution were analyzed with the Mann-Whitney's U test to compare between two groups while Kruskal-Wallis test was used for the comparison between the three groups; untreated, treated and control. For all tests a P value < 0.05 was considered significant.

Results

The study included 44 patients (21 males) and 20 healthy control subjects (10 males); age range from 15 to 60 years while the age range of the controls was 18 to 50 years. The duration of the disease was significantly higher (P= 0.006) in treated group with NB UVB phototherapy than untreated patients (Table 1).

Twelve patients (27.3%) had segmental type and 32 (72.3%) were non-segmental. The disease was active in 26 (59.1%) patients; the other 18 (40.9%) patients had stable vitiligo. A definite family history of vitiligo was present in 9 (20.5%) patients while 8 (18.2%) had a previous history of stress. Vitiligo Area Scoring Index (VASI) ranged from 0.05-21.15 among the patients. In total, twenty seven (61.4%) patients had skin phototype III and 17 (38.6%) had phototype IV (Table 1).

Flow cytometric analysis of the peripheral lymphocyte subpopulations revealed that the median values of CD4+ cells (helper T lymphocytes) was significantly lower in untreated vitiligo patients than in treated or control groups (Table 2; Figure 1), as well as CD4+/CD8+ ratio (Figure 2).

The level of natural killer cells (CD3-CD56+) was significantly higher in untreated than the other two groups (Table 2; Figure 3).

On the other hand, activated T-helper cells (CD4+CD25+) were significantly higher in both untreated and treated groups than in the control subjects (Table 2; Figure 4). The other median values of CD3+, CD8+, activated CD8+ and CD19+ were not statistically significant among the different groups (Table 2).

Table 1. Patients' characteristics

Variable	Untreated group N= 23	Treated group N= 21	*P value
Gender			
Female	12 (52.17)	11 (52.38)	NS
Male	11 (47.83)	10 (47.62)	
Age (years)	31 (17-56)	23 (15-60)	NS
Duration of disease (years)	2 (0.25-40)	5 (1.5- 30)	0.006
Vitiligo type			
Non-segmental	17 (73.91)	15 (71.43)	NS
Segmental	6 (26.09)	6 (28.57)	
Disease activity			
Active	17 (73.91)	9 (42.86)	0.04
Stable	6 (26.09)	12 (57.14)	
Family history			
Yes	6 (26.09)	3 (14.29)	NS
No	17 (73.91)	18 (85.71)	
History of Stress			
Yes	6 (26.09)	2 (9.52)	NS
No	17 (73.91)	19 (90.48)	
VASI	2.25 (0.5-21.15)	1.00 (0.05 -9.25)	NS
Skin phototype			
III	14 (60.9)	13 (61.9)	NS
IV	9 (39.1)	8 (38.1)	

Data were expressed as number (percentage), or median (range)., P>0.05 is not significant., NS= not significant

Abbreviation: VASI; Vitiligo Area Scoring Index

Table 2. Flow cytometric analysis of peripheral lymphocytes

Variable (%)	Untreated group	Treated group	Control	*P* value
CD3	64.6 (48.4-74.6)	64.1 (55.3-73.4)	66.95 (57.3-78.3)	NS
CD4	36 (23.7-46.9)	40 (32.4-51)	41.5 (31.9-51.6)	0.01*
CD8	27.3 (20-55.6)	23.6 (17.5-35)	26.5 (18.8-31.5)	NS
CD4/CD8	1.3 (0.5-1.8)	1.6 (1-2.4)	1.6 (1-2.4)	0.002*
CD4/CD25	7 (1.4-23.7)	5.5 (2.2-13.2)	3.8 (2.4-8)	0.002**
CD8/HLA-DR	28.2 (12-55)	19 (4.8 -44.6)	20.95 (6.1-41)	NS
CD19	8.2 (4.2-19.7)	7.3 (2.7-15)	5.15 (2.8-19.4)	NS
CD56	10 (1.2-14)	4.5 (1.4-19.7)	5.9 (2-13.1)	0.001*

Values were expressed as median and (range). Abbreviations: CD; Cluster of differentiation, CD3; T-lymphocytes, CD4; T-helper cells, CD8; T-cytotoxic cells, CD4/CD25; Activated T-helper cells, HLA; Human leukocytes antigen, CD8/HLA-DR; Activated T-cytotoxic cells, CD19; B-lymphocytes; CD56; Natural killer cells. *P*>0.05 is not significant. NS= not significant.

