# High levels of serum hyaluronic acid in adults with dermatomyositis

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## ABSTRACT

**Background/objectives:** Hyaluronic acid (HA) is rarely described in dermatomyositis (DM). Thus, we determined any clinical association of serum levels of hyaluronic acid (HA) in patients with dermatomyositis (DM).

**Materials and Methods:** This cross-sectional single-center analysis 75 DM and 75 healthy individuals, during the period from January 2012 to July 2013. An anti-HA antibody assay was performed using specific ELISA/EIA kits, according to the manufacturer's protocol.

**Results:** The patients with DM and control subjects had comparable demographic distributions (p>0.05). The median time duration between disease diagnosis and initial symptoms was 6.0 [3.0-12.0] months, with a median DM disease duration of 4.0 [1.0-7.0] years. The median level of serum HA was significantly increased in patients with DM compared to the control group [329.0 (80.0-958.0) versus 133.0 (30.0-262.0) ng/mL, respectively; p<0.001]. Additional analysis involving patients with DM showed that the serum level of HA did not correlate with age, duration between disease diagnosis and initial symptoms, disease duration, disease status, serum muscle enzyme levels or cumulative prednisolone dose (p>0.05). Serum HA also did not correlate with gender, ethnicity, auto-antibodies or drug use (p>0.05), but did correlate with cutaneous features, such as photosensitivity (p=0.001), "shawl" sign (p=0.018), "V-neck" sign (p=0.005) and cuticular hypertrophy (p=0.014).

**Conclusions:** A high level of serum AH was observed in DM compared to healthy individuals. In DM, HA did not correlate to demographic, auto-antibodies and therapy parameters. However, HA correlated specifically with some cutaneous features, suggesting that this glycosaminoglycan could be involved in modulating cutaneous inflammation in this population. More studies are necessary to understand the correlation between AH and patients with DM.


**Keywords:** Cutaneous features; Dermatomyositis; Glycosaminoglycan; Hyaluronic acid; Idiopathic inflammatory myopathies.

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## INTRODUCTION

Dermatomyositis (DM) is a rare systemic idiopathic inflammatory myopathy associated with high morbidity and functional disabilities. The annual incidence of DM is 0.5 to 8 cases per million inhabitants, with a two to one female to male ratio[1]. The disease has a bimodal age distribution, affecting individuals from 5 to 15 and from 45 to 55 years of age, although the average age of diagnosis occurs at approximately 40 years of age, and the disease can affect individuals of any age[1].

In addition to progressive symmetrical muscle weakness, DM is characterized by classic skin lesions, such as heliotrope rash and/or Gottron's papules[1,2]. Moreover, other cutaneous manifestations can be present, including periungual telangiectasia, mechanic's hand, skin ulcers, vasculitis, "V-neck" sign, "shawl" sign, calcinosis cutis and Raynaud's phenomenon[1,3].

Hyaluronic acid (HA) is a glycosaminoglycan that comprises the extracellular connective tissue matrix. HA is distributed in various tissues, such as synovial fluid, ophthalmological vitreous humor, umbilical cord, connective tissues and cartilage[4,5]. HA is produced mainly by fibroblasts, is transported by the lymph and is rapidly metabolized by the liver[4,5]. Moreover, there is evidence of the role of HA in regulating the immune

response by stimulating the expression of inflammatory genes in various immune cells at the site of injury. HA is active in regulating the inflammatory response through cell recruitment, cytokine release and cell migration[5]. HA also stimulates the release of inflammatory factors, such as TNF-alpha and IL-1β, and other cytokines by fibroblasts, which assist in the inflammatory response[4,5].

An increased level of serum HA has been reported in systemic autoimmune diseases, including rheumatoid arthritis, systemic sclerosis, psoriatic arthritis and systemic lupus erythematosus[6-14]. Patients with rheumatoid arthritis, for instance, have elevated concentrations of glycosaminoglycans, including HA in the blood and synovial fluid[9], which positively correlate with articular destruction[9]. Yoshizaki *et al*.[11] observed a high level of serum HA in patients with systemic sclerosis compared to healthy individuals, and HA correlated with disease severity and immunological abnormalities in this population. In psoriatic arthritis, there is also positive correlation between the increased levels of serum HA and cutaneous manifestations, but not with articular involvement[12].

Because HA is rarely described in DM[17,18], we aim to evaluated the serum level of this glycosaminoglycan in a large sample of adult patients with DM in comparison to healthy individuals.

## MATERIALS AND METHODS

The present cross-sectional study was performed at a single center and included 75 patients with DM (Bohan and Peter criteria)[2] followed at a myopathy unit, from January 2012 to July 2013. Patients with clinically amyopathic DM, cancer associated myositis, and acute and/or chronic infections (viral, bacterial or fungal) were excluded. As a control group, 75 age- and gender-matched adult healthy volunteers were recruited during the same period. The study was approved by the local Ethics Committee.

