Incidence of Antithyroid Antibodies in Vitiligo Patients

Un Cheol Yeo, M.D., Jae Hoon Chung, M.D.,*
Hwan Tae Sung, M.D., Eil Soo Lee, M.D., Myung-Hee Shin, M.D.**

Department of Dermatology, Internal Medicine, Center for Health Promotion**, Samsung Medical Center, Seoul, Korea*

Background **: Vitiligo is considered as an autoimmune disorder due to the generation and presence of autoantibodies directed against melanocyte antigens in the patients` sera. Previous studies have revealed an increased incidence of organ-specific autoantibodies in vitiligo patients. A number of studies have demonstrated an increased frequency of thyroid autoantibodies in vitiligo patients and vitiligo is commonly seen in patients with clinical thyroid diseases.**

Objective **: The aim of this study is to investigate the incidence of antithyroid antibodies in vitiligo patients and to correlate the presence of these antibodies with factors such as sex, age, activity of the disease, duration of the disease and the type of vitiligo. Another aim of this study is to compare the incidence of abnormal thyroid function in those who have antithyroid antibody and those who don't.**

Methods **: One hundred and fifty seven vitiligo patients who visited vitiligo clinic in Samsung medical center from January of 1995 to November of 1996 were enrolled in this study. Detection and titration of antithyroid antibodies were performed by immunoradiometric assay.**

Results **: Among 157 patients tested, 17(10.8%) patients had antithyroglobulin antibodies and 10(6.4%) patients had antimicrosomal antibodies. Five patients had both antibodies. Statistically meaningful data are as follows ; 1) Antimicrosomal antibody appeared less frequently in patients of childhood-onset. 2) Antithyroglobulin antibody was detected more frequently in active disease. Fifty nine out of 157 patients were examined for thyroid function. Four out of 22 patients with antithyroid antibody had abnormal thyroid function. None out of 37 patients without antithyroid antibody had abnormal thyroid function.**

Conclusion **: The incidence of antithyroid antibodies according to onset age and activity is contradictory to previous reports, therefore large scaled study will be necessary to draw a conclusion.(Ann Dermatol 9:(2) 132~138, 1997).**

Key Words : Antimicrosomal antibody, Antithyroglobulin antibody, Vitiligo

The pathogenesis of vitiligo is unknown, althogh there is evidence to suggest that it may be due to an autoimmune process associated with specific auto antibodies against melanocytes. The autoimmune pathogenesis has been suggested due to following features ; the association of vitiligo with other autoimmune conditions[1-3], the presence of organ-specific autoantibodies in the patients' sera[4-5], the detection of autoantibodies in the first-degree relatives of subjects with vitiligo[6], the association of the disease with HLA-DR4 or HLA-DR1[7-9] and the presence of antimelanocyte autoantibodies in patients' sera[10-14]. The cytotoxic action of antimelanocytic autoantibody on melanocytes is reportedly via the involvement of both cell mediated and humoral immunity[15]. Recently, in vivo destruction of melanocytes by the IgG fraction of serum from patients with vitiligo has been reported[16].

In the past, several authors described an association of vitiligo with autoimmune disorders and the presence of different tissue autoantibodies. Recently Schallreuter et al[17] confirmed that earlier results of a prevalence of thyroid disease and the

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Reprint request to : Un Cheol Yeo, M.D., Department of Dermatology, Samsung Medical Center, Seoul, Korea

Table 1. Age distrivution of vitiligo patients

Age	Male	Female
0~10	14	13
11~20	11	10
21~30	9	13
31~40	12	21
41~50	7	13
51~60	11	13
61~	6	4
Total	70	87

Table 2. Incidence of autontibodies and thyroid disease in vitiligo patients

Type of autoantibody	Vitiligo(N=157)
Antithyroglobulin	17(10.80%)
Antimicrosomal	10(6.4%)
Thyroid disease	6(3.8%)

Table 3. Incidence of autoantibodies and thyroid disease in vitiligo patients according to sex

