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Inflammatory Biomarkers Increase with Severity of Upper Extremity Overuse Disorders

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Abstract

Musculoskeletal disorders (MSDs) from overuse are common occupational health problems that cause pain, functional loss, and loss of work time. Our goal was to determine if a relationship exists between the severity of early onset overuse-related MSDs of the upper extremity and serum levels of interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), and C-reactive protein (CRP). Twenty-two subjects affected with upper extremity MSDs due to overuse for no longer that 12 weeks were stratified according to the severity of upper extremity signs and symptoms as determined by an upper body musculoskeletal assessment (UBMA). Nine asymptomatic subjects also participated. Serum cytokines were analyzed using enzyme linked immunosorbent assays (ELISA), and CRP was analyzed using laser nephelometry technique. CRP was strongly correlated, and TNFα, IL-1β and IL-6 were moderately correlated with UBMA scores. Only CRP and TNFα were significantly associated with UBMA scores in an ordinal logistic regression analysis in which age and body mass index (BMI) were covariates. These results are of clinical importance as they suggest that early onset overuse-related MSDs may have an inflammatory component. The possibility of using a combination of serum biomarkers to follow the progression of overuse-related MSDs or their response to therapeutic intervention may be of interest to clinical practitioners and should be the focus of future research.

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Abbreviations Used

BMI: Body mass index
CRP: C-reactive protein
ELISA: Enzyme Linked-Immuno-Sorbent Assay
IL-1β: Interleukin-1 beta
IL-6: Interleukin-6
MSDs: Musculoskeletal disorders
NSAIDs: Non-steroidal anti-inflammatory drugs
TNFα: Tumor necrosis factor alpha
UBMA: Upper body musculoskeletal assessment

Introduction

According to the most recent US Department of Labor survey of occupational injuries and illnesses, which reports on US private industry injury and illness records from 2004, musculoskeletal disorders (MSDs) accounted for 402,700 (32%) of the injuries and illnesses with days away from work.[1] Service industries accounted for 69% of all lost work day MSDs, and goods producing industries accounted for 31% of all MSD cases. Upper extremity MSDs have a relatively high impact on number of days away from work. For example, carpal tunnel syndrome is associated with the highest number of lost work days (median of 28 days), and injuries to the shoulder and wrist accounted for median lost work days of 17 and 14, respectively. In addition, exposure to repetitive motion, such as grasping tools, scanning groceries, and typing, resulted in the longest absences from work by exposure type (median of 20 days).

Despite epidemiological evidence linking the onset and severity of MSDs to repetitive and forceful tasks.[2,3] there is still much to learn about the underlying pathophysiology of these disorders. Previously, we conducted a series of studies using a rat model to explore the early onset of overuse MSDs in which we found motor behavioral and physiological tissue changes similar to that seen in humans with overuse MSDs.[4–7] We have shown a dose-dependent relationship between reach rate (i.e., level of overuse exposure) and the concentrations of the pro-inflammatory cytokines interleukin-1 alpha (IL-1α) and IL-1β in serum.[4,5] This body of work leads us to hypothesize that there is a relationship between severity of early onset upper extremity MSDs due to overuse and the serum concentration of inflammatory markers, such as pro-inflammatory cytokines.

The cytokines IL-1, tumor necrosis factor alpha (TNFα) and IL-6 are intercellular signaling polypeptides produced by most injured cells as well as activated immune cells, including activated monocytes and macrophages.[6, 8] Cytokines are the chief stimulators of acute phase response proteins, and contribute to the development and remediation of signs and symptoms of acute and chronic inflammation, such as the recruitment of immune cells. IL-1 and TNFα can potently stimulate immune and stromal cell production of other cytokines and chemokines as well as most mechanisms of inflammation, including phagocyte proliferation and activation, adhesion and angiogenesis.[8] IL-6, a pleiotropic cytokine, has many pro-inflammatory effects that overlap those of IL-1 and TNFα. For example, IL-6 induces all positive acute phase response proteins.[9] IL-6 also increases in response to exercise independent of muscle damage.[10] Interstitial muscle and peritendon IL-6 production and subsequent release into the blood stream is relative to the intensity and duration of the exercise and occurs as a result of muscle glycogen depletion.[10] The circulating IL-6 then acts in a hormone-like fashion to regulate lipolysis and fat oxidation.[11] IL-1β and TNFα are not influenced by glycogen content in muscle, and thus are not elevated in serum during exercise unless tissue damage has occurred.[11]

