Original paper

Assessment of ADAM17 and ADAM10 proteins with CXCL10 and thyroid autoimmunity in vitiligo pathogenesis

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Abstract

Introduction: Vitiligo is an acquired chronic pigmentation disorder. The etiopathogenesis is still not fully understood.
Aim: To research the correlation of ADAM proteins, shown to be associated with autoimmune diseases like rheumatoid arthritis and lupus erythematosus, with vitiligo also considered to be an autoimmune disease.
Material and methods: The study included a patient group of 45 patients with the diagnosis of vitiligo and a control group of 45 healthy adults. The ADAM10 and ADAM17 protein serum levels and CXCL10 and thyroid autoantibody anti-TG and anti-TPO levels along with FT3, FT4, and TSH hormone levels were determined with the ELISA method. Statistical analysis of results was made with the SPSS 22.0 program.
Results: In vitiligo patients, the ADAM10 levels (2.34 ±0.80 pg/ml) were statistically significantly low compared to the control group (10.29 ±1.71 pg/ml) ($p < 0.05$), while the ADAM17 levels (128.51 ±14.37 pg/ml) were statistically significantly high compared to the control group (16.30 ±6.31 pg/ml) ($p < 0.05$). Additionally, the CXCL10 levels were observed to be statistically significantly higher in the patient group (275.11 ±62.36) than in the control group (107.08 ±33.12). Thyroid autoimmunity test results (anti-TG, anti-TPO, and TSH) were shown to be different to a statistically significant degree in the patient group compared to the control group ($p < 0.001$, $p < 0.000$, $p = 0.003$, respectively). Statistical analyses used the Kolmogorov-Smirnov, Mann-Whitney *U* test, and the independent *T*-test.
Conclusions: We obtained data that are important in terms of understanding the pathogenesis. ADAM10 and ADAM17 proteins may be new targets for future therapeutic approaches.

Key words: vitiligo, ADAM10, ADAM17, CXCL10, thyroid autoimmunity.

Introduction

Vitiligo is an idiopathic acquired disorder characterized by depigmented macula and patches with clear boundaries occurring at any age. Skin is affected as a result of the destruction of functional melanocytes. A variety of hypotheses have been examined like genetic, autoimmune, oxidative stress, viral infection, neural, and self-destruction, for the etiopathogenesis of vitiligo. Among these hypotheses, autoimmunity is thought to play a basic role in the pathogenesis of vitiligo [1, 2].

The Th1-mediated IFNa-CXCL10 pathway is known to play an important role in the autoimmune hypothesis for vitiligo [1]. The incidence of autoimmune diseases is increased in vitiligo patients and their first-degree relatives. Both clinical and subclinical thyroid diseases are reported more commonly in vitiligo patients compared to healthy people [3].

The ADAM (a disintegrin and metalloprotease) protein family is found in the cell membrane and includes zinc-dependent metalloproteinases from many regions [4]. ADAMs are a multifunctional gene family contributing to the homeostasis of the extracellular matrix, transduction of specific intracellular signals, organogenesis, inflammation, reshaping tissue, adhesion, and cell migration [5].

Aim

In our study, based on the autoimmune theory for vitiligo disease with etiopathogenesis and clinical progress still unknown, the target was to assess the correlation of ADAM10 and ADAM17 levels with CXCL10 and thyroid autoimmunity, to determine new markers to monitor clinical progress in line with our data and to gain new approaches in terms of treatment for the literature.