* Significant when the untreated group was compared with the treated and control groups

** Significant when both untreated and treated groups were compared with the control group

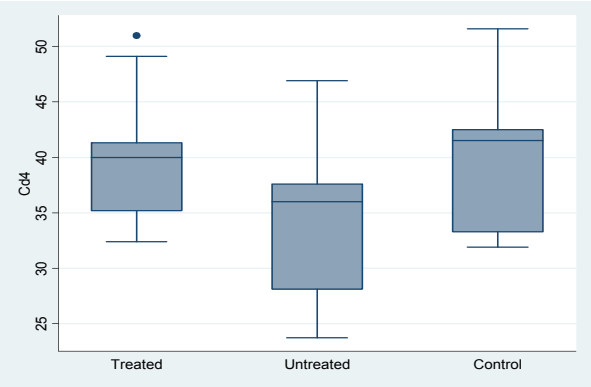

Figure 1. Showed a lower percentage of peripheral CD4+ T cells in untreated vitiligo patients when compared with treated and control groups (*P*=0.01).

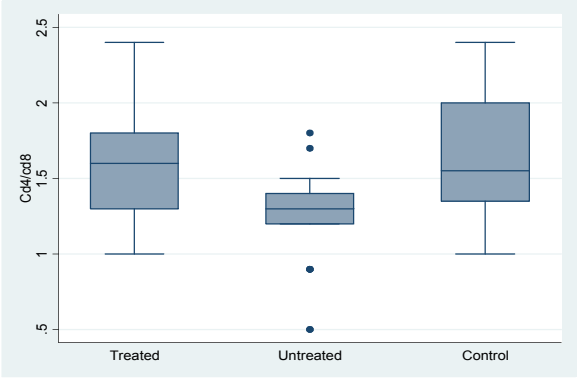

Figure 2. Showed reduced CD4+/CD8+ ratio in untreated vitiligo patients when compared with treated and control groups (*P*=0.002).

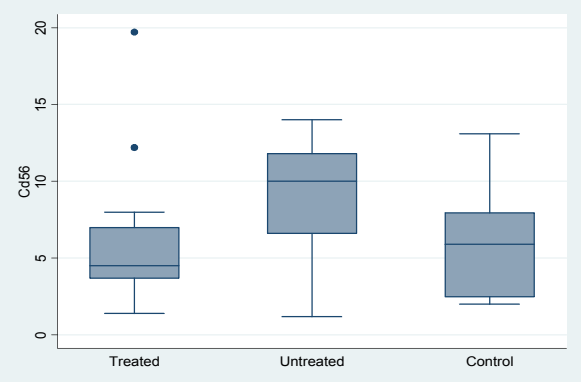

Figure 3. Showed a higher percentage of peripheral NK cells in untreated vitiligo patients when compared with treated and control groups (*P*=0.001).

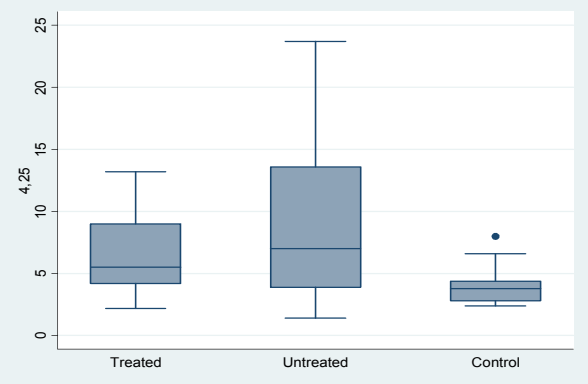

Figure 4. Showed a higher percentage of activated CD4+ cells in both of untreated and treated vitiligo patients when compared with the control group (*P*=0.002).