Type of autoantibody	Male(N=70)	Female(N=87)	p-value
Antithyroglobulin	6(8.57%)	11(12.64%)	0.414
Antimicrosomal	3(4.29%)	7(8.04%)	0.514*
Thyroid disease	0(0.00%)	6(6.90%)	0.034*

* : Statistical test by Fisher's exact test
Others : Statistical test by X^2 test

Table 4. Incidence of autoantibodies and thyroid disease in vitiligo patients according to age of onset

Type of autoantibody	Below 15 years old(N=39)	Above 15 years old(N=118)	p-value
Antithyroglobulin	4(10.26%)	13(11.02%)	1.00*
Antimicrosomal	0(0.00%)	19(8.47%)	0.004*
Thyroid disease	2(5.13%)	4(3.39%)	0.639*

* : Statistical test by Fisher's exact test

Table 5. Incidence of autoantibodies and thyroid disease in vitiligo patients according to duration

Type of autoantibody	Less than 1 year(N=76)	More than 1 year(N=81)	p-value
Antithyroglobulin	7(9.21%)	10(12.31%)	0.528
Antimicrosomal	4(5.26%)	6(7.41%)	0.747*
Thyroid disease	4(5.26%)	2(2.47%)	0.431*

* : Statistical test by Fisher's exact test
Others : Statistical test by X^2 test

presence of thyroid autibodies, whereas other diseases were a random event. High frequency of thyroid dysfunction in patients with vitiligo was further evidenced using ultrasonography of thyroid and thyroid function test[18].

The aim of this study is to investigate the incidence of antithyroid antibodies in vitiligo patients and to correlate the presence of these antibodies with factors such as sex, age, activity of the disease, duration of the disease and the type of vitiligo. Another aim of this study is to compare the incidence of abnormal thyroid fuction in those who have antithyroid antibody and those who don't.

Table 6. Incidence of autoantibodies and thyroid disease in vitiligo patients according to activity

Type of autoantibody	Active(N=127)**	Inactive(N=30)	p-value
Antithyroglobulin	17(13.39%)	0(0.00%)	0.044*
Antimicrosomal	7(5.51%)	3(10%)	0.404*
Thyroid disease	4(3.15%)	2(6.67%)	0.322*

* : Statistical test by Fisher's exact test

** : Active stage means onset within one year or development of new lesions within one year

Table 7. Incidence of autoantibodies and thyroid disease in vitiligo patients according to type

Type of autoantibody	**Localized(N=44)	Generalized(N=113)	p-value
Antithyroglobulin	3(6.82%)	14(12.39%)	0.401*
Antimicrosomal	1(2.27%)	9(7.96%)	0.285*
Thyroid disease	3(6.82%)	3(2.65%)	0.350*

* : Statistical test by Fisher's exact test

** : Localized type includes focal type and segmental type

MATERIAL AND METHODS

Patients

The patients group consisted of 87 women and 70 men aged from 1 to 74 who visited the vitiligo clinic in Samsung medical center from January of 1995 to November of 1996(Table 1). None of the patients had received any treatment except topical streoids at least 1 year prior to participation in this study.

Methods

We examined all the patients whether they are related to thyroid disease through history taking and medical recording. Patients' blood samples were used for analysis. Detection and titration of antithyroid antibodies were performed by the technique of immunoradiometric assay using TMAb (Biocode Inc. Belgium) for antimicrosomal antibody and TGAb IRMA (Biocode Inc. Belgium) for antithyroglobulin antibody. Titers 1:50 or greater were considered positive. Thyroid function test was done by using GammaCoat kit (Incstar co, USA).

Statistical analysis

We made statistical analysis of antithyroid antibody positivity according to sex, age, activity, duration and the type of vitiligo. The effect of each covariate on the antibody positivity was tested by x^2 test. Fisher's exact test was also used for the cases with expected numbers less than 5.

