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Circulating biomarkers in acute myofascial pain

A case–control study

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Abstract

The aims of the present study were to compare levels of circulating inflammatory biomarkers and growth factors between patients with myofascial pain syndrome (MPS) and healthy control participants, and to assess the relationship among inflammatory markers and growth factors in the two groups.

Biomarkers levels were assessed in patients (n=37) with myofascial pain complaints recruited from the hospital emergency department and non-MPS controls (n=21), recruited via advertisements in the hospital and community.

Blood levels of the cytokines, namely, interleukin-6 (IL-6), tumor necrosis factor (TNF), and interleukin-12 (IL-12), and the chemokine, namely, monocyte chemoattractant protein-1 (MCP-1), macrophage-derived chemokine (MDC), eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-8 (IL-8), and macrophage inflammatory proteins-1β (MIP-1β) were significantly higher in patients with MPS than controls. The results of the growth factor analyses revealed significantly higher levels of fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) in MPS patients versus controls. The pattern of correlation coefficients between cytokines and growth factors differed considerably for MPS patients and controls with far fewer significant positive coefficients observed in the controls. Serum inflammatory and growth factor biomarkers were elevated in MPS patients.

Inflammatory biomarkers and growth factor levels may play an important role in the onset and maintenance of MPS and therefore may be useful in the diagnosis and treatment of MPS. Understanding the mechanisms of inflammation in MPS necessitates future research.

Abbreviations: ANCOVA = analysis of covariance, BMI = body mass index, CGRP = calcitonin gene-related peptide, EDTA = ethylendiaminetetraacetic acid, FGF-2 = fibroblast growth factor-2, GM-CSF = granulocyte-macrophage colony-stimulating factor, IL-1β = interleukin-1β, IL-10 = anti-inflammatory cytokine interleukin-10, IL-12 = interleukin-12, IL-1ra = interleukin-1 receptor antagonist, IL-6 = interleukin-6, IL-8 = interleukin-8, MCP-1 = monocyte chemoattractant protein-1, MDC = macrophage-derived chemokine, MIP-1α = macrophage inflammatory protein-1α, MIP-1β = macrophage inflammatory proteins-1β, MPS = myofascial pain syndrome, MTrPs = myofascial trigger points, PDGF = platelet-derived growth factor, SEM = standard error of the means, TNF-α = tumor necrosis factors, VEGF = vascular endothelial growth factor.

Keywords: acute pain, chemokines, cytokines, growth factors, myofascial pain

1. Introduction

Acute and chronic myofascial pain syndrome (MPS) is the most commonly diagnosed clinical pain disorder with a high prevalence, varying from 15% of patients in general medical clinics to 85% in pain management centers.[1–3] Despite the high prevalence of MPS, as there are no objective diagnostic criteria, it is often misdiagnosed or underdiagnosed and therefore very likely undertreated.[4] Furthermore, although patients with acute or chronic MPS are commonly seen in the emergency room, emergency physicians are not often trained to diagnose and treat MPS, leading to suboptimal management of these patients.[5] Current clinical diagnosis of MPS is made based on focal or regional pain and the presence of hypersensitive nodules—myofascial trigger points (MTrPs).[6,7] These trigger points are located in taut bands, groups of muscle fibers that elicit pain with palpation.[4] Gerwin [8] proposed that muscle pain may arise from ischemia-induced inflammation in the muscle as a result of capillary compression by the taut bands.

Previous studies reported that increased levels of local (i.e., intramuscular) biomarkers associated with pain and inflammation have been observed in the vicinity of active MTrPs.[9,10] Specifically, Shah et al[9,10] observed that levels of interleukin-1β

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(IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factors (TNF-α), bradykinin, calcitonin gene-related peptide (CGRP), substance P, and norepinephrine increased in real time within MTrPs with increased pain. Moreover, patients with MTrPs have elevated levels of inflammatory biomarkers in remote uninvolved intramuscular sites, suggesting that inflammation is not only a localized response but may also be a systemic response.[9]

Shah et al used a microdialysis technique to assess the local biochemical milieu of the muscle.[9,10] However, microdialysis is not commonly available in the clinical setting. In contrast, we measured the levels of the circulating biomarkers, which is feasible to carry out in the clinic.