All of the patients underwent a standardized interview and defined protocol to collect the following information:

a) Demographic data: current age, gender, and ethnicity;
b) Clinical features: duration between initial symptoms and disease diagnosis; constitutional symptoms; articular involvement, pulmonary involvement characterized by abnormalities on pulmonary computer tomography (interstitial lung disease); and skin changes (heliotrope, Gottron's papules, photosensitivity, calcinosis cutis, skin ulcers, vasculitis, "V neck" sign, "shawl" sign, periungual telangiectasia, and Raynaud's phenomenon). These cumulative parameters were determined as "yes" or "no". In addition, questionnaires were used to assess the current status of disease activity: (A) manual muscle strength testing - MMT-8[19,20]; (B) physician global assessment of disease activity (VAS)[21,22]; (C) patient VAS[21,22]; and (D) Health Assessment Questionnaire (HAQ)[21,23,24];
c) Laboratory data: sample sera were collected and centrifuged immediately at 3,000 rpm for 15 minutes, at 4 ºC, and stored at - 70 ºC. From these samples, the following laboratory parameters were analyzed: creatine phosphokinase (CPK: reference value: 24 - 173 U/L) and aldolase (1.0 - 7.5 U/L) using automatized kinetics; antinuclear antibody (ANA) using a HEp-2 cells; anti-Mi-2 antibody using a commercially available line blot test kit (Myositis Profile Euroline Blot test kit, Euroimmun, Lübeck, Germany) according to a previously described method[25]. The anti-HA antibody assay was performed using specific ELISA/EIA kits, according to the manufacturer's protocol.

Statistical analysis. The Kolmogorov-Smirnov test was used to evaluate the distribution of each parameter. The demographic and clinical features are expressed as the means and standard deviations (SD) for continuous variables or as the frequencies and percentages for categorical variables. The median (25 - 75th percentile) was calculated for continuous variables that were not normally distributed. Comparisons between the patients' and controls' categorical parameters and patients' categorical parameters and the interquartile distribution of serum HA serum level were made using Pearson's chi-squared test or Fisher's exact test. For continuous variables, the Mann-Whitney or Student's t-test were used. For correlations between serum AH and continuous variables, the Spearman correlation was used. Values of $p < 0.05$ were considered to be significant. All of the analyses were performed with the SPSS 15.0 statistics software (Chicago, USA).

## RESULTS

The present study included 75 adult DM patients and 75 adult healthy controls. The DM patients and con-

**TABLE I. DEMOGRAPHIC, CLINICAL AND LABORATORY FEATURES OF PATIENTS WITH DERMATOMYOSITIS AND HEALTHY INDIVIDUALS**

| | Controls N=75 | DM N=75 | P |
|---|---|---|---|
| Age (years) | 44.2 ± 14.6 | 44.5 ± 12.0 | 0.905 |
| Female gender | 58 (77.3) | 58 (77.3) | 1.000 |
| Caucasian ethnicity | 54 (72.0) | 52 (69.3) | 0.720 |
| Time between symptoms and diagnosis (mo) | – | 6.0 [3.0-12.0] | – |
| Disease duration (years) | – | 4.0 [1.0-7.0] | – |
| Clinical cumulative features | | | |
| Constitutional symptoms | – | 25 (75.8) | – |
| Articular involvement | – | 12 (36.4) | – |
| Pulmonary involvement | – | 13 (39.4) | – |
| Cutaneous involvement | – | 51 (60.0) | – |
| Laboratory features | | | |
| Creatine phosphokinase (U/L) | 112.0 [86.0-164.0] | 125.0 [72.0-275.0] | 0.190 |
| Aldolase (U/L) | 3.6 [2.8-4.4] | 4.0 [4.8-6.5] | <0.001 |
| Anti-Mi-2 antibody | – | 5 (6.7) | |
| Antinuclear antibody | – | 41 (54.7) | – |
| Hyaluronic acid (ng/mL) | 133.0 [30.0-262.0] | 329.0 [80.0-958.0] | <0.001 |

DM: dermatomyositis. The results are expressed as the means ± SD, mean [interquartile 25 – 75th] or percentage (%).

trol patients had a comparable mean age (44.2 ± 14.6 vs. 44.5 ± 12.0 years, respectively) and gender and ethnicity distributions ($p > 0.05$) (Table I). The median duration between disease diagnosis and initial symptoms was 6.0 [3.0 - 12.0] months, with a median DM disease duration of 4.0 [1.0 - 7.0] years.

The serum CPK level was similar in the DM and control groups, whereas the serum aldolase level was higher in the DM patients.

The median serum HA level was significantly increased in the DM patients compared to the control group [329.0 (80.0 - 958.0) vs. 133.0 (30.0 - 262.0) ng/mL, respectively; $p < 0,001$].

