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Original Article

Characterization of tyrosinase and selected autoantibodies in Chinese vitiligo patients

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Abstract: Background: Autoantibodies detected in vitiligo patients are most commonly directed against pigment cell. However, the correlation between the level of these autoantibodies and disease activity remains obscure, especially in Chinese vitiligo patients. Objectives: Tyrosinase, tyrosinase antibody and melanocyte antibody in 139 vitiligo patients and 20 control individuals were detected. Methods: Seven commercial ELISA kits were used for detecting tyrosinase, tyrosinase antibody and melanocyte antibody. Results: Tyrosinase and autoantibodies to tyrosinase and melanocyte were more common in active and non-segmental vitiligo patients. There was a significant negative association between disease duration and the level of tyrosinase and selected antibodies. A significant trend between more extensive involvement and higher level of tyrosinase and selected antibodies was also found. In addition, a good agreement among the kits for the detection of tyrosinase, tyrosinase antibody or melanocyte antibody in vitiligo patients was observed. Conclusion: Our studies suggest that autoantibodies to melanocyte are involved in the pathogenesis of vitiligo.

Keywords: Chinese vitiligo patients, tyrosinase, tyrosinase antibody, melanocyte antibody

Introduction

Vitiligo is a skin disorder that is caused by selective melanocyte destruction, resulting in partial loss of depigmentation on skin and mucosal surfaces [1-3]. Vitiligo is a common, disease which involves approximately 0.5%-2% of the global population [4]. The disease was classified based on distribution patterns of vitiliginous lesions into segmental vitiligo and non-segmental vitiligo including acrofacial, generalized, mucosal (multifocal), and sporadic vitiligo [5]. Vitiligo is a long-term skin condition characterized by patches of skin losing their pigment, which, if untreated, is typically progressive and irreversible.

Although the exact aetiology of vitiligo remains obscure, autoimmunity has been suggested to play a role in the development of the disease, as some studies have shown that antimelanocyte autoantibodies are often present in the sera of vitiligo patients [6-8]. Further evidence for an autoimmune involvement in vitiligo development, autoantibodies, tyrosinase, gp100, from patients with vitiligo can destroy melanocytes both in vitro and in vivo [9, 10]. However, there has been a considerable controversy on the positive rate of tyrosinase and their relevance to the pathogenesis of vitiligo. Various studies show that the incidence of tyrosinase in vitiligo patients is quite different [11-13].

Although several earlier studies have reported the clinical and demographic characteristics of vitiligo patients [14-16], none has characterized the relationship between serological profile of individuals and the disease. The identification and characterization of their target antigens could be a landmark for uncovering the pathogenic mechanism. In this study we established the clinical, demographic details and the results of serological in a selected population of vitiligo patients from China, and evaluated the data for any correlations between clinical and demographic details. Seven commercial ELISA kits for detecting tyrosinase, tyrosinase antibody or melanocyte antibody (MC-Ab) were evaluated regarding their ability to detect them.

Table 1. Demographic and clinical details of vitiligo patients

Clinical or demographic detail	Non-segmental vitiligo	Segmental vitiligo	*P*-value
sex			
male	72/108	19/31	0.58
female	36/108	12/31	0.58
Age (years)			
Mean ± SD	33.2±11.3	32.5±11.8	0.75
<28	38/108	12/31	0.53
≥28	70/108	19/31	0.53
Onset age (years)			
Mean ± SD	26.7±11.3	28.1±11.9	0.54
<25.5	54/108	15/31	0.74
≥25.5	66/108	16/31	0.74
Disease duration (years)			
Mean ± SD	6.5±7.2	4.3±7.4	0.14
<1	12/108	13/31	8.19E-5***
≥1	96/108	18/31	8.19E-5***
Vitiligo activity			
active	86/108	29/31	0.07
stable	22/108	2/31	0.07