Discussion

Vitiligo is a psychologically distressing disorder with a presumed autoimmune etiology. Narrowband ultraviolet B (NB UVB) therapy is a relatively safe and effective mode of therapy for generalized vitiligo in adults and in children (Parsad *et al.,* 2010). Although, many studies were previously conducted to evaluate the possible implications of autoimmunity in the pathogenesis of the disease, few studies have evaluated the effect of phototherapy on the immune profile of the treated patients. To our knowledge, this is the first study to assess the possible immunomodulatory effect of NB-UVB phototherapy in patients with vitiligo.

In this study, we identified significant lower values of T-helpers and T-helper/T cytotoxic ratio in untreated patients while no significant differences in the total T, B or cytotoxic T-lymphocytes were observed in the different groups of participants. These data not only corroborated results reported by many previous studies, but also, supported the previous assumption that vitiligo is associated with immunological alterations (Grimes *et al.,* 1986; Halder *et al.,* 1986; Gunduz *et al.,* 2004; Basak *et al.,* 2008). In contrast, our findings disagreed with other studies that report an increased number of the peripheral T-helpers and an elevated T-helper/T cytotoxic ratio in studied patients (Soubiran *et*

al., 1985, D'Amelio *et al.* 1990, Al-Fouzan *et al.,* 1995; Pichler *et al.,* 2009).

The significant higher percentage of activated T-helpers (CD4+/CD25+) in our study shown in both untreated and treated patients aligned with the experience of Mahmoud and his colleagues (2002) who reported higher percentages of peripheral CD4+/CD25+ and CD4+/CD45RO+ (memory T-cells) in patients with non-segmental vitiligo.

Although, both natural killer cell activity and abnormalities in the circulating NK cells were previously described in patients with vitiligo, reports from previous studies were largely discordant (Mozzanica *et al.,* 1992). Many studies reported normal levels of total NK cells count in the peripheral blood of patients with vitiligo however, this study as well as others described an increased total count of NK cells (Halder *et al.,* 1986; Abdel-Naser *et al.,* 1992; Mahmoud *et al.,* 2002; Lin *et al.,* 2003; Gunduz *et al.,* 2004; Basak *et al.,* 2008; Pichler *et al.,* 2009).

The marked discrepancies in the published data about vitiligo-associated peripheral lymphocytes imbalances may be attributed to the differences in study techniques, clustering of patients at different stages of the disease in the same study group. In addition, the obvious limitation of this approach that it did not fully characterize cell populations that may selectively influence the pathogenesis of skin lesions as skin homing CLA+ lymphocytes (Antelo *et al.,* 2010).

NB UVB therapy has emerged as one of the most effective treatment options in vitiligo over the last decade. The mechanism of action of NB-UVB phototherapy is not fully elucidated. Similar to PUVA therapy, NB-UVB may exert its effects through the induction of local as well as systemic immunosuppression in an attempt to stop destruction of the melanocytes. Subsequently, the melanocytes of the outer hair root sheaths are stimulated to proliferate and migrate outwards to re-pigment the affected skin (Cui *et al.,* 1991; Norris *et al.,* 1994). Noteworthy, the comparable values of T-helper cells and T-helper/T-cytotoxic ratio observed in studied treated patients and controls may suggest that NB-UVB has an up- regulating effect on the immune profile of the treated patients. This controversy may be explained by the lack of data about the immune profile of the patients before treatment. However, Antelo and his coworkers (2010) noticed no effect of PUVA on the patients' immune profile by reporting insignificant difference of T-helper/T cytotoxic ratio and the proportion of CLA+ T cells between the patients and the control subjects, before and after PUVA phototherapy. Although, the difference between our result and the former study could not be soundly explained, the heterogenicity in vitiligo-associated peripheral lymphocytes imbalances remains a possible explanation.

Limitations of this study were the small number of participants, lack of analysis of T-lymphocytes subpopulations that selectively influence the pathogenesis of skin lesions as skin homing CLA+ lymphocytes and lack of information about immunological profiles of vitiligo patients before treatment.