Furthermore, there is strong evidence to suggest that in adults with muscle injury, serum levels of growth factors are elevated and may be involved in skeletal muscle injury and repair processes. The growth factors include fibroblast growth factor-2 (FGF-2)[11–13] and vascular endothelial growth factor (VEGF),[14] which is involved in muscle repair mechanisms. Since platelet-derived growth factor (PDGF), FGF-2 and VEGF are also involved in skeletal muscle capillary formation,[15,16] allowing restoration of the blood flow to the injured tissue, it is possible that these growth factor levels increase in response to injury in skeletal muscle. We hypothesize that in acute MPS the levels of circulating cytokines, cell signaling molecules that mediate inflammation, and chemokines, small chemotactic cytokines that direct migration of inflammatory cells to the sites of inflammation, are elevated compared with healthy control subjects. We also hypothesize that growth factors are significantly associated with inflammatory markers. The main objectives of the present study were to assess the levels of circulating inflammatory biomarkers in acute MPS patients admitted to a hospital emergency department within 24 hours of sustaining a musculoskeletal injury and compare them to those of non-MPS controls, and to assess whether their levels are associated with growth factors.

2. Methods

A total of 37 patients with MPS and 21 healthy controls participated in a case–control study. The study protocol was reviewed and approved by the Ethics Review Board of McMaster University, Hamilton Health Sciences and St. Joseph's Healthcare. The research in this study was conducted in accordance with the Declaration of the World Medical Association. Informed consent was obtained from each study participant. We recruited patients who presented to the emergency department of 3 teaching hospitals in Hamilton, Ontario. Non-MPS controls were recruited via advertisements placed on notice boards posted in the hospital and community.

2.1. Participants

Patient participants were recruited into the study if they were presented with myofascial pain within 24 hours of presenting to the emergency department. The diagnosis of MPS was determined by a physiatrist with 20 years of clinical experience after a thorough assessment (history and physical examination) by applying the published diagnostic criteria.[7,8,17] Inclusion criteria for the patients recruited into this study were as follows: women or men between the ages of 20 and 65 years diagnosed with primary MPS in the upper trapezius and neck (regional pain, acute onset), presented to the emergency department within 2 to 24 hours of symptom onset, musculoskeletal complaints (pain, myofascial trigger point), and Glasgow Coma Scale score 13 or higher. Exclusion criteria were as follows: pregnancy; recent trauma (prior to the study); comorbidities including diabetes, arthritis, multiple sclerosis, neurological disorder, dystrophy, and conditions associated with inflammation (e.g., active infection and malignancies); stroke; current participation in a contact sport; smoking; alcohol or drug abuse; and insufficient command of the English language to provide informed consent. Our objective was to recruit patients presenting at the emergency department who had primary MPS within 24 hours of onset and no other injuries. Patients were matched with controls for body mass index (BMI). The non-MPS participants were included in the study if they were symptom free for 6 months and their physical examination was normal. Review of study participants' medical history confirmed that none of the participants underwent chemotherapy or radiotherapy treatments or had cervical radiculopathy.

Systemic venous blood samples were drawn at the antecubital fossa on the day of presentation to the emergency department within 2 to 24 hours of symptom onset in MPS patients and during the visit in controls.

2.2. Biomarker analysis

Blood samples were collected in the emergency room into chilled tubes containing ethylendiaminetetraacetic acid (EDTA). The samples were then immediately centrifuged at 2056 g for 15 minutes at 4°C and the plasma was stored at −80°C until used for analysis. The biomarker analysis included the inflammatory cytokines TNF-α, IL-6, and interleukin-12 (IL-12); the chemokines, IL-8, eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1α, and macrophage inflammatory proteins-1β (MIP-1β); the anti-inflammatory cytokines interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra); and growth factors FGF-2, VEGF and PDGF.

Plasma cytokines and chemokines were assayed using a multiplex kit (LINCO Research Inc., St. Charles, MO), as described previously.[18] The samples were loaded into a 96-well plate, together with appropriate standards and controls, and were run in duplicate. Antibody-immobilized beads were added to the wells. The beads were conjugated to antibodies for the antigens in the panel. Following an incubation period, the unbound beads were removed and an antibody detection cocktail solution was added to the wells, together with streptavidin–phycoerythrin for visualization. The plate was run on a Luminex machine and the cytokines and chemokines were quantified.