The acute phase response is a general response designed to aid tissue repair and facilitate a return to physiological homeostasis. C-reactive protein (CRP) is a sensitive acute phase reactant produced by hepatocytes in the liver.[12, 16] CRP is a prototypic inflammatory marker of underlying low grade inflammation[12, 13] that is beneficial in identifying individuals with unstable angina pectoris underlying coronary artery disease,[14] or risk of future stroke.[15, 16] CRP is also elevated in smokers, elderly at risk for mortality, and patients with metabolic disease and diabetes.[17] Although controversial, there is emerging evidence that CRP is not only a marker of the acute phase response but may also be causal in the pathogenesis of inflammatory disease.[17, 18]

Kramer et al.[19] developed a tool to quantify the severity of work-related MSDs known as the Upper Body Musculoskeletal Assessment Instrument (UBMA). The UBMA takes a regional approach to diagnosis using clinically common tests resulting in a single composite outcome score that quantifies severity of work-related MSDs irrespective of specific diagnosis and may be useful in clinical diagnosis and evaluation of progress toward remediation. Test-retest reliability of the UBMA has been shown to be excellent (ICC = 0.88 for side of workplace equipment use and ICC = 0.94 for side opposite equipment use) among patients with upper extremity MSDs due to overuse.[19] In addition, the UBMA was shown to distinguish between a group of healthy controls and a group of workers with MSDs for both the side of equipment use and the side opposite equipment use ($p < 0.001$).[19]

In this study, we have utilized the UBMA in combination with biochemical assays of human serum to determine if a relationship exists between the severity of early onset overuse MSDs of the upper extremity and circulating inflammatory mediators and markers. Twenty-two subjects affected with upper extremity MSDs due to overuse for no longer than 12 weeks and 9 healthy, asymptomatic subjects participated. Serum was collected and examined for the presence of IL-1β, IL-6, TNFα, and CRP. We found that each of these proteins was progressively elevated with increasing UBMA score. However, only CRP and TNFα were significantly associated with UBMA score in an ordinal logistic regression model.

Materials and Methods

Subjects

Subjects were recruited from an outpatient physical therapy clinic, Chestnut Hill HealthCare facilities in Philadelphia, Pennsylvania, for participation in this study. Both the Temple University Institutional Review Board and Chestnut Hill Hospital Institutional Review Board approved all procedures. The research was carried out in accordance with the Declaration of Helsinki (2000) in the World Medical Association. All subjects signed an informed consent after full explanation of the purpose, nature and risk of all procedures. Individuals with a history of upper extremity MSDs due to overuse of no longer than 12 weeks duration were included in the study. A standardized demographic questionnaire covering medical history and medication use was administered. Exclusion criteria included a history of inflammatory disease (e.g., lupus, rheumatoid arthritis, diabetes, non-medically controlled hypertension), cancer, known coronary artery disease, disease processes that required ongoing treatment with steroids or non-steroidal anti-inflammatory drugs (NSAIDs), and cigarette smoking. Subjects were also excluded if they were medically unable or unwilling to refrain from taking NSAIDs or full dose aspirin for a period of 7 days. Individuals with a history of hypertension were included if the hypertension was currently controlled with medication. The study's demographic questionnaire included characteristics such as age, gender and race. Information was obtained related to the subject's referring diagnosis, occupation and/or primary activity related to the development of MSD. Body mass index (BMI) was calculated as weight (in kg) divided by the square of height (in m).

Subjects were examined by the same physical therapist using the UBMA as described by Kramer et al.[19] Twenty-two symptomatic subjects were recruited using a stratified, non-random sampling procedure where the strata were predefined UBMA score ranges (50–74, 75–99, and ≥100; n = 6–9 subjects per stratum). This sampling procedure was undertaken to obtain a full range of UBMA scores. Nine healthy, asymptomatic subjects were also included, and their UBMA scores were all less than 50.

Biochemical Analysis of Serum

Subjects were asked to refrain from performing strenuous exercise and taking NSAIDs or full dose (325 mg) aspirin for 7 days prior to the drawing of blood samples. Subjects taking low dose aspirin (81 mg/day) were included in this study. Venous blood samples were drawn from all subjects between 7 a.m. and 12 noon, and were collected into sterile Enzyme Catalyzed Therapeutic Activation (ECTA) tubes (5×10^{-3} mol/l; Labco, Califon, NJ) containing aprotinin (bovine lung 15–30 U/ml; Sigma (St. Louis, MO) at 0.67 U/ml. Serum was immediately separated from cells and platelets by centrifugation at 400 g for 7 minutes and then at 10,000 g for 7 minutes at 4°C. Following removal of appropriate quantities of serum for C-reactive protein (CRP) analysis, the aqueous phase was aspirated and stored in 150 microliter aliquots at –80°C until assayed as described below. Aliquots were assayed in batches to avoid any thawing and refreezing.

CRP was measured by a modification of the laser nephelometry technique (Berhing Diagnostics, GMGH, Deerfield, Illinois) according to standard protocols by the Chestnut Hill Hospital Pathology Lab. The CRP assay was standardized according to the World Health Organization First International Reference Standard. Data are expressed in mg/dL to be consistent with conventional units of reporting in the clinical literature.

