Serum hyaluronic acid in polymyositis: high serum levels tend to correlate with disease activity

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ABSTRACT

Objective: Polymyositis (PM) is a rare systemic idiopathic inflammatory myopathy. Hyaluronic acid (HA) is closely linked to inflammatory cellular reactions and disease activity. Increased serum levels of HA have been reported in several inflammatory diseases, but currently, there are no studies analysing the HA in PM. Thus, clinical association of HA with PM in patients was determined in the present study.

Methods: The present cross-sectional study was performed at one centre from 2012 to 2013 and included 35 consecutive adult patients with PM (Bohan and Peter criteria, 1975) and 38 adult healthy volunteers. The serum HA was assessed with anti-HA antibody, using the specific ELISA/EIA kits according to the manufacturer's protocol.

Results: The average age, distribution of females and ethnicity were comparable in patients with PM and the control group. Regarding disease status, patients with PM had a median patient visual analogue score (VAS) of 2 [0-6], physician VAS of 1 [0-3], MMT-8 of 74 [68--80] and HAQ of 0.48 [0.00-1.14]. The serum levels of HA were also significantly increased in patients with PM (390±412ng/mL) compared to healthy subjects (129±119ng/mL), p=0.001. In an additional analysis, the serum levels of HA did not correlate with PM demographic data (gender and ethnicity), current organ involvement or autoantibodies and were not been influenced by the use of prednisolone and/or immunosuppressives by the PM patients. However, there was a positive correlation between serum levels of HA and VAS (patient and physician), and a negative correlation between serum levels of HA and MMT-8.

Conclusion: High serum levels of HA were observed in patients with PM and tended to correlate with PM disease activity. Additional studies are needed to assess this correlation, as well as to understand the mechanism involved in the pathogenesis of PM by HA.

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Keywords: Disease activity; Hyaluronic acid; Idiopathic inflammatory myopathies; Polymyositis.

INTRODUCTION

Polymyositis (PM) is a rare idiopathic systemic inflammatory myopathy. The disease is characterised by progressive symmetrical muscle weakness with high morbidity and functional disability[1-3].

The annual incidence of PM is 2.2 to 7.7 cases per million individuals, affecting predominantly females (2 women: 1 man). The disease primarily affects individuals between the ages of 45 and 55 years, although the average age of diagnosis occurs at approximately 40 years of age and the disease can affect patients of any age[1-3].

Hyaluronic acid (HA) is a glycosaminoglycan comprising the extracellular connective tissue matrix. It is distributed in various tissues, such as synovial fluid, vitreous humour of the eye, the umbilical cord, loose connective tissue and cartilage. It is produced mainly by fibroblasts, transported by the lymph and is rapidly metabolised by the liver[4]. Moreover, there is evidence of the role of HA in the regulation of the immune response by stimulating the expression of inflammatory genes in various immune cells at the site of injury, and in regulating the inflammatory response through cell recruitment, cytokine release and cell migration[5]. In addition, HA stimulates the release of inflammatory factors, such as TNF-α and IL-1β, and cytokines produced by fibroblasts, which assists the inflammatory response[6].

Increased serum levels of HA have been reported in several diseases, including liver disease, malignant diseases and different systemic autoimmune diseases[7,8-11]. For instance, in rheumatoid arthritis, HA has been reported to be an important regulator of the

course of arthritis because of its inflammatory properties[8]. High levels of HA were also observed in patients with systemic sclerosis, being correlated with cutaneous manifestations, mainly in the early stages of the disease[9]. In systemic lupus erythematosus, increased levels of HA are associated with discoid cutaneous manifestations, which may contribute to drug resistance and disease severity[7]. In relation to dermatomyositis, there have been reports of patients with a positive correlation between the disease and serum HA activity[4,10]. In psoriatic arthritis, the increased serum levels of HA are highly positively correlated with skin involvement when compared to the normal population[11].

However, currently there are no studies analysing the role of HA in PM. Similar to other systemic inflammatory diseases, HA could be involved in PM. Thus, any clinical association of HA in this population was investigated in the present study.

MATERIALS AND PATIENTS

The present cross-sectional study was performed at one centre and included 35 patients over 18 years of age, diagnosed with PM (fulfilling the Bohan and Peter criteria excluding the cutaneous criteria[12]) and followed at an inflammatory myopathies unit during the period of January 2012 to July 2013.