***denotes P<0.0001.

Materials and methods

Patients

139 patients who had no other autoimmune disorders and no family history of autoimmune disease were involved in this study. The patients were diagnosed with vitiligo and treated between Jan 2017 and Jan 2018 at our hospital. Informed consent was obtained from each patient before the collection of blood for the autoantibody study. The study was conducted in accordance with the Declaration of Helsinki Principles and sanctified by Ethics Committee of air force medical center (NO 20161114002) on 14 Nov, 2016. 108 patients (72 male, 36 female; mean age: 33 years with range 4-60 years; mean disease duration: 6.5 years with range <0.1-40 years; mean age at onset: 26.7 years with range <1-54 years) was classified as non-segmental (Generalized, 9; Acrofacial, 29; Mucosa, 3; Sporadic, 67). 31 patients was classified as segmental (19 male, 12 female; mean age: 32 years with range 18-60 years; mean disease duration: 5.7 years with range <0.1-30 years; mean age at onset: 28.3 years with range <11-57 years). 24 patients had stable vitiligo (no disease progression/new lesions over the preceding 6 months) and 115 had active disease (the appearance of new lesions or enlargement of pre-existing lesions during the preceding 3 months). Percentage of body surface area (BSA) was used as the rule of nines as in burn assessment. Twenty healthy individuals (15 males, 5 females; mean age: 29 years with range 21-34 years) were taken for comparison in this study. The individuals were free of any infections and had no present or past history of vitiligo or autoimmune disorders.

ELISA tests

Patient serum was separated from whole blood samples (10-20 ml) by centrifugation at 3000 g for 15 min before storage at -80°C. The levels of serum tyrosinase, tyrosinase antibody or MC-Ab autoantibodies were determined by ELISA. The levels of serum tyrosinase were analyzed in three kits (kitA1: JL13940, Shanghai Jiang Lai Biotechnology Co., Ltd; kitB1: G-06109X, Shanghai GTx international corporation; kitC: CSB-EL025394HU, Wuhan Huamei Biological Engineering Co., Ltd). The levels of serum tyrosinase antibody were analyzed in two kits (kitA2: JL13563, Shanghai Jiang Lai Biotechnology Co., Ltd; kitB2: YS02939B, Shanghai GTx international corporation). The levels of serum MC antibody were analyzed in two kits (kitA3: JL19463, Shanghai Jiang Lai Biotechnology Co., Ltd; kitB3: YS-T10393, Shanghai GTx international corporation). All procedures were performed according to the manufacturer's directions. All assays were performed in triplicate.

Statistical analysis

SPSS 17.0 and MedCalc 15.2.2 were used for statistical analysis. Pearson or spearman correlation was used to evaluate the relationship between the results of the methods. Student's t test was performed to evaluate the difference of the antibodies between the serum of patients and healthy individuals. Student's t test was performed to evaluate the difference of the antibodies between the serum of active and stable vitiligo patients. *P*-value was considered significant if below 0.05.

Table 2. Demographic and clinical details of Non-segmental patients

Clinical or demographic detail	Sporadic	Generalized	Acrofacial	Mucosa	*P*-value
sex					
male	43/67	6/9	22/29	1/3	0.4
female	24/67	3/9	7/29	2/3	0.4
Age (years)					
Mean ± SD	32.5±11.7	39.2±11.7	33.4±10.4	28.0±6.6	0.33
Onset age (years)					
Mean ± SD	25.8±11.6	29.1±11.6	27.0±7.2	26.7±11.3	0.76
Disease duration (years)					
Mean ± SD	6.7±7.2	10.1±12.6	5.4±4.6	1.0±1.0	0.2
Vitiligo activity					
active	53/67	9/9	21/29	3/3	0.33
stable	14/67	0/9	8/29	0/3	0.33

Table 3. Relation between disease activity and tyrosinase, tyrosinase antibody and MC-Ab

	KitA1	KitB1	KitC	KitA2	KitB2	KitA3	KitB3
active							
Mean ± SD	4.8±4.5	3.4±2.5	16.2±11.1	24.3±23.1	3.0±2.4	39.0±42.2	2.9±2.4
stable							
Mean ± SD	2.6±0.9	1.9±2.3	11.1±9.3	14.0±9.7	1.6±1.1	21.6±9.3	1.2±1.1
P-value	0.021*	0.007**	0.027*	0.001**	<0.001***	0.047*	0.001**

*denotes P<0.005; **denotes P<0.001; ***denotes P<0.0001.

