Plasma Thiol Levels are Associated with Disease Severity in Nonsegmental Vitiligo

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Abstract

Background: Vitiligo is a depigmenting cutaneous disorder with complex pathogenesis. Thiol compounds are well-known organic structures that play a major role in melanogenesis. Aim: The aim of this study was to determine the association between plasma thiol level and disease severity in patients with nonsegmental vitiligo. Methods: A total of 73 patients with nonsegmental vitiligo (57 generalized and 16 localized type) and age- and sex-matched 69 healthy controls were enrolled in the study. Plasma levels of native thiols, disulfides, and total thiols were measured by a novel and automated assay. Disease severity of vitiligo was assessed with Vitiligo Area Scoring Index (VASI) score. The extent, stage, and spread of vitiligo of patients were evaluated according to the Vitiligo European Task Force (VETF) system. Results: The native and total thiol levels of vitiligo patients were higher than those of healthy control group ($P$≤0.001 and 0.001, respectively). The median VASI score of patients was 0.7 (0.02–28.30). Univariate analyses showed that plasma native thiol levels, VETF spread score, disease duration, and vitiligo type significantly correlated with VASI scores ($r=0.237$, $P=0.043$; $r=0.458$, $P=0.001$; and $P<0.001$, respectively). Stepwise multivariate analysis revealed that disease duration ($β=0.017$; $P=0.005$) and spread score ($β=1.301$; $P=0.001$) were found statistically significant as independent factors on VASI score. Conclusion: Although plasma native thiol level significantly correlated with VASI scores of patients, it is not a predictive factor for vitiligo severity.

Key Words: Disease activity, pathogenesis, thiol, vitiligo

Introduction

Vitiligo is a multigenic cutaneous disorder with complex pathogenesis. Although autoimmunity causing a melanocyte-specific response with adaptive immunity is currently considered as the main pathway, this theory alone does not explain the pathogenesis as a whole. Intrinsic defects within melanocytes including oxidative stress, melanocytorrhagy, neural mechanisms, adhesion defects, and inflammasomes are the other different mechanisms proposed to involve in vitiligo lesions.[¹]

Thiols, the organic compounds having sulfhydryl (-SH) groups are present as albumin thiols, protein thiols, and less as low-molecular-weight thiols such as cysteine, cysteinylglycine, glutathione (GSH), homocysteine and gamma-glutamylcysteine human plasma.[²,³] In a dynamic thiol/disulfide homeostasis, these compounds have disulfide bounds under oxidative conditions, which can be reduced to native thiols. Dynamic thiol/disulfide homeostasis regulates the maintenance of antioxidants, detoxification, apoptosis, and many cellular signal mechanisms involving cell division and cell growth.[⁴,⁵] Thiol compounds play a major role in melanogenesis,[⁶] Vitiligo presents with mainly depigmented patches, which is the reflection of impaired melanogenesis. The link between melanogenesis and thiol compounds suggested that there may be a link between plasma thiol levels and vitiligo severity. Currently, we can detect the plasma

What was known?

• Autoimmune theory alone does not explain the vitiligo pathogenesis as a whole
• Thiol compounds are well-known organic structures that play a major role in melanogenesis
• Increased levels of thiols are associated with pheomelanin production and pigment loss.

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levels of native and total thiols and disulfide levels by a simple, cheap, practical, and automatic novel assay.\(^6\)

In the present study, we investigated the association of plasma thiol levels and disease severity in patients with nonsegmental vitiligo.

**Materials and Methods**

The study was performed according to the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of the hospital. All participants signed written informed consent form.

**Subjects**

This case–control study was carried out in the outpatient clinic of Dermatovenereology, Ataturk Training and Research Hospital, Ankara. The study enrolled 73 patients with nonsegmental and focal vitiligo diagnosed by dermatological and Wood lamp examination (33 men and 40 women) and 69 healthy controls (22 men and 47 women), matched for age and gender [Table 1].

Patients who were on topical therapy for 1 month or phototherapy for 3 months before enrollment and who had systemic disease except thyroid disease were excluded from the study. Patients and controls did not have other dermatological diseases, alcohol consumption, and any systemic treatment or antioxidants. Pregnant and nursing individuals were not included in the study. None of the patients had segmental vitiligo.