Individuals with cancer-associated myositis, other systemic-associated autoimmune disease, or acute or chronic infections (viral, bacterial or fungal) were excluded.

As a control group, 38 adult healthy volunteers were included in the study.

The study was approved by the local Ethics Committee, and informed consent was obtained from all individuals.

All patients submitted to a standardised interview to collect the following information. Moreover, any missing data were obtained using an ongoing electronic database protocol carried out for all patients at 1- to 3--month intervals. Protocols consisted of an extensive clinical and laboratory evaluation, including those relevant to this study:

a) Demographic data: the current age of the patient, gender and ethnicity;
b) Clinical data: the time from first symptoms to disease diagnosis, the time since the disease diagnosis, current organ involvement (heart, lung and articulation), and treatment (corticosteroid and/or immunosuppressives). In addition, questionnaires were used to assess the current status of disease activity: (A) Manual Muscle Testing - MMT-8[13,14]; (B) physician visual analogue scale (VAS)[15,16]; (C) patient visual analogue scale (VAS)[15,16]; (D) quality-of-life questionnaire: Health Assessment Questionnaire (HAQ)[15,17,18] and (E) laboratory data: creatine phosphokinase (reference value: 24-173 U/L), aldolase (1.0-7.5 U/L); aspartate aminotransferase (< 31 U/L), alanine aminotransferase (< 31 U/L), lactate dehydrogenase (240 - 480 U/L), C-reactive protein (< 5 mg/L) by nephelometry, and erythrocyte sedimentation rate (< 19 mm / 1st hour) by Westegreen method; and
c) Hyaluronic acid: The sample blood sera is collected and centrifuged immediately at 3000 rpm, for 15 minutes, at 4 ºC and stored at - 70 ºC. From these samples, the anti-HA antibody was analyzed with specific kits of ELISA / EIA (HA Sandwich ELISA, Echelon Biosciences Inc, Salt Lake City), according to the manufacturer's protocol using 25 L samples and standard curves to determine the concentration values.

Statistical analysis. The Kolmogorov-Smirnov test was used to evaluate the distribution of each parameter. The demographic and clinical features were expressed as the mean ± standard deviation (SD) or median [25th - 75th percentile] for continuous variables or as percentages for categorical variables. Means were compared using Student's t-test; medians, with Mann--Whitney U-test. Categorical data were compared using chi-square test or Fisher's exact test. The correlation between HA serum level and continuous variables used Spearman's correlations. Values of $p < 0.05$ were considered significant. All of the analyses were performed with the SPSS 15.0 statistics software (Chicago, IL, USA).

RESULTS

The demographic, clinical and laboratory characteristics of 35 patients with PM and 38 healthy individuals are shown in Table I. The average age, the percentage of females and ethnicity were comparable in both groups. The median time from first symptoms to disease diagnosis was 8 months [5.0-24.0] and the median time since disease diagnosis was 4 years [2.0--10.0].

Regarding disease status, patients with PM had a me-

TABLE I. DEMOGRAPHIC, DISEASE STATUS PARAMETERS AND SERUM LEVEL OF THE HYALURONIC ACID OF PATIENTS WITH POLYMYOSITIS AND HEALTHY CONTROL INDIVIDUALS

Parameters	Controls N=38	Polymyositis N=35	P
Current age (years)	46.3±10.7	47.7±11.6	0.507
Female gender	30 (78.9)	27 (77.1)	1.000
Ethnicity (Caucasian)	27 (71.1)	21 (63.2)	0.720
Time from first symptoms to disease diagnosis (mo)	–	8.0 [5.0-24.0]	–
Time since disease diagnosis (years)	–	4.0 [2.0-10.0]	–
Disease status			
Patient's VAS	–	2 [0-6]	–
Physician's VAS	–	1 [0-3]	–
MMT-8	–	74 [68-80]	–
HAQ		0.48 [0.00-1.14]	–
Creatine phosphokinase (U/L)	120 [86-156]	186 [110-869]	0.049
Aldolase (U/L)	3.6 [3.2-4.4]	5.3 [3.7-9.1]	0.003
Aspartate aminotransferase (U/L)	20 [17-24]	26 [16-39]	0.019
Alanine aminotransferase (U/L)	19 [15-23]	29 [18-47]	0.053
Lactate dehydrogenase (U/L)	349 [309-399]	469 [365-544]	0.028
Hyaluronic acid (ng/mL)	129±119	390±412	0.001

VAS: Visual Analogic Scale; MMT: Manual Muscle Testing; HAQ: Health Assessment Questionnaire. Results are expressed as mean ± standard deviation (SD), median [interquartile 25th - 75th] or percentage (%).

dian patient VAS of 2 [0-6], physician VAS of 1 [0-3], MMT-8 of 74 [68-80] and HAQ of 0.48 [0.00-1.14]. Moreover, the serum level of muscle enzymes was slightly increased in patients with PM as shown in the Table I, but this increase was statistically significant in relation to the control group ($p < 0.050$).