Results

Demographic and clinical details of vitiligo patients

139 vitiligo patients were included in this study. Their demographic and clinical details were summarized in **Table 1**. Among the patients, Non-segmental vitiligo was the most common clinical sub-type (108/139). Additionally, among the cases of non-segmental vitiligo, 67 had sporadic vitiligo, 29 had acrofacial vitiligo, 9 had generalized vitiligo and 3 had mucosal type vitiligo. There was no significant difference on the mean duration of disease and the mean age of vitiligo onset of the clinical sub-types (segmental or non-segmental). Compared with segmental vitiligo patients', most of Non-segmental patients' disease duration was more than one year. The demographic and clinical details of Non-segmental patients were summarized in **Table 2**.

Relation between disease activity and tyrosinase, tyrosinase antibody or MC-Ab

Sera from patients with active vitiligo (n = 115) and stable vitiligo (n = 24) were evaluated for tyrosinase, tyrosinase antibody or MC-Ab antibody reactivity to examine the relation between the activity of vitiligo and the level of tyrosinase, tyrosinase antibody or MC-Ab antibody. All of them in active vitiligo patients were higher than those in stable vitiligo patients. The results were shown in **Table 3**.

Relation between disease extent and tyrosinase, tyrosinase antibody or MC-Ab

The association was examined in 139 patients in whom information on the extent of body surface area involved was available. As shown in **Table 4**, there is a significant trend between more extensive involvement and higher level of tyrosinase, tyrosinase antibody and MC-Ab antibody.

Relation between disease duration and tyrosinase, tyrosinase antibody or MC-Ab

The relation between disease duration and the level of tyrosinase, tyrosinase antibody and MC-Ab antibody was examined in 139 patients. There was a significant negative association between disease duration and the level of tyrosinase, tyrosinase antibody and MC-Ab antibody, the results was shown in **Figure 1**.

Table 4. Relation between disease extent and tyrosinase, tyrosinase antibody and MC-Ab

	KitA1	KitB1	KitC	KitA2	KitB2	KitA3	KitB3
disease extent							
r	0.195	0.281	0.256	0.173	0.236	0.202	0.433
P-value	0.022*	0.001**	0.002**	0.042*	0.005**	0.017*	<0.0001***

*denotes P<0.005; **denotes P<0.001; ***denotes P<0.0001.