RESULTS

Seventeen (10.8%) patients had antithyroglobulin antibodies and ten (6.4%)patients had antimicrosomal antibodies. Five patients had both antibodies. Consequently, 22 patients showed antithyroid antibody positivity. Six (3.8%) patients were detected to be related to thyroid disease (Table 2). Incidence of antithyroid antibodies and thyroid disease was analyzed according to sex (Table 3), age of onset (Table 4), duration (Table 5), activity (Table 6) and type of the vitiligo (Table 7). Statictically meaningful data are as follows ; 1) Thyroid disease has high incidence in female patients. This is a well known phenomenon. 2) Antimicrosomal antibody appeared less frequently in patients of childhood-onset. 3) Antithyroglobulin antibody was detected more frequently in active disease. Fifty nine out of 157 patients were examined for thyroid function. Four out of 22 patients with antithyroid antibody had abnormal thyroid function. None out of 37 patients without antithyroid antibody had abnormal thyroid function.

DISCUSSION

Vitiligo has been reported in association with several endocrinopathies of autoimmune nature including thyroid diseases, adrenal insufficiency, diabetes mellitus and autoimmune type chronic active hepatitis[17]. In addition, vitiligo has been described in association with alopecia areata, lupus erythematosus, scleroderma, myasthenia gravis, chronic subcutaneous candidiasis, pernicious anemia, atopic eczema, psoriasis, hearing abnormalities, eye involvement and melanoma. Recently, Schallreuter et al [17] examined 321 patients with vitiligo to confirm the frequencies of associated diseases that had been described to be associated with vitiligo so far. The data obtained revealed increased incidence of thyroid disease in vitiligo(7.8%) and congenital melamocytic nevi(6.2%), whereas other diseases were a random event.

Several investigators reported the correlation of thyroid disease and vitiligo in Korean patients. Park et al[19] reported one case of thyroiditis among 326 patients with vitiligo. Kim et al[20] reported 20 cases of vitiligo(6.38%) among 293 cases of autoimmune thyroid diseases and 2 cases of vitiligo(0.88%) among 227 cases of non-autoimmune thyroid diseases. And Kim et al insisted that vitiligo preceeded thyroid disease in the cases who had vitiligo and thyroid disease. Hwang et al[21] examined autoantibodies such as antithyroglobulim antibody, antimicrosomal antibody, antinuclear antibody, rheumatoid factor in 381 vitiligo patients. Positive rate for autoantibodies were as following ; antithyroglobulin antibody 21.3%, antimicrosomal antibody 10.0%, antinuclear antibody 1.6%, rheumatoid factor 1.3%. Among 90 patients who had positive antithyroid antibody, 27 patient were tested for thyroid function and 4 patients showed hyperthyroidism. Patients with autoantibodies showed delayed onset of the disease, but there was no statistical difference in antibody positivity according to sex, duration of vitiligo, type of vitiligo. Hann et al[22] investigated organ specific actoantibodies in 226 vitiligo patients and 120 healthy controls. They correlated the presence of autoantibodies with factors such as sex, onset of disease, activity of disease, family history, duration of disease and type of vitiligo. The incidence of antibody positivity were as following ; antithyroglobulin antibody 1.8%, antimicrosomal antibody 7.1%, antinuclear antibody 12.4%, antismooth muscle antibody 25.7%, antigastric parietal cell antibody 3.5% and antimitochondrial antibody 0%. Detection and titration of antibodies to thyroglobulin and microsome were performed by the technique of indirect agglutination using Serodia^{R}-ATG(Fugirebio Inc. Tokyo, Japan). Titers over 1:100 were considered positive. Positivity of antithyroid antibodies were increased in vitiligo patients than controls(antithyroglobulin antibody 0%, antimicrosomal antibody 5.0%). Positivity of antithyroid antibodies were not related to sex, onset of disease, activity of disease, duration of disease. Incidence of the antithyroglobulin antibody was higher in vitiligo patient with the segmental type than in those with the nonsegmental type. Incidene of the antimicrosomal antibody was higher in vitiligo patients with family history than those without family history. But, both results couldn`t be explained.