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Material and methods

Our study is a clinical experimental study and it was approved by the Clinical Research Ethics Committee of our University, dated 30.05.2018 and numbered 2018–2011. The study group comprised patients attending our clinic from June 2018 to October 2019. The study included a patient group of 45 vitiligo patients and a control group of 45 healthy adults. Inclusion criteria for the patient group were as follows: no systemic inflammatory disease and/or any systemic medication treatment, no acute or chronic infection, no history of malignancy, and age 18 years or older. The patient group comprised randomly-selected patients abiding by the criteria who accepted participation in the study. The patient group was also grouped as segmental and non-segmental vitiligo patients and according to previous treatments for vitiligo. The control group comprised people with no vitiligo lesions on dermatologic examination, with no systemic inflammatory disease and/or any systemic medication treatment, no acute or chronic infection, no history of malignancy, and aged 18 years or older. The control group comprised randomly-selected healthy adults abiding by the criteria who accepted participation in the study. Before the study, each subject was informed about the aim of the study, and written consent was obtained. After the blood samples were centrifuged, serum samples were stored in a freezer at –80°C until analysis. The ADAM10, ADAM17, CXCL10, and thyroid markers FT3, FT4, TSH, anti-TG, and anti-TPO levels were analysed with the ELISA method. After collecting the data, statistical analyses were completed and a significant assessment of the control and study groups was performed.

Statistical analysis

In vitiligo patients, ADAM10, ADAM17, CXCL10, FT3, FT4, TSH, anti-TG, anti-TPO, and demographic data analysis results were statistically assessed. It was determined that ADAM10 and ADAM17 results did not show normal distribution. After the Kolmogorov-Smirnov test ($p < 0.05$), the Mann-Whitney *U* test was applied to determine significance between the groups. Values with $p < 0.05$ were accepted as significant. The data obtained as a result of CXCL10 analysis were determined to show normal distribution ($p > 0.05$). The *T*-test was applied to determine significance and it was found to be statistically significant ($p < 0.05$). When thyroid autoimmunity tests are assessed, anti-TG, anti-TPO, and TSH results did not show normal distribution ($p < 0.05$). The Mann-Whitney *U* test results found that the differences between the groups were statistically significant ($p < 0.001$, $p < 0.001$, $p = 0.003$, respectively). For the FT3 and FT4 parameters, after the Kolmogorov-Smirnov test ($p > 0.05$), the significance of difference was determined with the independent *t*-test ($p > 0.05$). Values of $p < 0.05$ were accepted as significant. Results are given as mean ± standard deviation.

Table 1. Variation in ADAM10, ADAM17 and CXCL-10 levels (pg/ml)

Parameter	ADAM 10	ADAM 17	CXCL 10
Control	10.29 ±1.71*	16.30 ±6.31*	107.08 ±33.12*
Vitiligo	2.34 ±0.80[a]	128.51 ±14.37[a]	275.11 ±62.36[a]

Groups marked 'a' are seen to be statistically significant when compared to groups marked '' (p < 0.05). Results are presented as mean ± SD.*

Results

ADAM10 levels were lower in vitiligo patients (2.34 ±0.80 pg/ml) compared to the control group (10.29 ±1.71 pg/ml) ($p < 0.05$), while ADAM17 levels were high in vitiligo patients (128.51 ±14.37 pg/ml) compared to the control group (16.30 ±6.31 pg/ml) ($p < 0.05$). Additionally, when CXCL10 levels are investigated, the difference between the control group (107.08 ±33.12) and the patient group (275.11 ±62.36) was observed to be statistically significant (Table 1).

Variation in thyroid autoantibodies was observed in 66% of vitiligo patients. As a result, considering the variation in autoimmunity and thyroid markers in vitiligo patients with a tendency toward hypo/hyperthyroidism, it is possible to say the process may be associated with inflammatory parameters as a result of ADAM10, ADAM17, and CXCL10 levels (Table 2).