2.3. Statistical analysis

All statistical analyses were performed using the statistical package SPSS (version 19.0, SPSS Inc., Chicago, IL). The Shapiro–Wilk test of normality and graphical inspection of a Q–Q plot were performed to determine whether the data were normally distributed. An independent sample *t*-test or Mann–Whitney U test was performed to examine the differences in inflammatory markers and growth factors between patients with MPS and non-MPS controls. Data are presented as mean ± standard error of the means (SEM) or as medians and interquartile ranges (25th and 75th percentiles). An analysis of covariance (ANCOVA) was performed to compare the levels of

Table 1

Baseline characteristics of patients with MPS and non-MPS control group.

	MPS group	non-MPS controls
Age, y	42 ± 2.7	48 ± 2.4
Sex (men, %)	51%	56%
BMI (mean)	28.7 ± 1.4	26.3 ± 1.2

BMI = body mass index, MPS = myofascial pain syndrome.

biomarkers in patients with MPS and healthy controls, while controlling for sex and age (as these variables may affect the level of biomarkers). The Pearson product–moment correlation coefficient or the Spearman rank correlation coefficient was performed to assess the relationship between inflammation markers and growth factors. A *P*-value ≤ 0.05 was considered statistically significant.

3. Results

Baseline characteristics of patients with MPS and non-MPS control groups are presented in Table 1, including years of age, sex, and BMI.

3.1. Inflammatory mediator levels in patients with MPS and healthy controls

The levels of the cytokines IL-6, TNF, and IL-12 were significantly higher in patients compared with those of healthy controls (Fig. 1A, B). With the exception of MIP-1α, the levels of all the chemokines, including MCP-1, MDC, eotaxin, GM-CSF, IL-8, and MIP-1β were significantly higher in patients with MPS compared with those of healthy controls (Fig. 2A, B). The levels of the anti-Inflammatory IL-1ra and IL-10 were also higher in patients with MPS compared with those of the healthy controls. Median IL-1ra levels were 117.8 (IQR: 77.2, 505.3) in patients with MPS versus 6.0 (IQR: 3.5, 48.5) in healthy controls ($P < 0.001$). Median IL-10 levels were 4.4 (IQR: 1.2, 14.5) in patients with MPS versus 1.3 (IQR: 0.4, 1.8) in healthy controls ($P < 0.05$). Similarly, growth factors FGF-2, PDGF, and VEGF levels were significantly elevated in patients compared with those of healthy controls (Fig. 3A, B). Within 24 hours of admission, most inflammatory markers in patients with MPS were elevated 30 to 70 times than those of the healthy cohort. The ANCOVA revealed that the differences in the levels of biomarkers between the MPS patients and healthy controls were not significantly affected by sex and age.

3.2. Correlation between growth factors and inflammatory mediators

The pattern of correlation coefficients between cytokines and growth factors differed considerably for MPS patients and controls with far fewer significant positive coefficients observed in the controls. In patients with MPS, there was a strong positive correlation with the levels of growth factor FGF-2 and GM-CSF ($r = 0.75$, $P < 0.001$) and MIP-1β ($r = 0.70$, $P < 0.001$) and moderate correlation with IL-12 ($r = 0.52$, $P < 0.001$)

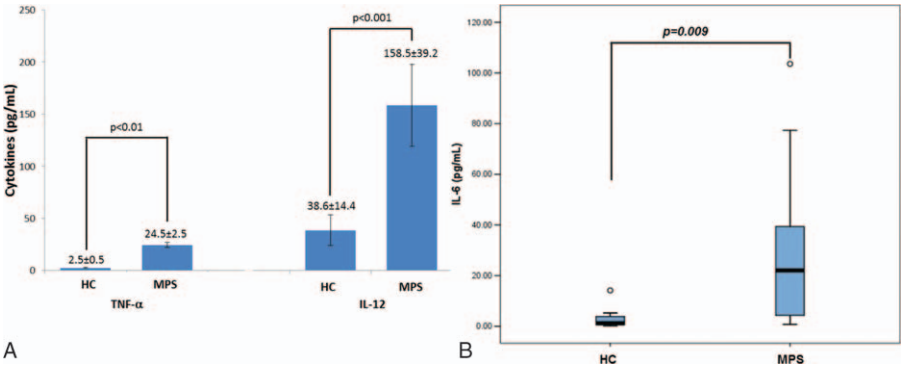

Figure 1. (A, B) Levels of cytokines in patients with MPS and healthy controls. The levels of the cytokines IL-6, TNF, and IL-12 were significantly higher in patients compared with those of healthy controls. Data are presented as mean ± SEM or as medians and interquartile ranges (25th and 75th percentiles). IL-6 = interleukin-6, IL-12 = interleukin-12, MPS = myofascial pain syndrome, TNF = tumor necrosis factor, SEM = standard error of the means.