For analysis of cytokines, serum samples were allowed to thaw on ice. Commercially available Enzyme Linked-Immuno-Sorbent Assay (ELISA) kits (OptEIA ELISA kits for human serum; catalog numbers 55911, 550799, 5550610, respectively; BD Biosciences, San Diego, CA) with high sensitivity for serum cytokine levels were used to determine the circulating levels of the three cytokines, IL-1β, IL-6, and TNFα. Each sample was run in duplicate for ELISA assays as a measure to control pipetting error. A mean was calculated from the duplicate values for further analysis. Data are expressed in pg/ml serum to be consistent with conventional units of reporting in the clinical literature.

Statistical Analysis

A comparison between UBMA scores for the asymptomatic and symptomatic subjects was performed using a Mann-Whitney U test. In addition, the number of single and multiple site impairments in symptomatic subjects was compared to that in asymptomatic subjects using a Chi square test. Comparisons of BMI and age between symptomatic and asymptomatic subjects were performed using independent samples t-tests. Spearman's rank correlation tests were used to determine correlations between each inflammatory biomarker and UBMA score. Ordinal logistic regression analysis with backward elimination was used to determine which combination of the inflammatory biomarkers, age and BMI were associated with UBMA score. SAS (SAS Institute, Inc., Cary, NC) was used for the statistical analyses. P-values of less than 0.05 were considered significant for all analyses.

Results

General characteristics of the subjects, existence of medically controlled hypertension, occupation and/or primary activity related to MSD, are given in Table 1, as are the UBMA scores. Table 2 summarizes the referring diagnoses, symptom duration and medications used during the time of the study for each subject. All of the referring diagnoses involved disorders of the upper extremity. The average duration of symptoms was 51.7 ± 18.9 (mean ± SD) days with a range from 17 to 76 days. Independent samples t-tests showed no significant differences between the asymptomatic and symptomatic subjects with respect to age ($p = 0.23$) and BMI ($p = 0.65$). A Mann-Whitney U test showed that UBMA score was significantly higher in the symptomatic group as compared to the asymptomatic group ($p < 0.0001$). Table 3 summarizes the UBMA results describing the number and type of impairments. The frequency of single site impairments (i.e., those related to a single anatomical region) was greater in symptomatic than in asymptomatic subjects (7 and 53, respectively), as was the frequency of multiple site impairments (i.e., those related to multiple anatomical regions; 1 and 28, respectively). Chi square analysis confirmed this difference between symptomatic and asymptomatic groups in single and multiple site impairments (χ^2 = 348; .001$\chi^2(1)$ = 10.83).

Spearman's rank correlation tests showed that all of the biomarkers were positively correlated with UBMA score. As shown in Figure 1, CRP was the most highly correlated with a Spearman's rank correlation coefficient of $r_s = 0.81$ ($p < 0.0001$). IL-1β was a moderately correlated biomarker with a Spearman's rank correlation coefficient of $r_s = 0.70$ ($p < 0.0001$), as were TNFα (rs = 0.66; $p < 0.0001$) and IL-6 ($r_s = 0.52$; $p = 0.003$).

Mean values for the serum levels of each biomarker are shown in Table 4 and reflect the correlational findings of increasing biomarker level with increasing UBMA score. Ordinal logistic regression analysis using the cut points for UBMA score indicated in Table 4 showed that CRP ($p = 0.0003$) and TNFα ($p = 0.0007$) were significantly associated with an increased UBMA score (Table 5). IL-1β and IL-6 were removed from the model with p values of 0.09 and 0.48, respectively. The covariates age ($p = 0.16$) and BMI ($p = 0.62$) were also removed from the model.

Discussion

Overall, there was a positive relationship between MSD severity, as reflected by UBMA score, and cytokine and CRP concentrations. All of the markers were positively correlated with UBMA: CRP was strongly correlated, and IL-1β, TNFα and IL-6 were moderately correlated. However, only CRP and TNFα were significantly associated with UBMA score in an ordinal logistic regression model. These results are consistent with the progressive increase in the levels of these markers with increasing severity of UBMA score.

Table 1. Demographic Characteristics of Subjects Ranked by Upper Body Musculoskeletal Assessment (UBMA) Score.
Means and standard deviations are shown for age and body mass index (BMI). Means and mean ranks, as determined for the Mann-Whitney U test, are shown for UBMA score.