In the present study, the significantly lower values of T-helper cells and T-helper/T-cytotoxic ratio and the elevated levels of natural killer cells (CD3-CD56+) in untreated vitiligo patients may suggest the role of cellular immunity in the pathogenesis of vitiligo. Narrowband UVB phototherapy may have an immunomodulatory effect in the course of vitiligo disease. Further studies are needed to ascertain the role of autoimmunity in melanocytes destruction.

References

1. Abdel-Naser MB, Ludwig WD, Gollnick H, Orfanos CE (1992). Non-segmental vitiligo: decrease of the CD45RA+ T-cell subset and

evidence for peripheral T-cell activation. Int. J. Dermatol; 31: 321–326.

2. Ahmed I, Nasreen S, Bhatti R (2007). Alopecia areata in children. J Coll Physicians Surg Pak; 17: 587-590.
3. Al-Fouzan A, Al-Arbash M, Fouad F, Kaaba SA, Mousa MA, al-Harbi SA (1995). Study of HLA class I/II and T lymphocyte subsets in Kuwaiti vitiligo patients. Eur J Immunogenet; 22:209-213.
4. Al-kahateeb A, Fain PR, Thody A, Bennett DC, Spritz RA (2003). Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Res; 16: 208–214.
5. Antelo DP, Filgueira AL, Cunha JMT (2010). Reduction of skin-homing cytotoxic T cells (CD8+–CLA+) in patients with vitiligo. Photodermatology, Photoimmunology & Photomedicine; 27: 40–44.
6. Basak PY, Adiloglu AK, Koc IG, Tas T, Akkaya VB (2008). Evaluation of activatory and inhibitory natural killer cell receptors in non-segmental vitiligo: a flow cytometric study. J. Eur. Acad. Dermatol. Venereol; 22: 970–976.
7. Boone B, Ongenae K, Van Geel N, Vernijns S, De Keyser S, Naeyaert JM (2007). Topical pimecrolimus in the treatment of vitiligo. Eur J Dermatol; 17: 55-61.
8. Cui J, Shen LY, Wang GC (1991). Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol; 97: 410–416.
9. Cui J, Arita Y, Bystryn JC (1995). Characterization of vitiligo antigens. Pigment Cell Res; 8: 53-59.
10. D'Amelio R, Frati C, Fattorossi A, Aiuti F (1990). Peripheral T cell subset imbalance in patients with vitiligo and in their apparently healthy first-degree relatives. Ann Allergy; 65 (2):143-145.
11. Farrokhi S, Hojjat-Farsangi- M, Noohpisheh MK, Tahmasbi R, Rezaei N (2005). Assessment of the immune system in 55 Iranian patients with vitiligo. J Eur Acad Dermatol Venereol; 19: 706-711.
12. Fitzpatrick TB (1975). Soleil et peau. J Med Esthet; 2: 33-34.
13. Gilhar A., Zelickson B, Ulman Y, Etzioni A (1995). In vivo destruction of melanocytes by the IgG fraction of serum from patients with vitiligo. J Invest Dermatol; 105: 683-686.
14. Gunduz K, Ozturk G, Terzioglu E, Sebik F (2004). T cell subpopulations and IL-2R in vitiligo. J Dermatol; 31: 94–97.
15. Halder RM, Walters CS, Johnson BA, Chakrabarti SG, Kenney Jr JA (1986). Aberrations in T lymphocytes and natural killer cells in vitiligo: a flow cytometric study. J. Am. Acad. Dermatol; 14: 733–737.
16. Hamzavi I, Jain H, McLean D, Shapiro J, Zeng H, Lui H (2004). Parametric modeling of narrowband UV-B phototherapy for vitiligo, using a novel quantitative tool: the Vitiligo Area Scoring Index. Archives of Dermatology; 140(6): 677–683.
17. Hartmann A, Brocker EB, Hamm H (2008). Occlusive treatment enhances efficacy of tacrolimus 0.1% ointment in adult patients with vitiligo: results of a placebo-controlled 12-month prospective study. Acta Derm Venereol; 88: 474-479.
18. Koga M, Tango T (1988). Clinical features and course of type A and type B vitiligo. Brit J Dermatol; 118:223-228.
19. Lang KS, Caroli CC, Muhm A, Wernet D, Moris A, Schittek B, Knauss-Scherwitz E, Stevanovic S, Rammensee HG, Garbe C (2001). HLA-A2 restricted, melanocyte-specific CD8+ T lymphocytes detected in vitiligo patients are related to disease activity and are predominantly directed against MelanA/MART1. J Invest Dermatol; 116: 891-897.
20. Lin IC, Chen YF, Li SY, Yang PY (2003). Imbalance of peripheral leukocyte and lymphocyte subpopulations in segmental vitiligo in Taiwanese. Journal of Dermatological Science; 33:189-191.
21. Mahmoud F, Abdul H, Haines D, Al-Saleh C, Khajeji M, Whaley K (2002). Decreased total numbers of peripheral blood lymphocytes with elevated percentages of CD4+, CD45RO+ and CD4+ CD25+ T-helper cells in non-segmental vitiligo. J. Dermatol; 29:68–73.
22. Mozzanica N, Villa ML, Foppa S, Vignati G, Cattaneo A, Diotti R, Finzi AF (1992). Plasma alpha-melanocyte-stimulating hormone, beta-endorphin, met-enkephalin, and natural killer cell activity in vitiligo. J Am Acad Dermatol; 26: 693–700
23. Norris DA, Horikawa T, Morelli JG (1994). Melanocyte destruction and repopulation in vitiligo. Pigment Cell Res; 7: 193–203.