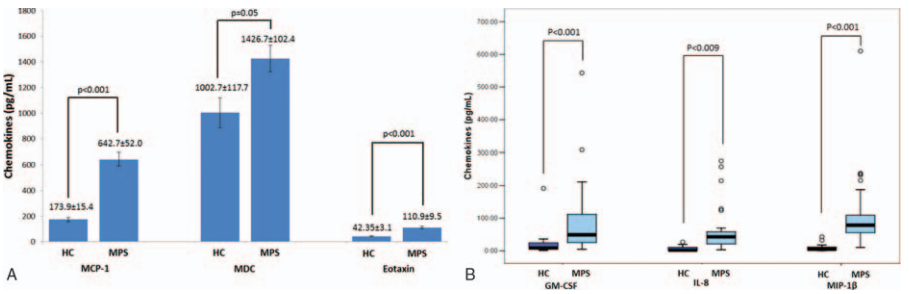

Figure 2. (A, B) Levels of chemokines in patients with MPS and healthy controls. The levels of the chemokines MCP-1, MDC, eotaxin, GM-CSF, IL-8, and MIP-1β were significantly higher in patients with MPS compared with those of healthy controls. Data are presented as mean ± SEM or as medians and interquartile ranges (25th and 75th percentiles). GM-CSF = granulocyte-macrophage colony-stimulating factor, IL-8 = interleukin-8, MCP-1 = monocyte chemoattractant protein-1, MDC = macrophage-derived chemokine, MIP-1β = macrophage inflammatory proteins-1β, MPS = myofascial pain syndrome, SEM = standard error of the means.

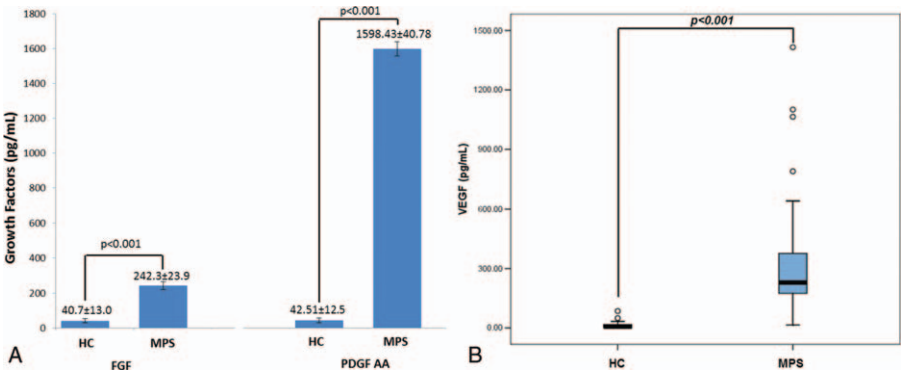

Figure 3. (A, B) Growth factors levels in patients with MPS and healthy controls. Growth factors FGF-2, PDGF, and VEGF levels were significantly elevated in patients compared with those of healthy controls. Data were presented as mean ± SEM or as medians and interquartile ranges (25th and 75th percentiles). FGF-2 = fibroblast growth factor-2, MPS = myofascial pain syndrome, PDGF = platelet-derived growth factor, SEM = standard error of the means, VEGF = vascular endothelial growth factor.

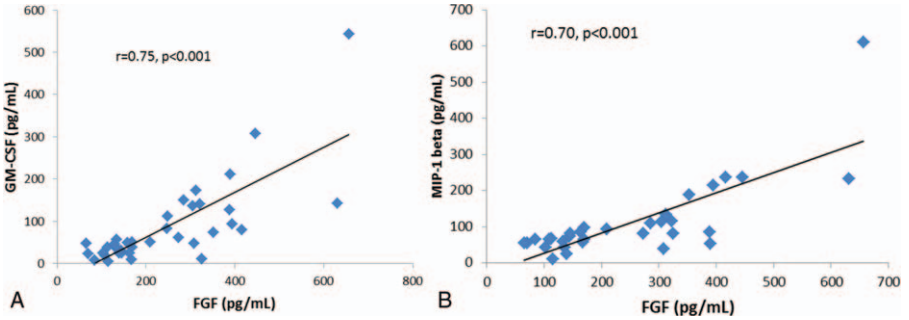

Figure 4. (A, B) Relationship between inflammatory markers and growth factors in patients with MPS. In patients with MPS, there were strong positive correlations between the levels of growth factor FGF-2 and GM-CSF and MIP-1β. FGF-2 = fibroblast growth factor-2, GM-CSF = granulocyte-macrophage colony-stimulating factor, MIP-1β = macrophage inflammatory proteins-1β, MPS = myofascial pain syndrome.