UBMA Score	Gender	Age	Race/hypertension	BMI (kg/m²)	Occupation/Primary Activity Related to MSD
Asymptomatic Subjects					
10	F[a]	47	Caucasian	19.2	Nurse
11	F[a]	49	Caucasian	23.0	Biomedical Engineer
12	M	46	Caucasian	22.0	Maintenance Engineer
16	M	29	Caucasian	24.6	Physical Therapist
22	F	37	Caucasian	20.3	Clerk
30	F	19	Caucasian	16.8	Administrative Assistant
31	M	30	Caucasian	25.2	Clerk
31	F[a]	46	Caucasian	24.0	Biomedical Engineer
41	F[a]	48	African-American	29.3	Hospital Administrator
39.0 (Mean)		39.0 (Mean)		22.7 (Mean)	
5.0 (Mean rank)		10.8 (SD)		3.7 (SD)	
Symptomatic Subjects					
50	M	29	Caucasian	24.4	Nurse
51	F	28	Caucasian	20.6	Engineer
52	F[a]	69	Caucasian	20.1	Gardener, Potter
56	F[b]	26	Caucasian	16.9	Recreation Therapist
56	M	33	Caucasian	26.2	Clerk
59	F	26	Caucasian	25.0	Nurse
66	M	42	Caucasian	23.5	Stockroom Clerk
66	F	34	Hispanic	20.4	Nurse (retired 1 month)
78	M	25	Caucasian	21.0	Engineer
78	F	31	Caucasian	27.0	Cardiologist
83	F	39	Caucasian	23.4	Engineer
84	M	73	Caucasian	27.3	Gardener, House Painter
86	F	39	Caucasian	22.1	Teacher
88	M	29	Caucasian	24.9	Cable TV Installer
90	M	49	Caucasian	24.0	Laboratory Technician
91	F[a]	62	Caucasian	21.1	Computer Programmer
102	M	66	Caucasian	23.5	Woodworker
105	M	54	Caucasian	19.7	Manager
106	M	74	African-American	26.9	Artist (painter)
109	M	57	Caucasian	19.1	Consultant
119	F[a]	56	Caucasian	32.6	Manager
124	F[a]	54	Caucasian	24.0	Cook
81.6 (Mean)		45.2 (Mean)		23.4 (Mean)	
20.5 (Mean rank)*		16.7 (SD)		3.5 (SD)	

[a]Post-menopausal subject; [b]subject menstruating at the time of serum collection; [c]History of hypertension that is currently controlled medically.
*Significantly different from asymptomatic subjects by Mann-Whitney U test ($p < 0.0001$).

The results from the UBMA showed the presence of local signs of pain, tenderness, peripheral nerve irritation, weakness and limited motion that progressively increased in number with increasing serum CRP, IL-1β and TNFα levels. The number of cases with involvement of multiple anatomical sites also increased with increased MSD severity and increased serum CRP, IL-1β and TNFα levels. These results are similar to those of Ravaglia et al.[20] showing that at least CRP has a strong association with functional impairment. These results are also similar to those from an earlier study of IL-1β using a rat model of MSD developed in our laboratory.[4, 5] It seems intuitive that the more severe the MSD, the more sites are injured, the larger the acute phase response and thus the larger the concentration of serum inflammatory mediators and markers. Therefore, we suggest that the systemic inflammatory response is associated with local signs and symptoms of inflammation in humans as in our rat model. It is likely that the systemic response is initiated by a local response and is proportionally amplified in the presence of greater tissue injury and inflammation (i.e., greater number of physical signs, symptoms and impairments). The increase in the number of affected anatomical sites may be either a cause or an effect of the systemic inflammatory response.

In contrast to IL-1 and TNFα, circulating IL-6 has a more complex relationship with MSD severity in this study. The pleiotropic effects of IL-6 may help explain these results. IL-6 is a tightly regulated cytokine that is not normally detected in serum unless there is trauma, infection, cellular/tissue stress, or during or following muscle glycogen-depleting exercise intensities.[10, 11, 21] In the case of infection or inflammation, IL-6 is an early cytokine responder.[21, 22]

Table 2. Referring Diagnoses, Symptom Duration and Medication Use Ranked by Upper Body Musculoskeletal Assessment (UBMA) Score