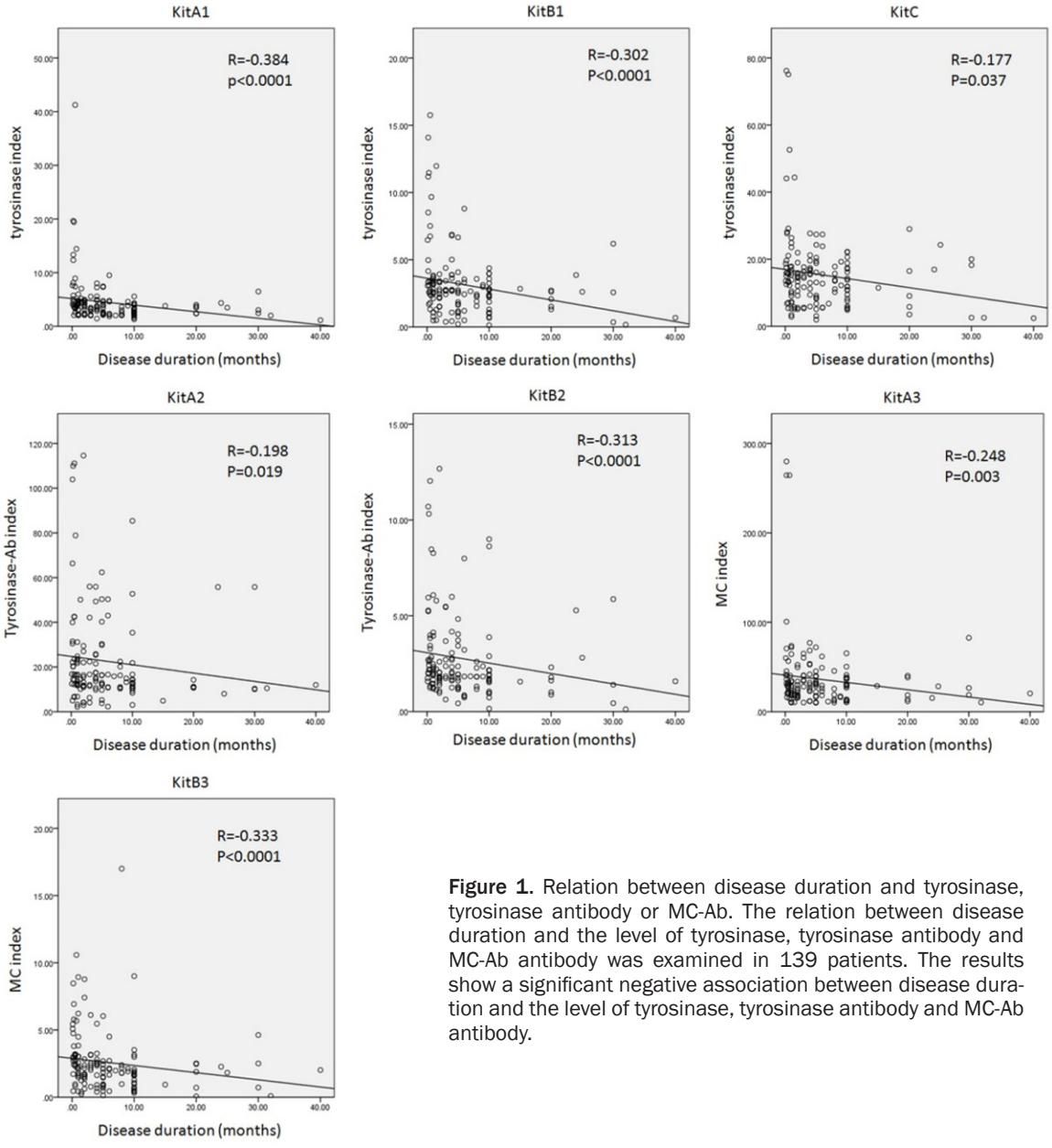


Figure 1. Relation between disease duration and tyrosinase, tyrosinase antibody or MC-Ab. The relation between disease duration and the level of tyrosinase, tyrosinase antibody and MC-Ab antibody was examined in 139 patients. The results show a significant negative association between disease duration and the level of tyrosinase, tyrosinase antibody and MC-Ab antibody.

Relation between disease classification and tyrosinase, tyrosinase antibody or MC-Ab

The association was examined between disease classification and the level of selected antibodies. There was a significant correlation between disease classification and the level of tyrosinase, tyrosinase antibody and MC-Ab antibody, all of them in non-segmental vitiligo patients (n = 108) were higher than that in seg-

Table 5. Relation between disease classification and tyrosinase, tyrosinase antibody and MC-Ab

	KitA1	KitB1	KitC	KitA2	KitB2	KitA3	KitB3
segmental							
Mean ± SD	3.1±1.3	1.4±1.4	10.0±6.3	16.3±13.0	2.0±1.4	15.6±5.5	1.4±1.4
non-segmental							
Mean ± SD	4.8±4.7	2.9±2.4	16.8±11.6	24.3±23.4	3.0±2.4	41.9±42.5	2.9±2.4
P-value	0.002*	<0.0001***	<0.0001***	0.016*	0.004**	0.043*	<0.0001***

*denotes P<0.005; **denotes P<0.001; ***denotes P<0.0001.