The demographic and clinical variables of patients were noted on an evaluation sheet designed according to the report of Vitiligo European Task Force (VETF).\(^6\)

Clinical scoring was performed according to the Vitiligo Area Scoring Index (VASI)\(^10\) and the extent, stage, and spread of vitiligo of patients were evaluated according to the VETF system.\(^9\)

**Blood samples and analysis of plasma thiol and disulfide levels**

Venous blood samples of all participants were collected in tubes containing EDTA after fasting overnight. Plasma samples were separated from cells by centrifugation at 1500 rpm for 10 min. The samples were run immediately. In this novel assay, sodium borohydride (NaBH\(_4\)) was used to reduce the disulfide bonds to the thiol groups. The unused NaBH\(_4\) remnants are completely removed by formaldehyde. This prevented the extra reduction of the 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) and further reduction of the formed disulfide bond, which were produced after the DTNB reaction. The total thiol content of the sample was measured using modified Ellman reagent. Native thiol content was subtracted from the total thiol content and half of the obtained difference gave the disulfide bond amount. After manual spectrophotometric optimization studies, parallel tests were applied using an automated analyzer (Cobas c501, Roche). Values were presented as \(\mu\text{mol/L}.\)

**Statistical analysis**

Normality of the continuous variables was evaluated with Shapiro–Wilks test. Normally distributed continuous variables were expressed as mean ± standard deviation, and were compared using the Student t-test for two independent groups. Continuous variables that were not normally distributed were expressed as median (minimum–maximum) and were compared using the Mann–Whitney U-test for two independent groups. The association between continuous variables was explored by Pearson (normally distributed) or Spearman (nonnormally distributed) correlation analyses. Categorical variables were presented by frequency and percentage. Comparisons between two categorical variables were performed using the Chi-square analysis. Linear regression analysis was used to correlate VASI with other demographic and clinical variables. All statistical analyses were performed using IBM SPSS software version 21.0; (IBM, Armonk, NY USA), and significance was set considered statistically significant \(P<0.05\).

**Results**

The gender and ages of patients and healthy participants were similar at the examination \((P=0.318 and 0.145, respectively); [Table 1]. Of the patients, 78.1% had generalized vitiligo, and 21.9% had localized type. Demographic and clinical features of all patients are given in Tables 2 and 3. The native and total thiol levels of vitiligo patients were higher than those of healthy control group \((P<0.001 and 0.001, respectively) [Table 4]. The median VASI score of patients was 0.7 \((0.02–28.30). Univariate analysis demonstrated that plasma native thiol levels, VETF stage score, disease duration, affected body surface area, and vitiligo type significantly correlated with VASI scores \((r=0.237, P=0.043; r=0.487, P<0.001; r=0.458, P<0.001; r=0.834, P<0.001; and P<0.001, respectively) [Tables 5 and 6]. Multiple linear regression analyses were used to assess multivariate relations among native thiol, age, gender, vitiligo duration, spread score, and vitiligo type [Table 7]. Since both VASI and stage score were closely related with pigmentation.

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**Table 1: Comparison of some demographic features of vitiligo patients and healthy controls**

| Variable                | Patients (n=73) | Controls (n=69) | \(P\)  |
|-------------------------|----------------|----------------|-------|
| Age at visit (years)    | 33.5±9.6       | 35.5±12.2      | 0.318 |
| Gender, n (%)           |                |                |       |
| Male                    | 33 (45.2)      | 22 (31.9)      | 0.145 |
| Female                  | 40 (54.8)      | 47 (68.1)      |       |

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Table 2: Demographic and clinical characteristics of vitiligo patients

| Variables                          | n (%)   |
|-----------------------------------|---------|
| Types of vitiligo                 |         |
| Generalized vitiligo              | 57 (78.1) |
| Vulgaris                          | 40 (54.8) |
| Acrofacial                        | 17 (23.3) |
| Localized vitiligo                | 16 (21.9) |
| Co-existing other findings        |         |
| Premature hair graying            | 27 (37.0) |
| Alopecia areata                   | 1 (1.4)  |
| Treatment history                 |         |
| Untreated patients                | 27 (37.0) |
| Treated patients                  | 46 (63.0) |
| Topical corticosteroid            | 23 (31.5) |
| Topical calcineurin inhibitor     | 19 (26.0) |
| Phototherapy                      | 6 (8.2)  |
| Systemic corticosteroid           | 1 (1.4)  |
| Oral antioxidant                  | 1 (1.4)  |
| Disease activity in the past 6 months |       |
| Active vitiligo                   | 63 (86.3) |
| Stable or regressive vitiligo     | 10 (13.7) |
| Previous episode of repigmentation| 29 (39.7) |
| Depigmentation on scars           | 2 (2.7)  |
| Stress as onset factor            | 59 (80.8) |
| Pruritus before depigmentation    | 14 (19.2) |
| Patients with autoimmune thyroiditis | 30 (41.1) |
| Presence of Koebner’s phenomenon  | 34 (46.6) |
| Smokers                           | 19 (26.0) |
| Skin phototype                    |         |
| Type 2                            | 30 (41.1) |
| Type 3                            | 36 (49.3) |
| Type 4                            | 7 (9.6)  |
| Family history of vitiligo        | 21 (28.8) |
| Family history of premature hair graying | 29 (39.7) |
| Family history of autoimmune disease | 17 (23.3) |