UBMA Score	Referring Diagnosis	Symptom Duration (Days)	Medications
Asymptomatic Subjects			
10	N/A	N/A	
11	N/A	N/A	HCTZ
12	N/A	N/A	Flonase
16	N/A	N/A	
22	N/A	N/A	Flonase
30	N/A	N/A	Mvi
31	N/A	N/A	
31	N/A	N/A	Tylenol
41	N/A	N/A	Mvi
Symptomatic Subjects			
50	Median neuropathy	61	Atenelol
51	Lateral epicondylitis	52	
52	Wrist tendonitis	18	Tylenol, Pravachol[a]
56	Wrist sprain	30	
56	Wrist strain	45	Motrin
59	Myalgia	33	
66	Overuse syndrome	66	
66	Wrist extensor strain	61	Birth Control Pills
78	Rotator cuff tendonitis, wrist strain	66	
78	Lateral epicondylitis	72	Birth Control Pills
83	Carpal tunnel syndrome	29	Birth Control Pills
84	Early compartmental syndrome	17	Low Dose Aspirin, Mvi, Plavix
86	Overuse syndrome forearm	32	Tylenol, Neurontin
88	Tendonitis	72	
90	Tendonitis	48	Tylenol PM, Neurontin
91	Carpal tunnel syndrome	55	Prilosec
102	Carpal tunnel syndrome	64	
105	Cervical strain, pronator teres syndrome	71	Low Dose Aspirin, Pravachol[a]
106	Bicipital tendonitis, hand edema	68	Mvi, Lopressor, Dilantin
109	Bicipital tendonitis	34	
119	Wrist tendonitis	67	Zocor[a]
124	Carpal tunnel syndrome	76	Tylenol, Pravachol[a]
		51.7 (mean) 18.9 (SD)	

HCTZ = hydrochlorothiazide; Mvi = multivitamins; N/A = not applicable.
[a]Indicates a statin medication.

Table 3. Impairments Identified by Upper Body Musculoskeletal Assessment (UBMA) in Asymptomatic and MSD Groups

Impairment	# Single Site Impairments	# Multiple Site Impairments
Asymptomatic Subjects		
Resisted MMT	0	0
PROM	1	0
Trigger Point	5	1
Median Tinel	1	N/A
Finkelstein	0	N/A
Phalen	0	N/A
Adson	0	N/A
Grasp/Pinch	0	N/A
Total	7	1
Symptomatic Subjects		
Resisted MMT	10	9
PROM	7	10
Trigger Point	10	17
Median Tinel	6	N/A
Finkelstein	4	N/A
Phalen	7	N/A
Adson	3	N/A
Grasp/Pinch	15	N/A
Total	53*	28*

N/A = not applicable because none of these tests are administered to multiple sites.
MMT = manual muscle test. PROM = passive range of motion.
*Significantly higher observed frequency than expected from the frequency in asymptomatic subjects as determined by χ^2 analysis ($p < 0.001$).

Pro-inflammatory effects of IL-6 include induction of cell growth and proliferation, inflammation, and acute-phase responses.[22, 23] It also appears to have anti-inflammatory actions such as inducing increases in circulating levels of IL-1 receptor antagonist and soluble TNF receptor.[22, 24] These activities would have a potent anti-inflammatory effect by suppressing the actions of circulating IL-1 and TNFα. We would expect the IL-6 concentrations in the serum of our subjects to fluctuate depending upon whether IL-6 is inducing pro- or anti-inflammatory effects, and would further hypothesize that symptomatic subjects less severely affected (i.e., those with UBMA scores in the 50–75 range) would benefit the most from the anti-inflammatory functions of IL-6, such as attenuating IL-1β and the highly cytotoxic TNFα. Indeed, in our subjects, serum levels of TNFα are within range of control values at UBMA scores between 50 and 75 (Figure 1C), as are IL-1β levels in 3 of 8 subjects (Figure 1B). Among more severely affected subjects (i.e., those with UBMA scores above 100), IL-6 may augment the inflammatory response, or it may be unable to downregulate the production of other proinflammatory cytokines.

Our serum results differ from those in a study by Freeland et al.[25] in which serum and tenosynovial tissues were examined in 41 patients with idiopathic carpal tunnel syndrome (most with abnormal nerve conduction velocity changes). Serum was collected 1 week prior to carpal tunnel release surgery, while tendosynovial tissue was collected at the time of surgery. Their findings of increased serum malondialdehyde, a marker of cell injury resulting from reperfusion, an absence of serum IL-1 and IL-6, as well as increased malondialdehyde, prostaglandin E2 and IL-6 in the tendosynovial tissues, led them to hypothesize that idiopathic carpal tunnel syndrome has a non-inflammatory ischemia-reperfusion etiology that results in fibrosis of carpal tunnel tissues. We also found fibrosis of carpal tunnel tissues in our rat model between 9 and 12 weeks, a few weeks after the onset of the inflammatory response.[7] The fibrotic carpal tunnel changes were accompanied by significant declines in median nerve

