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EXPRESSION OF NATURAL KILLER ACTIVATING RECEPTOR (NKP46) IN NON-SEGMENTAL VITILIGO

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ABSTRACT

Introduction: NKp46 is unique amongst the natural cytotoxicity receptors (NCRs) and is considered the most specific NK cell marker. NKp46 is clearly important for anti-viral immunity, malignancy and autoimmune diseases. **Aim of work:** The aim of this study was to assess the level of NKp46 (lesional and peri-lesional) in vitiligo patients to assess its possible role in the pathogenesis of vitiligo. **Patients & methods:** The present case-control study included 20 vitiligo patients and 20 healthy age and sex matched controls. Skin biopsies were taken from all participants to examine the levels of NKp46. **Results:** The results showed that NKp46 levels were significantly higher in patients (lesional and peri-lesional) than in controls. lesional NKP46 levels were also significantly higher than its peri-lesional levels. Higher lesional (but not the peri-lesional) NKp46 levels were found in patients with positive family history. No significant correlations were detected between NKp46 and age, sex, extent and duration of disease. **Conclusion:** The present study suggests a possible role for NKp46 receptors in the pathogenesis of vitiligo.

KEYWORDS: Vitiligo, Natural Killer Cells, NKp46.

INTRODUCTION

Vitiligo is an acquired idiopathic depigmentary skin disease with partial or complete loss of melanocytes.[1] The pathogenesis of this disorder is uncertain but seems to depend on the interaction of genetic, immunological, neurological,[2] and possibly other factors such as oxidative stress.[3]

The autoimmune theory is suggested based on the observed association between vitiligo and other autoimmune diseases, such as hypothyroidism and diabetes.[4-8] Also, infiltration of T-lymphocytes was observed in vitiligo lesions[9] and melanocyte-specific antibodies were found in the blood of vitiligo patients. So, the role of adaptive immunity could be concluded from all these findings,[10-12] but there are also early suggestions that innate immunity is disturbed in vitiligo.[13] A more recent study using transcriptional analysis showed abnormalities in innate immunity in both lesional and non lesional vitiligo skin.[14]

Natural killer (NK) cells are part of the innate immune system. They differentiate like T- and B-lymphocytes, from the common lymphoid progenitor, in the bone marrow.[15] They are CD3-ve lymphocytes and don't rearrange antigen receptors.[16] Natural killer cells are identified by the expression of CD16 and CD56, even though NKp46 (CD335) has been suggested as an alternative marker.[17]

In addition to its primary role in innate immunity, Natural killer (NK) cells were connected to autoimmune diseases.[18] They were found deficient in the peripheral blood of many autoimmune diseases e.g. SLE,[19] autoimmune thyroid disease (20). Also, natural killer cells accumulation was found in the affected tissues of autoimmune diseases e.g. Type I diabetes,[21] alopecia areata,[22] juvenile dermatomyositis,[23] and rheumatoid arthritis.[24]

Natural killer cell responses are controlled by the interactions between different activating and inhibitory receptors. The activating NK cell receptors recognize tumor-, pathogen-, stress-induced, and self-ligands. The most prominent NK cell activating receptors are the natural cytotoxicity receptor (NCR) family. NKp46 is unique amongst the NCRs. It is the most specific NK cell marker and is considered a major activating receptor. It is expressed on both resting and activated NK cells.[25]

It was found that the murine activating receptor NKp46 (NCR1) is essential for the development of type-1 diabetes (T1D) as autoimmune disease.[26]

The aim of this study was to measure the level of NKp46 in vitiligo patients in comparison with healthy controls in order to verify their possible role in the pathogenesis of vitiligo.

PATIENTS AND METHODS

The current case-control study was conducted in the outpatient clinic Kasr Al-Ainy, Cairo University, after the approval of the ethical committee of the Dermatology department. A total of 20 patients (all over 16 years old) with a confirmed diagnosis of generalized (>5%) non segmental vitiligo, and 20 age and sex matched controls were included in this study (table 1).

Vitiligo patients with other dermatological and/or systemic diseases were considered ineligible to be included. Patients were kept off any topical or systemic treatment for vitiligo for at least 2 month prior to inclusion.

After the signing of an informed consent by the patients, all included patients were subjected to history taking, physical examination and skin biopsy from lesional & perilesional areas. Skin biopsies were taken with a 3 mm punch and were incubated in PBS buffer (PH7.2-7.4, rapidly frozen with liquid nitrogen. After melting, samples were maintained at 2-8℃. PBS was added then homogenized by homogenizer, then centrifugation was performed for 20-min at the speed of 3000 r.p.m. Supernatant was obtained and stored at -80 C^0 till utilization.

Estimation of Human Natural cytotoxicity triggering receptor 1 (NCR1/CD335) in skin biopsy samples (quantitative detection of NCR1/CD335 level) was performed using a commercially available enzyme linked immunosorbent assay (ELISA) kit provided by Sun Red Biotechnology following the manufacturer's recommendations.

Statistical method

Data were coded and entered using the statistical package SPSS version 22. Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test (27). For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 (28). Correlations between quantitative variables were done using Pearson correlation coefficient (29). P-values less than 0.05 were considered as statistically significant.

RESULTS

NKp46 levels were higher in patients (lesional and peri-lesional) than in controls with statistically significant difference (p=<0.001and 0.019respectively) (table2), Also There were statistically significant difference between lesional and peri-lesional NKP46 levels in patients (p=0.002) (table 3).