An additional analysis involving patients with DM showed that the serum HA level did not correlate with age, duration between disease diagnosis and initial symptoms, disease duration, disease status, serum muscle enzyme levels or the cumulative prednisolone dose (Table II). The serum AH serum level also did not correlate with gender, ethnicity, or auto-antibodies. However, the level correlated with cutaneous features, such as photosensitivity, "shawl" sign, "V-neck" sign and periungual telangiectasia (Table III).

Regarding drugs, the serum HA level did not correlate with prednisolone or immunosuppressive treatment (Table III).

## DISCUSSION

In the present study, we observed a high serum HA level in a large sample of patients with DM. Moreover, there was a tendency for HA to positively correlate with cutaneous manifestations.

Yoshinoya *et al.*[8] showed high levels of serum glycosaminoglycans, including HA, in rheumatoid arthritis, and the serum HA level correlated specifically with disease severity.

Chang *et al.*[15] observed an accumulation of HA and sulfate chondroitin specifically in cutaneous lesions of patients with lupus erythematosus or DM. These authors suggested that these glycosaminoglycans could be involved in the pathogenesis of cutaneous inflammation in these diseases.

Faaber *et al.*[16] studied the function of HA in patients with systemic lupus erythematosus. These authors showed cross-reactivity of anti-DNA antibodies with HA and sulfate chondroitin, leading to anti-proteoglycan activity and consequently promoting inflammatory changes in these tissues, leading to arthralgia, arthri-

**TABLE II. SPEARMAN CORRELATION BETWEEN DEMOGRAPHIC, CLINICAL AND LABORATORY FEATURES OF PATIENTS WITH DERMATOMYOSITIS AND SERUM HYALURONIC ACID**

| | | r | P |
|---|---|---|---|
| Age (years) | 44.5 ± 12.0 | 0.2196 | 0.059 |
| Time between symptoms and diagnosis (mo) | 6.0 [3.0-12.0] | -0.0571 | 0.629 |
| Disease duration (years) | 4.0 [1.0-7.0] | -0.0643 | 0.584 |
| Current disease status | | | |
| Patient VAS | 2 [0-5] | 0.0021 | 1.000 |
| Physician VAS | 1 [0-4] | 0.5190 | 1.000 |
| MMT-8 | 80 (78-80) | -0.0724 | 0.537 |
| HAQ | 0.14 [0-12.9] | 0.1702 | 0.145 |
| Laboratory features | | | |
| Creatine phosphokinase (U/L) | 125.0 [72.0-275.0] | -0.0615 | 0.600 |
| Aldolase (U/L) | 4.0 [4.8-6.5] | -0.0662 | 1.000 |
| Treatment | | | |
| Prednisolone: cumulative dose (g) | 13.3 [8.0-23.2] | 0.0254 | 0.930 |

VAS: visual analog scale; MMT-8: manual muscle testing; HAQ: healthy assessment quality. The results are expressed as the means ± standard deviation, mean [interquartile 25-75$^{th}$].

**TABLE III. CORRELATION BETWEEN DEMOGRAPHIC, CLINICAL AND LABORATORY FEATURES OF PATIENTS WITH DERMATOMYOSITIS AND SERUM HYALURONIC ACID EXPRESSED IN INTERQUARTILES**

| | HA1 N=19 | HA2 N=19 | HA3 N=19 | HA4 N=18 | P |
|---|---|---|---|---|---|
| Female gender | 13 (68.4) | 16 (84.2) | 15 (79.0) | 14 (77.8) | 0.736 |
| Caucasian ethnicity | 11 (57.9) | 15 (79.0) | 15 (79.0) | 13 (72.2) | 0.455 |
| Cutaneous cumulative features | | | | | |
| Heliotrope | 16 (84.2) | 13 (68.4) | 12 (63.2) | 16 (88.9) | 0.218 |
| Gottron's papules | 18 (94.7) | 19 (100.0) | 19 (100.0) | 16 (88.9) | 0.184 |
| Calcinosis cutis | 4 (21.0) | 2 (10.5) | 0 | 5 (27.8) | 0.056 |
| Cutaneous ulcers | 1 (5.3) | 3 (17.8) | 2 (10.5) | 5 (27.8) | 0.253 |
| Vasculitis | 1 (5.3) | 4 (21.0) | 4 (21.0) | 6 (33.3) | 0.190 |
| Photosensitivity | 5 (26.3) | 10 (52.6) | 11 (57.9) | 16 (88.9) | 0.001 |
| "Shawl" sign | 0 | 0 | 3 (17.8) | 4 (22.2) | 0.018 |
| "V-neck" sign | 0 | 3 (17.8) | 5 (26.3) | 8 (44.5) | 0.005 |
| Cuticular hypertrophy | 4 (21.0) | 11 (57.9) | 9 (47.4) | 13 (72.2) | 0.014 |
| Raynaud's phenomenon | 4 (21.0) | 7 (36.8) | 9 (47.4) | 6 (33.3) | 0.402 |
| Laboratory features | | | | | |
| Anti-Mi-2 antibody | 2 (10.5) | 1 (5.3) | 1 (5.3) | 1 (5.6) | 0.852 |
| Antinuclear antibody | 8 (42.0) | 10 (52.6) | 10 (52.6) | 13 (72.2) | 0.601 |
| Treatment | | | | | |
| Prednisolone | 11 (57.9) | 10 (52.6) | 11 (57.9) | 10 (55.6) | 1.000 |
| Prednisolone (>20 mg/day) | 4 (21.0) | 6 (31.6) | 5 (26.3) | 5 (27.8) | 0.941 |
| Immunosuppressors * | | | | | |
| One | 7 (36.8) | 11 (57.9) | 14 | 7 (38.9) | 0.083 |
| Two or more | 5 (26.3) | 2 (10.5) | 3 (17.8) | 6 (33.3) | 0.240 |