(Fig. 4A, B). However, FGF-2 levels were negatively correlated with MCP-1 levels ($r = -0.47$, $P < 0.003$). There was also a significant moderate correlation between VEGF and TNF-α ($r = 0.55$, $P < 0.001$) (Fig. 5), IL-8 ($r = 0.47$, $P < 0.001$) and eotaxin ($r = 0.46$, $P < 0.05$), and a moderate correlation with MDC ($r = 0.33$, $P = 0.04$), IL-12 ($r = 0.40$, $P < 0.01$), and MIP-1β ($r = 0.32$, $P < 0.05$). There was a weak but significant correlation between PDGF and MDC ($r = 0.35$, $P < 0.03$) and TNF-α ($r = 0.35$, $P = 0.03$), Furthermore, there was also a significant positive correlation between FGF-2 and the anti-inflammatory markers IL-1ra ($r = 0.64$, $P < 0.001$), as well as between VEGF and IL-1ra ($r = 0.39$, $P < 0.1$).

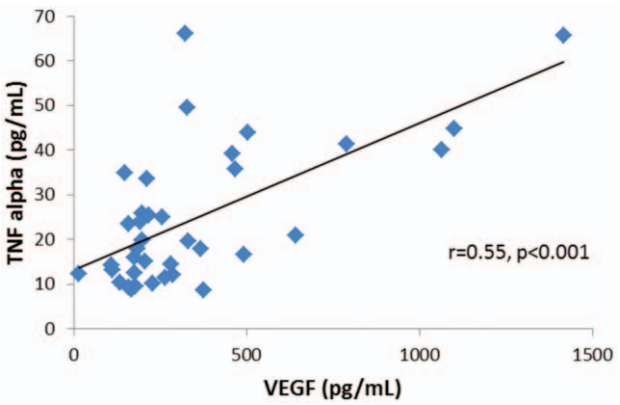

Figure 5. Relationship between inflammatory markers and growth factors in patients with MPS. In patients with MPS, there was a strong positive correlation between the levels of growth factor VEGF and TNF-α. MPS = myofascial pain syndrome, TNF-α = tumor necrosis factors, VEGF = vascular endothelial growth factor.

In contrast, the pattern of results was considerably different for the correlation matrix in the non-MPS controls, where far fewer significant coefficients were observed: FGF levels were significantly correlated with TNF-α ($r = 0.85$, $P < 0.001$), and negatively moderately correlated with IL-10 ($r = -0.53$, $P < 0.03$). VEGF levels were significantly correlated with TNF-α ($r = 0.88$, $P < 0.001$).

4. Discussion

Our results demonstrate higher levels of serum inflammatory, anti-inflammatory biomarkers, and growth factors in patients with clinical acute MPS as compared with non-MPS controls. These circulating blood biomarkers included the cytokines, namely, TNF-α, IL-6, IL-12, and the chemokines, namely, GM-CSF, MIP-1β, MDC, IL-8, MCP-1, and eotaxin. These results are consistent with findings showing local and remote intramuscular changes in inflammatory biomarkers associated with pain and inflammation, suggesting a systemic involvement.[9,10] Our findings suggest that inflammatory mediators may dissipate from the site of injury into the blood stream and manifest as a

systemic response. This finding is consistent with the concept of the skeletal muscle as a secretory tissue, releasing cytokines and other peptides, exerting autocrine, paracrine, and endocrine effects.[19–21] In addition, we found that the levels of anti-inflammatory biomarkers IL-1ra and IL-10 were elevated. This suggests that the inflammatory response is counterbalanced by the anti-inflammatory mediators, possibly to tightly modulate the inflammatory processes.