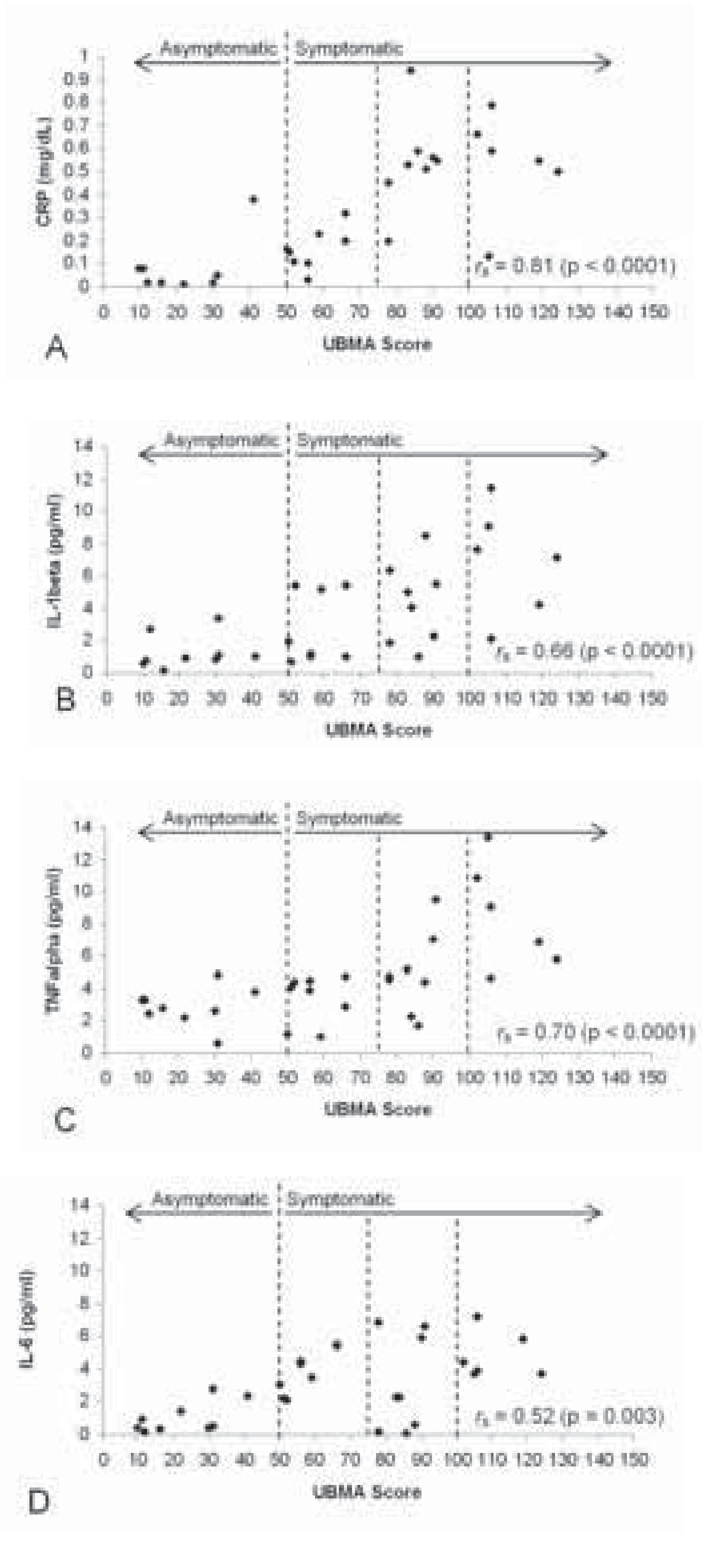

Figure 1. Inflammatory biomarker concentrations versus upper body musculoskeletal assessment (UBMA) scores for all symptomatic (n = 22) and asymptomatic (n = 9) subjects showing Spearman's rank correlation coefficients (r_s). **(A)** C-reactive protein (CRP); **(B)** interleukin-1β (IL-1Beta); **(C)** tumor necrosis factor α (TNF alpha); and **(D)** interleukin-6 (IL-6). Vertical dashed lines show cut points used in ordinal logistic regression. Horizontal arrows indicate ranges of scores for asymptomatic (left arrow) and symptomatic (right arrow) subjects.

Table 4. Summary of Mean (SD) Inflammatory Marker Concentrations in Serum for the Asymptomatic and Symptomatic Groups Calculated According the Cut Points Used in the Ordinal Logistic Regression Analysis.
CRP is expressed in mg/dL, and TNFα, IL-1β and IL-6 are expressed in pg/ml.

UBMA Score Range	CRP	TNFa	IL-1b	IL-6
0–49 (asymptomatic)	0.08 (0.12)	2.87 (1.18)	1.29 (1.04)	1.05 (0.94)
50–74	0.16 (0.09)	3.35 (1.49)	2.73 (2.18)	3.82 (1.32)
75–99	0.54 (0.20)	4.93 (2.49)	4.33 (2.50)	3.11 (2.93)
100–150	0.54 (0.22)	8.45 (3.32)	6.95 (3.35)	4.80 (1.42)

CRP = C-reactive protein, IL-1 = interleukin, SD = standard deviation, UBMA = upper body musculoskeletal assessment.