The results are expressed as percentages (%). AH: hyaluronic acid, expressed in interquartiles (HA1: 0-50 ng/mL; HA2: 51-329 ng/mL; HA3: 330-958; HA4: 959-15110 ng/mL).
*Immunosuppressors: azathioprine (2~3 mg/kg/day), methotrexate (20~25 mg/week, cyclosporine (2~3 mg/kg/day), mycophenolate mofetil (2~3 g/day), leflunomide (20 mg/day).

tis and skin rashes.

Elkayam *et al*.[12] observed that a high serum HA level was related to psoriatic arthritis, particularly to skin activity and not joint disease. HA turnover is increased in psoriasis associated with an elevated serum HA level, and the turnover most likely occurs in the skin, as in suction blisters, in patients with active untreated psoriasis, which contain high concentrations of HA in the fluid. In another study, Lundin *et al*.[14] showed that the HA concentration in the blister fluid from active skin lesions in psoriatic arthritis patients was greatly increased compared to the control group.

The role of serum HA in DM has been rarely described in the literature[17,18]. Kubo *et al*.[17] reported two cases in which there was a positive correlation between the serum HA level and DM disease activity. In one of the DM patients analysed, the serum HA level decreased after using corticosteroid therapy. In the second patient, who also suffered from cancer, the HA level decreased after the surgical resection of a mammary carcinoma and subsequent chemotherapy. These same authors observed in a subsequent study[18], involving 40 patients with DM, that the serum HA level was higher in DM patients compared to patients with systemic lupus erythematous, rheumatoid arthritis or systemic sclerosis. As a limitation, the authors did not include a control group and did not exclude DM patients with neoplasia.

In our study, we observed a high serum AH level in a large sample of patients with defined DM. Different than previous, we excluded not only patients with cancer associated myositis but also clinically amyopathic DM, as well as chronic infections and/or current treatments (viral, bacterial or fungal) that could interfere with serum HA expression. Our data showed that HA did not correlate with laboratory features, drug treatment with prednisolone or current disease status. However, HA positively correlated with DM cutaneous features, such as photosensitivity, "shawl" sign, "V-neck" sign and peringual telangiectasia.

In healthy skin, HA is distributed mainly in the intercellular space of the papillary dermis, with intense staining and uneven distribution observed in the reticular dermis[6]. A prominent staining layer is found below the epidermis in the basement membrane zone[6]. HA has been shown to control the production and activation of matrix metalloproteinase in fibroblast and keratinocyte cell cultures[26]. HA plays an important role in regulatory processes, such as inflammation, wound healing and tumor progression[4].

Increased HA production has been reported in different skin diseases[13]. The proliferation and activation of fibroblasts has been reported in fibrous and inflammatory tissue in skeletal muscle[27]. This finding could explain the DM physiopathological mechanism and elevated serum HA level in active disease because HA plays an important role in the inflammatory process, associated with physiologic abundance in the skin.

As a limitation of the present study, our patients were receiving a consolidated treatment at the time of analysis. This fact may have prevented the identification of correlation between an elevated serum HA level and DM clinical manifestations and laboratory features. Other limitation is the anti-HA antibody assay, which was performed using ELISA/EIA kits (assay availability and also sensitivity and specificity of the method). More studies are necessary including also patients without any treatments (corticosteroid and/or immunossuppressives).

## CONCLUSIONS

In conclusion, we found an elevated serum HA level in DM. Furthermore, HA correlated specifically with some cutaneous features (photosensitivity, "shawl" sign, "V-neck" sign, and peringual telangiectasia), suggesting that this glycosaminoglycan could be involved in modulating cutaneous inflammation in this population. More studies are necessary to understand the correlation between HA and patients with DM features.

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