The study included 90 people with 45 patients and 45 controls. The mean age in the patient group was 40.75 ±17.0 years, with a median of 47.0 years (min.: 18.0, max.: 85.0). In the control group, the mean age was 39.1 ±13.6 years, with a median of 35.0 years (min.: 19.0, max.: 82.0). There was no statistically significant difference identified between the mean ages in the patient and control groups ($p = 0.004$). Sociodemographic data of vitiligo patients and control groups were analysed. Evaluations were analysed using the χ^2 test. It was observed that 28 of 45 vitiligo patients were female and 17 were male. The control group consists of 24 female and 21 male patients. Two-thirds of vitiligo patients do not have a family history, although it was observed in 13 patients ($p = 0.0001$). The mean age of onset for vitiligo in the patient group was 31.20 ±14.73 years and the mean vitiligo disease duration was 9.8 ±12.97 years. Vitiligo was observed non-segmentally in the patient group, localized in 23 patients, acrofacial in 10, and generalized in 12 patients ($p = 0.0001$). When patients who received treatment before were compared with patients who did not receive treatment, it was observed that 25 of 45 patients received treatment ($p = 0.011$), while 42 of 45 patients were currently treated ($p = 0.0001$).

Table 2. Thyroid hormone and autoimmunity parameters in vitiligo patients

Parameter	Anti-TG	Anti-TPO	FT3	FT4	TSH
Control	M: 1.92 ±0.64* m: 0.00 (0–12)	M: 12.86 ±6.47* m: 10.66 (5–35.29)	M: 3.14 ±0.38* m: 3.07 (2.56–4.04)	M: 1.21 ±0.19* m: 1.21 (0.80–1.78)	M: 1.42 ±0.53* m: 1.40 (0.51–2.48)
Vitiligo	M: 152.36 ±103.38[b] m: 215.13 (10–461)	M: 86.56 ±116.54[b] m: 59.80 (10.37–511.60)	M: 3.15 ±0.44[b] m: 3.12 (2.30–4.43)	M: 1.21 ±0.14[b] m: 1.20 (0.94–1.58)	M: 2.07 ±1.11[b] m: 1.95 (1.00–6.07)

M: mean ± SD; m: median (min.–max.) values. Groups marked "b" are seen to be statistically significant when compared to groups marked "" ($p < 0.05$).*

Discussion

Vitiligo is an idiopathic acquired disease characterized by depigmented macules and patches with clear boundaries occurring at any age. Studies to explain the pathogenesis of vitiligo have gained speed in recent periods.

The ADAM (a disintegrin and metalloprotease) protein family is found in cell membranes of all tissues and are zinc-dependent metalloproteinases belonging to many regions [4]. ADAMs have a duty in the destruction of proteins linked to the cell membrane and play an important role in growth factor receptor transactivation [6]. ADAMs are a multifunctional gene family contributing to extracellular matrix homeostasis, specific intracellular signal transduction, organogenesis, inflammation, reshaping of tissue, adhesion, and cell migration [5].

Of proteins in the ADAM family, ADAM10 and ADAM17 have the highest similarity in the proteolytic area and this mostly results in degradation functions of overlapping substrates. ADAM10 was shown to play a role in many paracrine signal mechanisms and is responsible for the degradation of many substrates including Notch receptors, cadherins, delta-like 1, IL-6R, CXCL16, and CD23 [7]. Among these substrates, cytokines and chemoattractants (VE-cadherin, TNF-α, IL6R, CXCL16, CX3CL1) play important roles in the immune system. As a result, ADAM10 is necessary for a healthy immune system; however, it represents a potential therapeutic target as it modulates immune disorders. Keratinocyte proliferation is regulated by epithermal growth factor- (EGF), Wnt- and Notch-signals [8–10]. Interestingly, the ADAM10-dependent Notch signal acts as a negative regulator of the proliferative activity in keratinocytes. In a mouse model deficient in ADAM10, keratinocytes resembled hyperproliferative lesions observed under conditions of Notch deficiency [11, 12]. Further, growth factors like amphiregulin, HB-EGF, and TGF-β are taken again by ADAM10 and increased in psoriasis [13, 14]. ADAM10 lowers the cell surface level of the Axl receptor, an important immune regulator and receptor tyrosine kinase. Axl is expressed at high rates in macrophages and binding of the ligand mediates anti-inflammatory signals through suppression of the Gas6, NF-κB pathway [15]. Additionally, in systemic lupus erythematosus (SLE) disease, there is a change in ADAM10 levels. In our study of the autoimmune inflammatory disease of vitiligo, ADAM10 levels appeared to be reduced. In this context, our results correlate with the literature.