Table 6. Relation between tyrosinase, tyrosinase antibody and MC-Ab levels in vitiligo patients

	KitA1/KitA2	KitA1/KitA3	KitA2/KitA3	KitB1/KitB2	KitB1/KitB3	KitB2/KitB3
r	0.365	0.335	0.404	0.498	0.291	0.672
P-value	9.763E-06***	5.415E-05***	8.285E-07***	4.395E-10***	5.652E-07***	1.07E-19***

***denotes P<0.0001.

mental vitiligo patients (n = 31). The results were shown in **Table 5**.

Relation between tyrosinase, tyrosinase antibody or MC-Ab levels in vitiligo patients

There was a significant association between the expression of tyrosinase, tyrosinase antibody and MC-Ab antibody in vitiligo patients, the results was shown in **Table 6**.

Comparison of different kits for detecting tyrosinase, tyrosinase antibody or MC-Ab in vitiligo patients

To reveal if different kits were presenting similar qualitative results, a scatter plot, displaying the results for each patient in each kit compared two by two, was constructed. As shown in **Figure 2** and **Table 7**, there was a good agreement among the kits for the detection of tyrosinase, tyrosinase antibody or MC-Ab in vitiligo patients. Comparison of the results using Bland-Altman analysis basing on assessment of differences (**Figure 2**) shows that 94%-96% of the values falls within a specified interval (mean ± 1.96 SD).

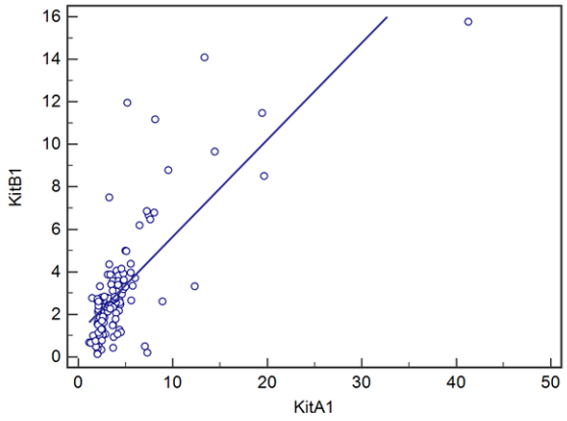
Expression of tyrosinase, tyrosinase antibody or MC-Ab in patients with vitiligo and controls

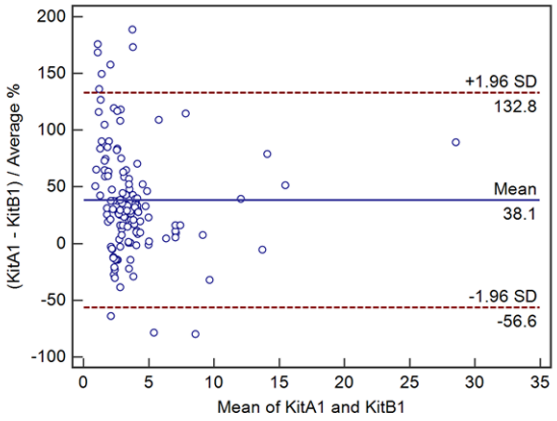
The expression level of tyrosinase, tyrosinase antibody and MC-Ab in patients with vitiligo and controls were detected by different ELISA kits. All of them in vitiligo patients were higher than that in healthy individuals. The results showed in **Table 8**.