We examined 157 vitiligo patients to assess the antithyroglobulin antibody, antimicrosomal antibody and thyroid function. Antithyroglobulin antibody was detected in 17 (10.8%) patients and antimicrosomal antibody was found in 10 (6.4%) patients. We measured autoantibody by immunoradiometric assay using TMAb and TGAb IRMA kit. Hann et al performed indirect agglutination test using Serodia- ATG kit. Hwang et al didn't indicate the method. Therefore, direct comparision of the incidence of antithyroid antibody in Korean vitiligo patients is impossible. The correlation of the presence of antithyroid antibodies with factors such as sex, onset, activity, duration and type of vitiligo was similarily done by us and Hann et al. Hann et al reported none of these factors are related to presence of antithyroid antibodies except that antithyroglobulin antibody was higher in vitiligo patients with the segmental type. According to our investigation, antimicrosomal antibody appeared less frequently in patients of childhood-onset and antithyroglobulin antibody was detected more frequently in active disease. Other factors had no statistical significance. These results are contradictory to each other and large scaled study will be needed to draw a conclusion in Korean patients. Antithyroid antibody positivity goes up with age and antithyroid antibodies are rarely present in children without thyroid disease. This might be related to low incidence of antimicrosomal antibody in vitiligo patients with onset below 15 in our study. Antithy-

roglobulin antibody had high incidence in patients with active disease in our study. If this phenomenon happens constantly in large scaled study, antithyroglobulin antibody can be used as an index for disease activity in vitiligo patients. In Hwang et al's study, among 90 patients who had positive antithyroid antibody, 27 patients were tested for thyroid fuction and 4 patients showed hyperthyroidism. In our study, Fifty nine out of 157 patients were examined for thyroid function. Four out of 22 patients with antithyroid antibody had abnormal thyroid function. None out of 37 patients without antithyroid antibody had abnormal thyroid function. Consequently, performance of thyroid fuction test is highly recommened if vitiligo patients shows presence of antithyroid antibody.

Thyroglobulin, the antigen of antithyroglobulin antibody, is the 670-kD protein synthesized in thyroid cells. Four to six B cell epitopes of thyroglobulin are known to be involved in the human autoimmune process. The cDNA for thyroglobulin includes a motif analogue to the enzyme acetylcholinesterase and antithyroglobulin antibodies do cross-react with acetylcholinesterase [23,24]. This finding suggests autoimmune thyroid disease may be related to myasthenia gravis. The low levels of antithyroglobulin antibodies are detected in up to 36% of normal adult women and 15% of normal adult men using sensitive assay[25]. Chung et al[26] reported antithyroglobulin antibodies are detected in 18.6% of 236 Korean normal people. Antithyroglobulin antibodies are rarely present in children without evidence of thyroid disease and the prevalence in normal person increases with age. The frequency and level of antithyroglobulin antibodies correlate with the presence of lymphocytic infiltration in thyroid gland. Over 90% of patients with Hashimoto's thyroiditis and 50% of Graves' disease have these antibodies. High antibody levels are often found in patients with hypothyroidism after thyroidectomy or ^{131}I ablation[27]. Thyroglobulin and antithyroglobulin antibody immune complexes have been detected along the basement membrane of thyroid follicular cells in patients with Hashimoto's thyroiditis. Its complex may produce tissue destruction by fixing complement or acting in conjuction with killer cells[28,29].

Antimicrosomal antibodies bind to nondenatured thyroid cytoplasm and fix complement in the presence of human thyroid membranes. This antigen is known to be thyroid peroxidase (TPO), glycoprotein present in the plasma membrane. Czarnocka et al[30] have also shown the identity of microsomal antigen and TPO. There are two forms of 107 and 101 kD by different splicing of mRNA and multiple B cell epitopes in human TPO [31,32]. Antimicrosomal antibodies are detected in 8-18% of normal adult persons [33-35]. In Koreans, antimicrosomal antibodies are detected in 15.7% of normal people, in 82.7% of patients with Graves' disease and in 82.0% of patients with Hashimoto's thyroiditis[26]. It has been reported that antimicrosomal antibodies may destroy thyroid cells by complement-dependent cytotoxicity or antibody-dependent cell mediated cytotoxicity [36,37]. Antimicrosomal antibodies also inhibit the function of TPO by binding to it although this effect is probably limited in vivo [38].