The serum level of HA was also statistically increased in patients with PM (390 ± 412 ng/mL) compared to healthy subjects (129 ± 119 ng/mL), $p = 0,001$.

In addition, there was no correlation between serum levels of HA and the following continuous parameters: age, the time from first symptoms to disease diagnosis, the time since disease diagnosis, HAQ, serum levels of muscle enzymes, C-reactive protein and erythrocyte sedimentation rate. However, there was a positive correlation between serum levels of HA and VAS (patient and physician), and a negative correlation between serum levels of HA and MMT-8.

The correlation between serum levels of HA expressed in interquartile and categorical parameters analysed in the present study are shown in Table III. The data show that serum levels of HA did not correlate with demographic data (gender and ethnicity) or autoantibodies. Moreover, the use of prednisolone and/or immunosuppressives (azathioprine 2-3 mg/kg/day, methotrexate 20-25 mg/week, cyclosporine 2-3 mg/kg/day and/or mycophenolate mofetil 2-3 g/day) did not affect the serum levels of HA.

The median disease duration until medication was started was 8 months [5.0-24.0]. The median duration of using medication at the time of HA analysis was 2 years [0.8-8.3].

DISCUSSION

The present study showed, for the first time, the correlation between serum HA levels and patients with PM. The results showed a high level of serum HA in PM, with a tendency to correlate with the activity of PM disease.

HA plays an important regulatory role in the immune response by stimulating the expression of inflammatory genes in several immunological cells present in lesion areas, promoting the recruitment of cells, cytokine release and cell migration[5]. Furthermore, HA stimulates the release of factors that assist the action of fibroblasts in the inflammatory response, such as TNF-α and IL-1β[6].

The presence of high levels of HA serum is descri-

TABLE II. CORRELATION BETWEEN DEMOGRAPHIC, DISEASE STATUS PARAMETERS OF PATIENTS WITH POLYMYOSITIS AND SERUM LEVELS OF THE HYALURONIC ACID

Parameters		r	P
Age (years)	44.5±12.0	0.3904	0.391
Time from first symptoms to disease diagnosis (mo)	6.0 [3.0-12.0]	0.0849	0.847
Time since disease diagnosis (years)	4.0 [1.0-7.0]	-0.0575	0.743
Disease status			
Patient's VAS	2 [0-6]	0.3424	0.044
Physician's VAS	1 [0-3]	0.4073	0.015
MMT-8	74 [68-80]	-0.3987	0.018
HAQ	0.43 [0.00-1.14]	0.2032	0.273
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
Aspartate aminotransferase (U/L)	26 [16-39]	0.1840	0.298
Alanine aminotransferase (U/L)	29 [18-47]	0.1922	0.294
Lactate dehydrogenase (U/L)	469 [365-544]	0.2884	0.175
Protein C-reactive (mg/L)	3.7 [1.1-7.5]	-0.023	0.896
ESR (mm/1st hour)	20.0 [8.0-33.0]	-0.118	0.593

ESR: erythrocyte sedimentation rate; VAS: Visual Analogic Scale; MMT: Manual Muscle Testing; HAQ: Health Assessment Questionnaire. Results are expressed as mean ± standard deviation (SD), median [interquartile 25th - 75th].

TABLE III. CORRELATION BETWEEN SERUM LEVELS OF THE HYALURONIC ACID EXPRESSED IN INTERQUARTILE AND DEMOGRAPHIC AND TREATMENT OF THE PATIENTS WITH POLYMYOSITIS