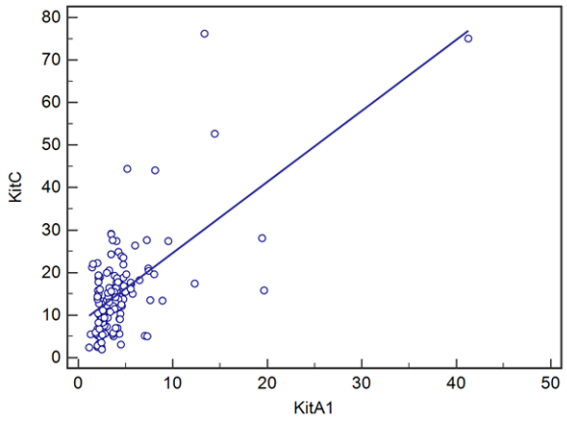
Discussion

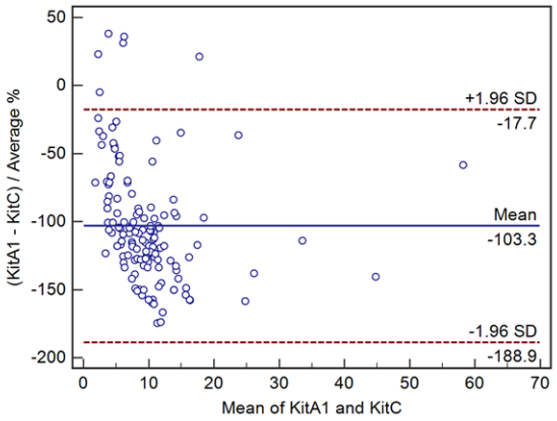
The presence of autoantibodies directed against pigmented cells in sera of patients with vitiligo is well established and there is a direct correlation between the level of these autoantibodies and disease activity [17, 18]. But the reports were still contradictory, it has been reported that patients with diffuse vitiligo had significantly higher titres of IgG anti-tyrosinase autoantibodies than patients with localized disease or healthy subjects [19]. But Dorđić found that lower levels of IgM anti-tyrosinase autoantibodies existed in vitiligo patients compared to controls [20]. Xie observed none of the vitiligo or control individuals had antibodies to tyrosinase [13]. Kroon reported 42.8% of the vitiligo patients showed an antibody against tyrosinase, MART1, MCHR1, gp100 or TH. However, the level of antibodies against melanocytes did not correlate with recent disease activity or other relevant disease parameters [21]. Eskandani observed lower tyrosinase activity in skin with lesion than in skin without lesion [22]. Cui reported cytolytic antibodies to melanocytes were more frequent in patients with active vitiligo than in those with inactive disease and more frequent in patients with vitiligo than control individuals [23].

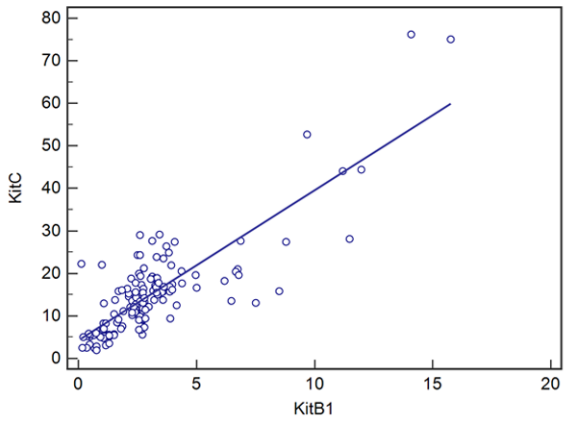
In this study, seven commercial ELISA kits were used to detect tyrosinase, tyrosinase antibody or MC-Ab in 139 vitiligo patients and 45 healthy individuals. Then the relation between clinical details and the levels of tyrosinase, tyrosinase antibody and MC-Ab was established. This study indicated that all the three