Table 3: The clinical characteristics of vitiligo patients

| Variables                          | Values |
|-----------------------------------|--------|
| Age at onset of vitiligo (years)  | 27.7±11.8 (5-51) |
| Vitiligo duration (years)         | 27.0 (0.1-32.0) |
| VASI score                        | 0.70 (0.02-28.30) |
| Total affected body surface area, % | 1.0 (0.1-28.1) |
| Staging score                     | 4 (1-11) |
| Spreading score                   | 1 (~1-5) |
| Progressive patients (spreading score >0) | 40 (54.8%) |
| Nonprogressive patients (spreading score ≤0) | 33 (45.2%) |

*Values are given as median (minimum–maximum) or mean±SD (minimum–maximum). SD: Standard deviation, VASI: Vitiligo Area Scoring Index

Discussion

Vitiligo is mainly considered to be an autoimmune disorder of the skin melanocytes whereas many nonautoimmune mechanisms such as oxidative stress and apoptotic pathways are also suggested to involve in the disease pathogenesis.[1] Researches about melanogenesis led new proposals about impairment of melanogenesis in vitiligo. Thiol compounds were well-known organic structures that played a major role in melanogenesis.[7,11,12] Glutathione (GSH), the active – SH compound of human skin extract, was found to be associated with the inhibition of melanogenesis in human epidermis.[12,14] van Scott et al. reported that the sulfhydryl content of vitiliginous skin was lower than that of the normal skin surrounding the lesions.[15] Although melanocyte death was considered to be involved in vitiligo, depigmented patches were reported to consist of both eumelanin and mainly pheomelanin.[16] The key factor for pheomelanogenesis was the presence of thiol groups of cysteine molecule.[7,11,12] Thiol groups chelated with the copper part of the tyrosinase enzyme and inhibited melanogenesis.[17] The thiol-dopaquinon reaction shifted eumelanogenesis to pheomelanogenesis. Increased levels of thiols including GSH were associated with pheomelanin production and pigment loss.[14]

Pheomelanogenesis was suggested to be an adaptive excretion mechanism which removed excess thiol compound, cysteine.[19] Pheomelanin had pro-oxidant features[20] whereas eumelanin served as a scavenger of reactive oxygen derivate.[21] Pheomelanogenesis resulted in the consumption of GSH which served as the main cysteine source and which acted as an important antioxidant.[11,12] Pheomelanin caused oxidative lipid injury in the cells[22] and induction of apoptosis.[23] Some researchers suggested that the inhibition of tyrosinase activity or other factors causing pheomelanogenesis were involved in vitiligo pathogenesis.[16]

There had been many investigations about the amount of some thiol compounds such as GSH and homocysteine and enzyme activities involving the production and catabolization of these products in vitiligo patients and their relationship with disease severity.[24-26] However, plasma thiol levels and its role in vitiligo had not been investigated before. Since thiol-containing compounds were not stable in a dynamic organism, we considered that the systemic impact of thiol compounds, rather than isolated effects of individual ones, might have an important impact on vitiligo pathogenesis. Using a novel, practical, and automated assay developed for further statistical analyses. After stepwise method, disease duration (β=0.017; P=0.005) and spread score (β=1.301; P=0.001) were found statistically significant as independent factors on VASI score.
Table 4: Serum thiol levels of vitiligo patients and control subjects

| Variables                | Patients (n=73) | Controls (n=69) | P    |
|--------------------------|----------------|----------------|------|
| Native thiol (μmol/L)    | 496.6±51.9     | 463.9±48.6     | <0.001|
| Disulfide (μmol/L)       | 18.5±8.1       | 18.8±6.1       | 0.807 |
| Total thiol (μmol/L)     | 533.5±58.4     | 501.4±51.4     | 0.001 |