Table 5. Summary of Final Step in the Backward Ordinal Logistic Regression Model Showing the Significant Variables, C-reactive Protein (CRP) and Tumor Necrosis Factor Alpha (TNFα)

Variable Name	OR	CI	p
CRP	>999.9	111.8–>999.9	0.0003
TNFα	2.2	1.4–3.4	0.0007

OR = odds ratio; CI = 95% confidence interval.

conduction velocity. Recent and as yet unpublished results from our rat model indicate that the serum cytokine levels return to normal after the changeover to a fibrotic tissue state. We hypothesize that our observed serum inflammatory responses in both this current human study and in our previous rat studies are signs of early onset of MSDs and are not indicative of later stages of MSDs. We would also like to note that this current study is not entirely comparable to Freeland's study. We had only 4 patients diagnosed with carpal tunnel syndrome in this study. The remainder of our symptomatic subjects had musculoskeletal overuse diagnoses. Further experimentation is needed to determine if the involvement of musculoskeletal tissues instead of or in addition to nerve tissue is necessary for an increase in serum cytokines.

There are many patient characteristics in addition to the presence of MSD that may affect the serum concentrations of these inflammatory biomarkers. While intense and prolonged exercise elevates IL-6 due to exercise-induced glycogen depletion,[10, 11] repetitive low intensity exercise does not appear to contribute to elevated serum IL-6 levels.[26] In a study using microdialysis catheters to measure IL-6 levels in the trapezius following 20 minutes of repetitive low-force exercise, interstitial muscle levels of IL-6 were elevated but not serum levels.[26] Conversely, in another study by Chan et al.,[11] serum levels of IL-6 increased significantly after 2.5 hours of high intensity, glycogen-depleting exercise. Circulating levels of IL-6 have been shown to remain increased for 48 hours after prolonged exercise.[27] Therefore, our subjects were asked to refrain from vigorous exercise for 7 days before having their blood drawn. While we did not ask them to refrain from light intensity exercise, it is unlikely that our measures were affected by low intensity physical activity, as suggested by Rosendal et al.[26]

The more severely affected symptomatic subjects show similar CRP levels as elderly subjects[28] and patients with non-medically controlled angina,[14] but greater than smokers.[15] Hypertension, obesity and high coffee intake contribute to levels of serum CRP and TNFα that are similar to those seen in our more severely affected subjects, and to serum levels of IL-6 seen in all of our symptomatic subjects.[29–31] The IL-6 levels in our MSD subjects are also similar to the very elderly, aged smokers, and aged patients with significantly reduced functional status,[32] although considerably higher than levels found by Hager et al.[9] in elderly subjects. All of our subjects had CRP and serum cytokine levels that were considerably lower than those seen in studies examining patients with severe trauma with risk for mortality, cardiogenic shock and sepsis.[33–35, 49]

Obesity is associated with an increase in serum CRP, TNFα and IL-6.[30, 36] In our study, the mean BMIs for the asymptomatic and MSD groups were below the threshold for obesity of 30 kg/m^2 defined by the American Heart Association (Table 1; americanheart.org). Only one of our symptomatic subjects would be considered obese. Therefore, BMI was not a significant covariate in our regression model, although a sample with a larger number of obese individuals might give a different result.

Medications may affect serum levels of inflammatory mediators. Statin medications have been shown to reduce serum levels of IL-1β[37] and CRP,[37, 38] but not TNFα.[39] Low dose aspirin has been shown to reduce IL-1β, but not CRP.[37] We did not exclude subjects who were prescribed and taking low dose aspirin (81 mg/day) and statin drugs. Four of our symptomatic subjects were prescribed statin therapy or low dose aspirin (Table 2). Since statins and aspirin tend to lower CRP and pro-inflammatory cytokine levels, their ingestion by any of our subjects would lower the concentration of the biomarkers, thereby diluting our main effects and, therefore, at least not falsely supporting our hypothesis.

Hormonal factors also affect the production of inflammatory mediators, and may help explain the increased susceptibility of women to overuse MSDs. Estrogens exacerbate inflammation making women more vulnerable to chronic inflammatory disorders.[40] Lack of estrogen, through naturally occurring or medically induced menopause, is associated with systemic increases in IL-6, and localized tissue increases in IL-1, IL-6 and TNFα.[41–43] In addition, the use of hormone replacement therapy might be associated with an enhanced thrombotic tendency.[44, 45] The evidence is equivocal about the relationship between pro-inflammatory cytokines and estrogen. We are uncertain as to the effect of not stratifying our female subjects by their hormonal status other than recording whether the subject was post menopausal or menstruating at the time of serum collection. Four of the 9 asymptomatic subjects were postmenopausal, whereas 5 of the 22 symptomatic subjects were postmenopausal or menstruating at the time of serum collection. The effect of this small number of subjects would tend to dilute our main effects, thus not falsely supporting our hypothesis.