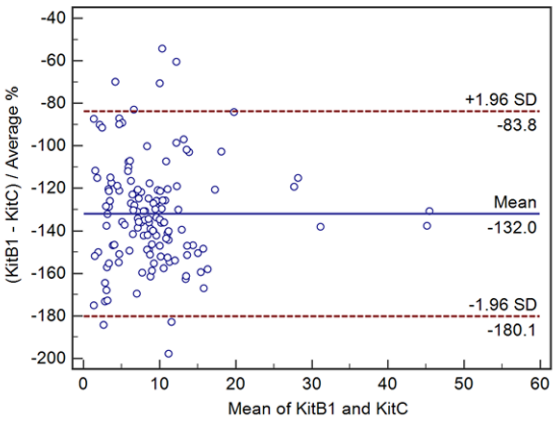

KitB1
KitA1

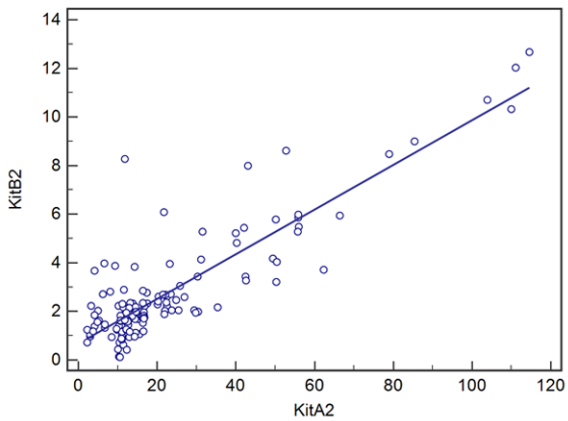

(KitA1 - KitB1) / Average %
Mean of KitA1 and KitB1
+1.96 SD
132.8
Mean
38.1
-1.96 SD
-56.6

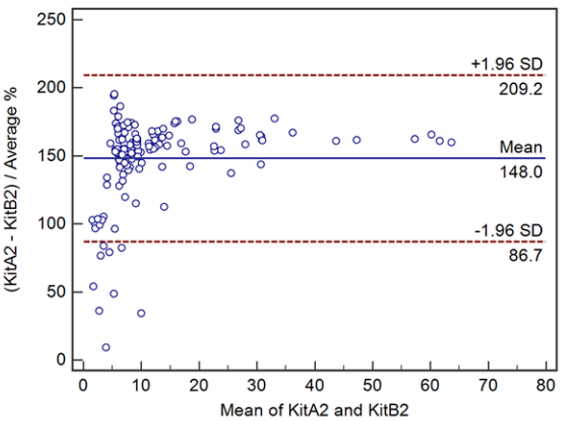

KitC
KitA1


(KitA1 - KitC) / Average %
Mean of KitA1 and KitC
+1.96 SD
-17.7
Mean
-103.3
-1.96 SD
-188.9


KitC
KitB1


(KitB1 - KitC) / Average %
Mean of KitB1 and KitC
+1.96 SD
-83.8
Mean
-132.0
-1.96 SD
-180.1


KitB2
KitA2


(KitA2 - KitB2) / Average %
Mean of KitA2 and KitB2
+1.96 SD
209.2
Mean
148.0
-1.96 SD
86.7