The explanation for the increase in these biomarkers may be related to either skeletal muscle injury, ischemic response, or both. This is supported by findings that the levels of IL-6 and creatine kinase (CK) increased [22] and the expression of MCP-1 was elevated in injured muscle tissue.[23] Inflammation-induced muscle pain may also be linked to ischemia/reperfusion injury. A proposed mechanism for the release of inflammatory mediators is the development of local muscle ischemia as a result of capillary compression by the taut bands.[8] Plasma IL-6 and IL-8 levels increase in response to ischemia in human skeletal muscle.[24] The mechanism underlying pain originating from MTrPs can be explained by myriad inflammatory mediators released from injured tissue, which as a result, increase the mechanical sensitivity of nociceptors, and subsequently decrease the mechanical threshold of the receptors. Consequently, the receptors respond to low pressure or non-painful stimuli.[25] The sensitized muscle nociceptors are connected to the nociceptive pathway in the central nervous system. Thus, activation of these nociceptors can elicit subjective pain and local tenderness when weak mechanical stimuli are applied to the muscle.[25] The severity of pain in patients with MPS and its relationship with the levels of inflammatory biomarkers should be assessed.

Interestingly, levels of growth factors FGF-2, VEGF, and PDGF were also elevated and they correlated significantly with the inflammatory markers. Future studies should examine whether growth factors are increased in response to the inflammatory process following skeletal muscle injury as well as their role in the skeletal muscle repair processes. Several growth factors are involved in the process of muscle regeneration and improving muscle force following injury. In animal models, studies have reported that VEGF improves restoration of muscle force by reducing connective tissue and increasing the relative amount of muscle fibers, fibrosis reduction, as well as improving skeletal muscle repair by modulating muscle tissue regeneration after acute trauma,[14] myoblast growth by promoting cell adhesion, proliferation, and wound healing activity after injury.[13] Studies have shown that FGF enhances muscle regeneration and improves muscle force after muscle strain injury.[11,12] The role of PDGF in the muscle tissues is less clear. Studies have shown that FGF-2 and PDFG-BB are synergistically acting on muscle repair after ischemic injury, increasing capillary growth, collateral formation, and allowing restoration of blood flow.[16] VEGF expression in the myocyte is also involved in skeletal muscle capillary formation.[15] There was a strong correlation between several inflammatory mediators and FGF, whereas inflammatory markers only moderately correlated with VEGF and PDGF. Future studies should investigate whether FGF is involved in skeletal muscle repair in response to the inflammatory process. Moreover, the role of growth factors in both acute and chronic conditions as well as in the transition from acute to chronic MPS should be addressed in future work.

In our study, patients were matched with controls for BMI as it may affect the levels of inflammatory cytokines.[26] We recruited our study participants from the emergency department in an effort to recruit patients whose diagnoses were within 24 hours of onset. This recruitment strategy is preferable to the recruitment from the primary care clinics, as general practitioners may not have as many acute MPS patients. A limitation to the current work is that we measured inflammatory biomarkers in acute MPS and not over time. There is a possibility that the elevation of some biomarkers peaked within 24 hours, whereas some continuously increased over time after the 24 hours. In addition, it is possible that confounding factors may have affected the levels of inflammatory markers. Future studies should examine the levels of local and systemic inflammatory biomarkers longitudinally, investigating whether patients with elevated levels of systemic inflammatory biomarkers are likely to develop chronic MPS. Our study aimed at exploring whether inflammatory biomarkers are elevated in MPS. However, the usefulness of specific markers in the diagnosis of MPS should be investigated. Furthermore, we cannot exclude the possibility that undiagnosed conditions may have affected the levels of inflammation in the study participants. Another limitation is that in this study we did not perform subgroup analysis according to the levels of stress, anxiety, and other psychological factors that may, in part, be responsible for the elevation in the biomarker levels. Future studies should examine the effects of psychological stress on the levels of systemic biomarkers in patients with MPS. It is important to note that while most of the bassline characteristics were similar, the age of the patients with MPS and non-MPS controls was slightly different. However, patients with MPS were younger than non-MPS controls so that age likely did not account for the elevated levels of inflammatory biomarkers in this patient population.

In conclusion, serum levels of inflammatory biomarkers and growth factors were significantly higher in MPS patients than in non-MPS healthy control subjects, suggesting that these are by-products of muscle injury. Although we specifically examined the levels of biomarkers in the patients with MPS in the upper trapezius and neck, the present findings suggest that these biomarkers may be useful for both diagnosis and treatment of MPS. While patients with acute or chronic MPS are commonly seen in the emergency room, emergency physicians are not often trained to diagnose and treat MPS, leading to suboptimal management of these patients. Biomarkers' assessment, in conjunction with the current diagnostic methods of manual palpation, may assist emergency room physicians to more accurately diagnose MPS. Furthermore, the present findings may shed light on the mechanisms of inflammation in this common musculoskeletal pain condition.

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