The prevalence of antithyroid antibodies goes up with age [39]. Low-level antithyroid antibodies are detected in normal persons without clinical thyroid disease and in patients after total thyroidectomy or ^{131}I ablation. Cross-reaction of antithyroid antibodies with non-thyroidal tissue is limited. These findings suggest that detection of antithyroid antibodies in patients with vitiligo may be an epiphenomenon in the autoimmune process and their role may be limited in the pathogenesis of vitiligo.

REFERENCES

1. El Mofty A, El Morf M. : Vitiligo : a symptom complex. Int J Dermatol 1019:237-44, 1980.
2. Macron C, Winter Kl, Traisman HS : Vitiligo and juvenile diabets melitus. Arch Dermatol 113:1515-9, 1977.
3. Harzoulis P, Kanakoudi-Tsakalidis F, Uyzantidis A : Auto-immunity and vitiligo(abstract). Arch Dermatol 114:1554-60, 1970.
4. Bettere C, Del Prete GF, Preserico A , Bersani G, Craccio F, Trisotto A et al : Autoantibodies in vitiligo. Arch Dermatol 112:1328, 1976.
5. Brostoff J, Bor S, Feiwel M : Autoantibodies in patients with vitiligo. Lancet 2:177, 1969.
6. Grimes PF, Halder R, Jones C : Autoantibodies and their clinical significance in black vitiligo population. Arch Dermatol 119:300-3, 1983.
7. Dunston GM, Halder RM : Vitiligo is associated with HLA-DR4 in black patients: apreliminary report. Arch Dermatol 126:56-60, 1990.

8. Poloy A, Tibor L, Kramer J, Anh-Tuan N, Kraszite E : HLA-DR1 is associated with vitiligo. Immunol Letters 27:59-62, 1991.
9. Foley LM, Lowe NJ, Mischelhoff E , Tiwari JL : Association of HLA-DR4 with vitiligo. J Am Acad Dermatol 8:39-42, 1983.
10. Moellmann GE, Krass P, Halaban R : On the subject of serum antibodies to melanocytes. J Invest Dermatol 84:333-4, 1985.
11. Galbraith GMP, Miller D, Emerson DL : Western blot analsis of serum antibody reactivity with human melanoma cell antigens in alopecia areata and vitiligo. Clin Immunopathol 48:317-24, 1988.
12. Naughton G, Reggiardo D, Bystryn JC : Correlation between vitiligo antibodies and extent of depigmentation in vitiligo. J Am Acad Dermatol 15:978-81, 1986.
13. Naughton GK, Eisinger M, Bystryn JC : Antibodies to normal human melanocytes in vitiligo. J Exp Med 158:246-51, 1983.
14. Hertz KC, Gazze LA, Charles AB, Kirkpatrick H, Katz SI : Auto-immune vitiligo-detection of antibodies to melanin producing cells. New Engl J Med 297:634-7, 1977.
15. Abdel-Naser MB, Kruger-Krasagakes S, Krasagakis K, Gollnick H and Orfanos CE : Further evidence for involvement of both cell mediated and humoral immunity in generalized vitiligo. Pigment cell res : 711-8, 1994.
16. Amos G, Brian Z, Yehuda U, Amos E : In vivo destruction of melanocytes by the IgG fraction of serum from patients with vitiligo. J Invest Dermatol 105: 683-6, 1995.
17. Schallreuter KU, Lemke R, Brandt O,Schartz R, Westhofen M, Montz R et al :Vitiligo and other diseases : Coexistence or true association ?. Dermatology 188:269-5, 1994.
18. Laszlo H, Michael H, Morten G, Henfick H and Mimi HM : High frequency of thyroid dysfunction in patients with vitiligo. Acta Derm Venereol 74:120-3, 1994.
19. Park KC, Youn JI, Lee YS : Clinical study of 326 cases of vitiligo. Kor J Dermatol 26(2):200-5, 1988.
20. Kim JA, Shong YK, Kim KH : Vitiligo in autoimmune thyroid disease. Kor J Dermatol 28(5):582-6, 1990.
21. Hwang YJ, Yang HY, Kim JH : It`s clinical analysis and autoantibodies. Kor J Dermatol 31(5):657-63, 1993.
22. Hann SK, Im SB, Kim HI, Kim HS, Lee YJ and Park YK : Increased incidence of antismooth muscle antibody in Korean vitiligo patients. J Dermatol 20:679-83, 1993.
23. Mercken L, Simons M-J, Swillens S, Massaer M, Vassart G: Primary structure of bovine thyroglobulin deduced from the sequence of its 8, 431-base complementary DNA. Nature 316 (6029): 647-51, 1985.
24. Hurel S, Wilkin TJ: Thyroglobulin antibodies cross-react with acetycholinesterase: a role in Graves' ophthalmopathy? Book of abstracts for the 17th annual meeting of the european thyroid Association, No. 80, Montpellier, France, September 11-6, 1988.
25. Ericsson UB, Christensen SB, Thorell JI: A high prevalence of thyroglobulin autoantibodies in adults with and without thyroid disease as measured with sensitive solid-phase immunosorbent radioassay. Clin Immunol Immunopathol 37 (2): 154-62, 1985.
26. Chung JH, Lee MS, Cho BY, Lee HK, Koh C-S, Min HK, Lee M: The analysis of the value of the thyroid autoantibody measured by radioimmunoassay. Kor J Nucl Med 21 (2): 133-41, 1987.
27. Lundell G, Jonsson J: Thyroid antibodies and hypothyroidism in 131I therapy for hyperthyroidism. Acta Radiol 12 (5):443-53, 1973.
28. Clagett JA, Wilson CB, Weigle WO: Interstitial immune complex thyroiditis in mice. The role of autoantibody to thyroglobulin. J Exp Med 140: 1439-56, 1974.
29. Kalderon AE, Bogaars HA: Immune complex deposits in Graves' disease and Hashimoto's thyroiditis. Am J Med 63: 729- 34, 1977.
30. Czarnocka B, Ruf J, Ferrand M, Carayon P, Lissitzky S: Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. FEBS Letters 190 (1) : 147-52, 1985.
31. Hamada N, Grimm C, Mori H, DeGroot LJ: Identification of a thyroid microsomal antigen by western blot and immunoprecipitation. J Clin Endocrinol Metab 61 (1): 120-8, 1985.
32. Hamada N, Portmann L, DeGroot LJ: Characterization and isolation of thyroid microsomal antigen. J Clin Invest 79 (3): 819- 25, 1987.
33. Yoshida H, Amino N, Yagawa K, Uemura K, Satoh M, Miyai K, Kumahara Y: Association of serum antithyroid antibodies with lymphocytic infiltration of the thyroid gland: studies of seventy autopsied cas-