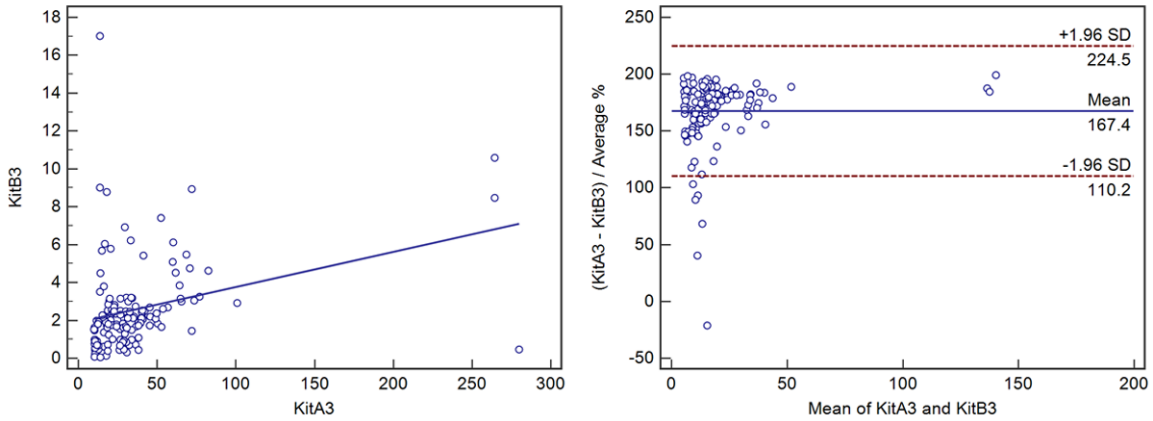


Figure 2. Comparison of different kits for detecting tyrosinase, tyrosinase antibody or MC-Ab in vitiligo patients. A scatter plot displaying the results for each patient in each kit compared two by two was constructed. Bland-Altman analysis was used to comparison the results of differences and shows that 94%-96% of the values falls within a specified interval.

Table 7. Comparison of different kits for detecting tyrosinase, tyrosinase antibody and MC-Ab in vitiligo patients

	KitA1/KitB1	KitA1/KitC	KitB1/KitC	KitA2/KitB2	KitA3/KitB3
r	0.668	0.456	0.726	0.687	0.381
P-value	2.515E-19***	1.756E-08***	5.306E-24***	1.013E-20***	3.755E-06***

***denotes P<0.0001.

Table 8. Expression of tyrosinase, tyrosinase antibody and MC-Ab in patients with vitiligo and controls

	KitA1	KitB1	KitC	KitA2	KitB2	KitA3	KitB3
vitiligo							
Mean ± SD	4.4±4.2	3.1±2.6	15.3±11.0	22.5±21.7	2.7±2.3	36.0±39.1	2.6±2.3
controls							
Mean ± SD	2.1±1.3	1.9±1.1	4.47±4.18	10.3±8.5	2.6±3.5	17.0±11.6	1.3±1.6
P-value	0.048*	0.042*	0.01*	0.001*	0.01*	0.049*	0.048*

*denotes P<0.005.

selected materials were more common in active and non-segmental vitiligo patients. Previous studies found that tyrosinase antibodies had no relevance to duration of vitiligo disease or the type of vitiligo (including only one segmental vitiligo patient) [11, 24], which seems to be contrary to the intuition that patients with higher autoantibodies tend to have longer disease duration. Hani reported that oxidized tyrosinase was significantly higher among vitiligo patients whose disease duration was longer than 10 years [17]. Here we found a significant negative association between disease duration and the level of tyrosinase, tyrosinase antibody and MC-Ab. The reason may be the difference of disease extent or recent disease activity of vitiligo patients. We also found a significant trend between more extensive involvement and higher level of selected materials. Autoantibodies was one of the reason of melanocyte loss and tyrosinase may cause autoimmune attack against pigment cell, antityrosinase was one marker of absence of melanocytes, so this maybe why the trend of tyrosinase, tyrosinase antibody and MC-Ab in vitiligo patients is consistent. In addition, a good agreement among the kits for the detection tyrosinase, tyrosinase antibody or MC-Ab in vitiligo patients was found. This shows that the results of different test kits have good consistency. In addition, the level of tyrosinase, tyrosinase antibody and MC-Ab in

vitiligo patients was higher than that in healthy individuals.

In summary, these results suggest that autoantibodies to melanocyte are involved in the pathogenesis of vitiligo. The simple ELISA as described may serve as a serological test for disease activity. The role of tyrosinase, tyrosinase antibody or MC-Ab in the pathogenesis of vitiligo needs further investigation.

Disclosure of conflict of interest

None.

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