The effect of aging on CRP and cytokine production has also been studied extensively. Roubenoff et al.[46] found that serum concentrations of IL-6, CRP and IL-1 receptor antagonist, but not IL-1β or TNFα, were increased in elderly subjects (mean age 79 years) compared to younger control subjects (mean age 39 years). In addition, the increased concentration of IL-6 was correlated with an increased serum concentration of CRP. The authors postulated a dysregulation of IL-6, CRP and IL-1 receptor antagonist with aging. However, since findings by Macy et al.[12] and Chrysohoou et al.[29] indicate a weak or absent association of CRP with age, perhaps the elevation of CRP in the elderly population studied by Roubenoff was due to inflammatory comorbidities, which were not reported. On the other hand, in a large study of community-dwelling elderly (aged 65 and older) by Cohen et al.,[32] elevation of IL-6 was correlated strongly with age independent of several selected disease states and disorders of aging. Similar results of increased proinflammatory cytokine activity after menopause have been found in numerous studies.[32, 47, 48] In our study, even though the mean age of our symptomatic subjects was higher than that of our asymptomatic subjects, the difference was not statistically significant. Despite there being 4 individuals among our symptomatic subjects greater than 65 years of age (Table 1), age was not found to be a significant covariate in the ordinal logistic regression model. While inclusion of a larger number of aged subjects might change this finding, the age group included in this study is certainly representative of working-age adults, which is a group greatly affected by overuse MSDs.[1–3]

Cardiovascular problems such as severe unstable angina,[14] underlying coronary artery disease, hypertension, and risk of future stroke[15, 16] are well known to result in elevation of CRP. CRP and proinflammatory cytokines have been shown to be among the first responders in many inflammatory and/or infectious conditions. For example, Fida et al.[49] evaluated the utility of using serum levels of IL-1α, IL-6, TNFα and CRP in differentiating sepsis from meningitis in children. They found that optimal cut-off points (i.e., the points that allow detection of as many true positive and as few false positive results as possible) were 0.70 mg/dL for CRP, 12.07 pg/ml for IL-1α, 5.43 pg/ml for IL-6, and 27.35 pg/ml for TNFα. Liuzzo et al.[50] evaluated the prognostic value of CRP in adults with unstable angina. Using a cut-off point for CRP of 0.3 mg/dL as a marker for subsequent cardiac events or the need for urgent angioplasty, they found a sensitivity of 90% and a specificity of 82%. Our findings also suggest a relationship between the severity of MSD and the plasma concentrations of CRP and the proinflammatory cytokines. Although serum concentrations among our subjects are below the cut-off points for severe conditions, such as sepsis, they are comparable to those for cardiac disease risk. If CRP is a mediator of future cardiac events, as some recent hypotheses in the literature suggest, then the elevated CRP levels induced by overuse leading to MSDs could contribute to future cardiac problems if left unchecked. Clearly, the clinical use of these measures in determining the specific cause of an inflammatory disease process has limitations and it is necessary to control for potentially confounding factors when using CRP and proinflammatory cytokines as disease markers. However, their usefulness in identifying an underlying inflammatory component may add significantly to the understanding of a disease process, and these biomarkers may prove useful in determining the exacerbation or remission of the inflammatory response in MSDs such as might occur with chronic risk factor exposure or clinical intervention.

Conclusion

Several key points are supported by these findings. First, early onset MSDs due to overuse are associated with a systemic inflammatory response as demonstrated by a direct positive relationship between serum concentrations of key markers of inflammation and symptom severity in subjects with early onset MSDs. Second, increases in serum biomarkers are associated with an increase in the number of local signs of pain, tenderness, peripheral nerve irritation, weakness and limited motion as well as the involvement of multiple anatomical sites among these subjects. Recent work in our animal model of repetitive motion injury shows that local tissue injury and inflammatory responses are linked to a systemic release of proinflammatory cytokines. Therefore, the results of the present study indicate that the inflammatory markers reflect an underlying low grade inflammatory process induced by overuse. Third, the magnitude of the systemic serum response in the patients with more severe MSD signs and symptoms is of the same order as several low grade chronic inflammatory conditions known to produce elevated serum pro-inflammatory biomarkers, although not as high as major organ trauma, serious infections or inflammatory diseases. Therefore, in any study that monitors the serum levels of pro-inflammatory cytokines and CRP, care must be taken to control for other confounding characteristics, diseases and injuries. Based on our results, MSDs should be counted among these confounding variables. Fourth, while the non-specificity of the serum cytokine and CRP response presents limitations to the use of these markers in the diagnosis of specific overuse MSD conditions, the potential use of multiple serum inflammatory marker levels as indicators of the exacerbation and resolution of MSDs in combination with the UBMA instrument or a similar assessment tool is compelling, may be of interest to clinical practitioners and should be the focus of future research.

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