	AH1 N=9	AH2 N=9	AH3 N=9	AH4 N=8	P
Female Gender	6 (66.7)	7 (77.8)	7 (77.8)	7 (87.5)	0.944
Caucasian	5 (55.6)	7 (77.8)	6 (66.7)	3 (37.5)	0.420
Disphagia	0	0	0	2 (22.3)	
Articular involvement	0	0	1 (11.1)	1 (11.1)	
Pulmonary involvement	2 (22.3)	1 (11.1)	1 (11.1)	0	
Cardiac involvement	0	0	0	0	1.000
Antinuclear antibody	5 (55.6)	5 (55.6)	3 (37.5)	4 (44.5)	
Anti-Jo-1 antibody	2 (22.3)	0	1 (11.1)	0	
Prednisolone					
Current using	4 (44.5)	6 (66.7)	6 (66.7)	3 (37.5)	0.593
Current doses > 20 mg/day	9 (100.0)	8 (88.9)	8 (88.9)	7 (87.5)	0.889
Immunosuppressives					
One	2 (22.2)	6 (66.7)	5 (55.6)	3 (37.5)	0.253
Two	8 (88.9)	5 (55.6)	7 (77.8)	6 (75.0)	0.487
Three	8 (88.9)	9 (100.0)	8 (88.9)	8 (100.0)	1.000

Results are expressed as percentage (%). AH: hyaluronic acid expressed in interquartile (AH1: 0-72 ng/mL; AH2: 73-284 ng/mL; AH3: 285-731 ng/mL; AH4: 732-1651 ng/mL); Immunosuppressives: azathioprine 2-3 mg/kg/day, methotrexate 20-25 mg/week, cyclosporine 2-3 mg/kg/day, mycophenolate mofetil 2-3 g/day

bed in other autoimmune diseases, such as rheumatoid arthritis, systemic sclerosis, systemic lupus erythematosus, dermatomyositis and psoriatic arthritis[7-11,19,20].

Regarding systemic lupus erythematosus, increased HA relates to disease activity as an immunomodulator, with specific molecular recruitment of immune cells to sites of inflammation[7].

Serum HA levels were also significantly increased in patients with psoriatic arthritis[11]. However, the correlation was significant only between HA levels and the degree of skin involvement. No correlation was demonstrated with articular involvement[11].

In dermatomyositis, few studies have been conducted, but the published ones correlated clinical manifestations of this disease with mildly elevated values of HA[10]. Kubo et al[4] reported two patients who presented a positive correlation between disease activity and high levels of HA. The same authors observed, in 40 patients with dermatomyositis, that serum HA was elevated when compared to patients with systemic lupus erythematosus, rheumatoid arthritis and systemic sclerosis[4]. In DM, there is a correlation between increased HA levels and cutaneous manifestations, such as photosensitivity. Therefore, it was concluded that the longitudinal measurement of the concentration of serum hyaluronate can be useful for estimating the activity of DM in patients whose initial serum hyaluronate is high[10].

These studies show the strong correlation between HA and disease activity[7-11,19,20]. However, they did not specifically analyse the role of immunosuppressives and/or glucocorticosteroid on the expression of HA. Previous studies showed that cyclosporine[21] and glucocorticosteroid[22], for instance, could decrease the level of HA expression. Our study showed that the serum level of HA was independent of drug therapy.

In the present study, an increase in the serum level of HA in patients with PM was also noted and had a tendency to correlate with disease activity. It most likely was not possible to find a strong positive correlation between the serum level of HA and PM disease activity because the majority of our patients were relatively stable in terms of clinical and laboratory data.

Similarly to other systemic autoimmune diseases, HA could play a role in inflammatory regulation. Moreover, HA could stimulate the expression of inflammatory genes in several immunological cells present in areas of muscle lesions, promoting the recruitment of cells, cytokine release and cell migration. Furthermore, HA could be related to clinical manifestations of autoimmune diseases that can cause impairment of proximal limb movement and cutaneous manifestations, such as discoid lesions and shawl sign, in addition to increased inflammatory response. However, more studies are needed to assess the mechanism of HA involved in the pathogenesis of PM, particularly its correlation with the clinical manifestations of the disease and their degree of severity because the demographic and laboratory parameters did not show a strong association with an increase in HA.

The small sample size is the major limitation of this study and it is due to our rigorous selection criteria for a relatively rare disease. Additionally, the inclusion of patients solely from a tertiary care centre may not represent the full PM spectrum and could result in an overestimation of disease or drug complications of a more severe disease (bias). Therefore, the external validity of our results needs to be confirmed.

CONCLUSIONS

It was observed that serum HA was elevated in patients with PM and showed a tendency to correlate with the activity of this disease. Additional studies are needed to assess this correlation, as well as to understand the mechanism involved in the pathogenesis of PM by HA and the possible prognostic role of HA in these patients.

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