ADAM17 known as tumour necrosis factor transforming enzyme (TACE) is a protease expressed in many areas participating in the shedding of a variety of transmembrane proteins like growth factor ligands, cytokines, and receptors to the external environment [6]. Studies have shown that ADAM17 has a role in shedding substrates like TNF receptor I and II, L-selectin, TGF-α, IL1 receptor, apart from TNF-α [16]. Psoriatic plaques are characterized by high TGF-α levels [13] and psoriatic arthritis synovial fluid was shown to have elevated levels of the cytokine receptors soluble TNF receptor I and II [17]. A study by Conway *et al.* [18] compared TACE and the matrix metalloproteinase inhibitor dual inhibitor GW3333 with anti-TNF agents in an arthritis model in mice and show that it reducesTNF-α production while it was as effective as anti-TNF agents. In conclusion, they proposed that TACE regulation may be a beneficial therapeutic agent for the treatment of inflammatory disease by controlling a variety of growth factors and cytokine shedding. On the other hand, it is known that a range of 'immune moderated inflammatory diseases' (IMID) where TNF-α plays a key role like psoriasis, rheumatoid arthritis, multiple sclerosis, and Crohn disease share clear common pathogenetic mechanisms. In a study of a mouse model by Sato *et al.* [19], TACE was shown to play a role in psoriasis pathogenesis by causing the release of TNF-α and EGFR ligands. The same study observed that TACE inhibition cancelled keratinocyte proliferation linked to EGFR ligand and *in vitro* VEGF production. Based on this, it was considered that ADAM17 may play a role in vitiligo pathogenesis.

In our study, ADAM17 levels were determined to be increased to a significant degree compared to the control group. As vitiligo is an inflammatory autoimmune disease, it was observed that correlated results were obtained in similar types in the literature. With the observation of variation linked to ADAM10 levels, a severe increase was observed in CXCL10 levels. A correlation was observed between this chemokine level included in the inflammatory process with ADAM10.

Melanocyte destruction in vitiligo is known to form as a result of variations in humoral and cellular changes. The Th1-mediated IFN-α-CXCL10 path plays a central role in directing autoimmunity in vitiligo. Studies have stated that CXCL10 may be a clinical marker showing vitiligo

progression [1]. The incidence of autoimmune diseases like autoimmune thyroid diseases, type 1 diabetes, pernicious anaemia, rheumatoid arthritis, Addison's disease, lupus, and Guillain-Barre syndrome is increased among vitiligo patients and their first-degree relatives [3]. Both clinical and subclinical thyroid diseases are stated to be more common in vitiligo patients when compared with healthy people. Autoimmune thyroid disease is characterized by high serum antibodies directed against thyroid-specific antigens like thyroperoxidase and thyroglobulin. The development risk of autoimmune thyroid disease is higher in vitiligo patients compared to patients without vitiligo (2.5 times) and the elevated risk of thyroid antibodies in vitiligo patients is five times higher than in patients without vitiligo [20]. In our study, we found that two-thirds of our patients had increased thyroid autoimmunity markers. In the literature, when investigated with ADAM10 levels, the reduction in ADAM10 levels and increases in ADAM17 and CXCL10 levels linked to chronic inflammation show hypothyroidism was observed to develop in patients. Considering treatments received and continuing, it is possible to say that vitiligo developed with hypothyroidism.

Conclusions

The assessment of vitiligo disease in our study emphasized the importance of metalloprotease levels one more time. By the literature, the correlation of CXCL10 with the autoimmune disease of vitiligo and the association of thyroid autoimmunity with vitiligo disease was shown in our study. These correlations were researched with ADAM proteins and vitiligo showing the pathogenesis may be significantly associated with ADAM proteins as in the autoimmune theory most supported by data in the literature. ADAM proteins may be promising new markers for the identification of disease progression or selection of new treatment targets.

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Conflict of interest

The authors declare no conflict of interest.

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