24. Ogg GS, Rod Dunbar P, Romero P, Chen JL, Cerundolo V (1998). High frequency of skin homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. J Exp Med; 188(6):1203–1208.

25. Ongenae K, Beelaert L, van Geel N, Naeyaert JM (2006). Psychosocial effects of vitiligo. J Eur Acad Dermatol Venereol; 20: 1-8.

26. Palermo B, Campanelli R, Garbelli S, Mantovani S, Lantelme E, Brazzelli V, Ardigó M, Borroni G, Martinetti M, Badulli C, Necker A, Giachino C (2001). Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. J Invest Dermatol; 117: 326-332.

27. Parsad D, Gupta S; IADVL Dermatosurgery Task Force (2008). Standard guidelines of care for vitiligo surgery. Indian J Dermatol Venereol Leprol; 74:S37–45.

28. Parsad D, Bhatnagar A and De D (2010). Narrowband ultraviolet B for the treatment of vitiligo. Expert Rev. Dermatol; 5(4): 445- 459.

29. Pichler R, Sfetsos K, Badics B, Gutenbrunner S, Berg J, Aub¨ock J (2009). Lymphocyte imbalance in vitiligo patients indicated by elevated CD4+/CD8+T-cell ratio. Wien Med Wochenschr; 159: 337–341.

30. Sandoval-Cruz M, García-Carrasco M, Sánchez-Porras R, Mendoza-Pinto C, Jiménez-Hernández M, Munguía-Realpozo P, Ruiz-Argüelles A (2011). Immunopathogenesis of vitiligo. Autoimmunity Reviews; 10: 762–765.

31. Soubiran P, Benzaken S, Bellet C, Lacour JP, Ortonne JP (1985). Vitiligo: peripheral T-cell subset imbalance as defined by monoclonal antibodies. Br J. Dermatol; 113: 124–127.

32. Taieb A Picardo M; VETF Members (2007). The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res; 20: 27–35.

33. Van den Boorn JG, Konijnenberg D, Dellemijn TA, van der Veen JP, Bos JD, Melief CJ, Vyth-Dreese FA & Luiten RM (2009). Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. J Invest Dermatol; 129 (9): 2220-2232.

34. Westerhof W, Nieuweboer-Krobotova L (1997). Treatment of vitiligo with UV-B radiation vs topical psoralen plus UV-A. Arch Dermatol; 133:1525-1528.