Table 5: Correlation analyses between Vitiligo Area Scoring Index score and some demographic and clinical features and laboratory data of vitiligo patients

| Variables                  | VASI score r | P    |
|----------------------------|--------------|------|
| Age at visit               | 0.117        | 0.324|
| Onset age                  | −0.210       | 0.076|
| Vitiligo duration          | 0.458        | <0.001|
| BMI                        | 0.087        | 0.467|
| Affected body surface area | 0.834        | <0.001|
| Stage score                | 0.487        | <0.001|
| Spread score               | 0.368        | 0.001|
| Native thiol levels        | 0.237        | 0.043|
| Disulfide levels           | 0.035        | 0.767|
| Total thiol levels         | 0.225        | 0.056|
| WBC                        | −0.109       | 0.358|
| Neutrophil count           | −0.082       | 0.489|
| Lymphocyte count           | −0.038       | 0.753|
| NLR                        | −0.054       | 0.648|
| MPV                        | −0.058       | 0.636|
| RDW                        | −0.061       | 0.606|
| sCRP                       | 0.130        | 0.286|

Table 6: Comparison of Vitiligo Area Scoring Index scores between some demographic and clinical parameters of vitiligo patients

| Variables                  | VASI score | P    |
|----------------------------|------------|------|
| Gender                     |            |      |
| Male (n=33)                | 0.90 (0.02-28.30) | 0.890|
| Female (n=40)              | 0.60 (0.05-25.28) |      |
| Vitiligo type              |            |      |
| Localized (n=16)           | 0.10 (0.05-1.50) | <0.001|
| Generalized (n=57)         | 1.05 (0.02-28.30) |      |
| Smoking                    |            |      |
| Smokers (n=19)             | 0.60 (0.02-9.00) | 0.402|
| Nonsmokers (n=54)          | 0.70 (0.05-28.30) |      |
| Skin type                  |            |      |
| Type 1 and 2 (n=30)        | 0.66 (0.05-11.25) | 0.918|
| Type 3 and 4 (n=43)        | 0.67 (0.02-28.30) |      |
| Koebnerization             |            |      |
| Koebner positives (n=34)   | 0.74 (0.02-28.30) | 0.495|
| Koebner negatives (n=39)   | 0.62 (0.05-25.28) |      |
| Thyroid disease            |            |      |
| autoimmune thyroiditis     | 0.58 (0.02-25.25) | 0.805|
| positive (n=30)            |            |      |
| autoimmune thyroiditis     | 0.70 (0.05-28.30) |      |
| negative (n=43)            |            |      |
| Activity in the last 6 months |        |      |
| Active (n=63)              | 0.63 (0.02-28.30) | 0.670|
| Inactive (n=10)            | 1.45 (0.08-4.69) |      |
| Activity due to spread score |            |      |
| Progressive (n=40)         | 1.03 (0.05-28.30) | 0.086|
| Nonprogressive (n=33)      | 0.54 (0.02-12.70) |      |

Conclusion

The present study was the first one which investigated the relationship between plasma thiol levels and vitiligo severity. Although plasma native thiol level closely correlated with VASI score of patients, it was not a predictive factor for vitiligo severity. Further studies are needed to determine whether systemic thiol metabolism is related to the etiopathogenesis of vitiligo.

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Nil.

Conflicts of interest
There are no conflicts of interest.
Table 7: Multivariate linear regression analysis showing independent variables associated with Vitiligo Area Scoring Index score

| Variables            | Multivariate analysis | Stepwise multivariate analysis |
|----------------------|-----------------------|-------------------------------|
|                      | P         | \( \beta \) | 95% CI | P         | \( \beta \) | 95% CI |
| Constant             | 0.933     | -0.656       | -16.142-14.830 | 0.815     | -0.165       | -1.570-1.239 |
| Age                  | 0.221     | 0.073        | -0.045-0.181 | 0.005     | 0.017        | 0.005-0.029 |
| Gender               | 0.775     | -0.302       | -2.403-1.798 | 0.001     | 1.301        | 0.555-2.048 |
| Vitiligo duration    | 0.014     | 0.016        | 0.003-0.029 |           |              |            |
| Spread score         | 0.002     | 1.254        | 0.469-2.040 |           |              |            |
| Native thiols        | 0.884     | -0.002       | -0.025-0.021 |           |              |            |
| Vitiligo type        | 0.745     | -0.437       | -3.100-2.227 |           |              |            |

\( \beta \): Beta coefficient, CI: Confidence interval

What is new?
- Plasma native thiol levels closely correlated with VASI scores. However, it is not a predictive factor for vitiligo severity.
- Circulating excess thiol compounds may have a potential role in vitiligo pathogenesis.

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