Regarding family history it was found that lesional NKp46 levels were higher in patients with positive family history while peri-lesional NKp46 levels were not significant as (P were 0.014 and 0.499 respectively) (table 4).

There was no statistically significant difference between Lesional or peri-lesional NKp46 between both sex (p= 0.072 and 0.239 respectively). Also previous exposure to psychic stress did not give statistically significant difference between lesional and peri-lesional NKp46 (p=0.797 and 0.720 respectively).No statistically significant correlations were detected between NKp46 and age, sex, extent and duration of the disease (table 5).

DISCUSSION

The present study demonstrated a significant increase in NKp46 in the skin microenvironment of melanocytes for both lesional and peri-lesional skin compared to controls. To our knowledge this is the 1st study to measure the specific NKp46 receptor in vitiligo.

The increased NKp46 levels in peri-lesional skin of vitiligo patients may indicate an unfavourable generalized melanocyte-microenvironment that acts as a bad soil for the melanocyte to survive. However, what causes this NK activation in vitiligo skin is not clear.

Going with our results, a study in 2012 which reported a significant increase in the proportion of NK cells in vitiligo lesions as demonstrated by the presence of CD3-NKG2D+ cells (Natural Killer Group 2 member D, activating receptor) not only in the LS (Lesional skin) but also in the normal-appearing NLS (Non lesional skin).[14]

Many older studies have also shown an increase in the number of circulating NK cells in the blood of vitiligo patients.[30-34]

Also through researches on mice, it was found that NKG2D receptors, present in both humans and mice were higher in vitiligo mice than normal.[35] And when percentages of natural killer cells (CD3-CD56+) were studied, they were found significantly higher in vitiligo patients than normal.[36]

Other contradictory studies found that $CD16^+CD56^+$ and $CD45RA^+$ cells which are peripheral blood natural killer (NK) cells,[37] and iNKT cells (another variant of NK cells).[38] were significantly reduced in vitiligo patients compared to healthy controls.

In our study, we also found that Lesional NKp46 levels were significantly higher in patients with positive family history. This finding is going with the well-known fact that vitiligo has genetic factors, and adds to the belief of the role of NK cells in vitiligo.

There was no significant correlation between Lesional and peri-lesional NKp46 levels and age, duration or extent of vitiligo.

In other autoimmune diseases like type I diabetes (which is often associated with vitiligo), NKp46 recognizes ligands expressed by islet β-cells, and that in the absence of NKp46 diabetes development is inhibited.[26]

Targeted immunotherapy carries a great promise for the treatment of many autoimmune and inflammatory diseases.[39] Recently, it was found that anti-NKp46 treatment significantly delayed diabetes early development in NOD (non-obese diabetic) models. Both, short-term and repeated long-term treatments with anti-NKp46 monoclonal antibodies resulted in an NKp46-specific impairment of NK function without NK depletion "Targeted immunotherapy".[40]

RECOMMENDATIONS

The new finding in our study that NKp46 level is higher in vitiligo patients than normal individuals, both in lesional and peri-lesional skin can be a path to detect that NKp46 has a role in vitiligo pathogenesis and progression. So, studies targeting immunotherapy for vitiligo can focus more on anti-NKp46 as a treatment that could provide a chance for a definitive treatment for vitiligo patients.

Table 1: Dermographic and clinical data of the studied groups.

Variables	Vitiligo patients N=20	Controls N=20
Age (years) **Range** **Mean ±SD**	 22 - 53 36.90 ±10.90	 28-55 39.30±7.71
Sex **Males N (%)** **Females N (%)**	 8 (40%) 12 (60%)	 10 (50%) 10 (50%)
Duration (years) **Range** **Mean ±SD**	 0.75 - 15 7.14±4.45	
Extent **Range** **Mean ±SD**	 10% - 45% 23.50±11.01	
Stress **positive N (%)** **Negative N (%)**	 9 (45%) 11(55%)	
Family history **positive N (%)** **Negative N (%)**	 4(20%) 16(80%)	
NKp46 levels (lesional) **Mean ±SD**	 5.21 ±1.09	
NKp46 levels (peri-lesional) **Mean ±SD**	 4.16± 0 .71	
NKp46 levels (Controls) **Mean ±SD**		 3.65 ± 0.62

Table 2: Comparison between patients and controls regarding NKp46 levels.

Variables	Patients		Control		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
Lesional NKp46 level	5.21	1.09	3.65	.62	< 0.001
Perilesional NKp46 level	4.16	.71	3.65	.62	0.019

Table 3: Comparison between patients regarding lesional and peri-lesional NKp46 levels.

	Patients		P value
	Mean	Standard Deviation	
Lesional NKp46 level	**5.21**	**1.09**	**0.002**
Perilesional NKp46 level	**4.16**	**.71**	

Table 4: Correlations between lesional and perilesional skin level of NKp46 with family history in patients.

	Family history				P value
	Yes		No		
	Mean	Standard Deviation	Mean	Standard Deviation	
Lesional CD 335 level	5.99	.41	5.01	1.13	0.014
Perilesional CD 335 level	4.38	.48	4.11	.75	0.499

Table 5: Correlation between lesional &peri-lesional NKp46 levels and age, duration & extent.

		Lesional NKp46 level	Perilesional NKp46 level
Age	Pearson Correlation	.326	.025
	P value	.161	.917
	N	20	20
Duration (years)	Pearson Correlation	.171	-.247-
	P value	.472	.293
	N	20	20
Extent (%)	Pearson Correlation	.092	-.113-
	P value	.700	.637
	N	20	20

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