es. J Clin Endocrinol Metab 46: 859-62, 1978.
34. Amino N: Antithyroid antibodies. In: Werner's the thyroid, 5th ed. JB Lippincott, Philadelphia, p267, 1986.
35. Prentice LM, Philips DIW, Sarsero D, Beever K, McLachlan SM, Smith BR: Geographical distribution of subclinical autoimmune thyroid disease in Britain: A study using highly sensitive direct assays for autoantibodies to thyroglobulin and peroxidase. Acta Endocrinol (Copenh) 123: 493-8, 1990.
36. Khoury EL, Hammond L, Botazzo GF, Doniac D: Presence of the organ-specific microsomal autoantigen on the surface of human thyroid cells in culture: Its involvement in complement-mediated cytotoxicity. Clin Exp Immunol 45: 316-8, 1981.
37. Bogner U, Schleusener H, Wall JR: Antibody-dependent cell mediated cytotoxicity against human thyroid cells in Hashimoto's thyroiditis but not Graves' disease. J Clin Endocrinol Metab 59: 734-8, 1984.
38. Okamoto Y, Hamada N, Saito H, Ohno M, Noh J, Ito K, Morii H: Thyroid peroxidase activity-inhibiting immunoglobulins in patients with autoimmune thyroid disease. J Clin Endocrinol Metab 68 (4): 730-4, 1989.
39. Dingle PR, Ferguson A, Horn DB, Tubmen J, Hall R: The incidence of thyroglobulin antibodies and thyroid enlargement in a general practice in northeast England. Clin Exp Immunol 1 (3): 277-84, 1966.