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Inflammatory Biomarker Gene Expression After a Repetitive Manual Task

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Abstract: Exposure to repetitive manual tasks in the workplace can induce tendon tissue injury and inflammation, which can later lead to pain and discomfort associated with lost workdays, chronic injury, and potential disability. The identification of gene signatures corresponding to early stages of tendon injury and inflammatory changes can be utilized as a screening tool before tissue damage to indicate the need for workplace interventions that can prevent pathologic progression. This project aims to identify and quantify the protein and gene expression changes in four inflammation-associated cytokine genes before and after the completion of a repetitive manual task. Sixteen healthy male college students between 18-25 years old with no history of previous exposure to forceful or repetitive manual tasks two months before the start of the study were recruited. The participants were asked to complete a brief health questionnaire and provide a baseline blood sample. Each of the subjects then performed a repetitive manual task daily for five consecutive days. Peripheral blood samples were collected at day 3, day 5 after completing the task, and a follow-up one week later. Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) was used to quantify the gene expression of C-reactive protein (CRP), interleukin-6 (IL-6), interleukin 1-beta (IL-1β), and prostaglandin-endoperoxide synthase 2 (PTGS2) in peripheral WBC. Preliminary results suggest increases in CRP and 1L-6 that return to baseline levels one-week post activity. No changes were observed in the expression of IL-1β throughout the experimental period. Levels of PTGS2 decrease beginning on day 3 and continue to decrease up to one week after completing the task. These preliminary results suggest that changes in the expression of inflammation-associated genes can be quantified

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from peripheral blood samples before inflammatory cytokines are expressed at a clinical level. Furthermore, preliminary results provide the basis for a continuing project aimed to identify markers of pre-inflammatory tendon tissue injury.

Keywords: Tendon, repetitive task, inflammation, mRNA, qRT-PCR.

1. Introduction and Background.

Work-related musculoskeletal disorders (WMSDs) are comprised of an extensive collection of degenerative and inflammatory conditions affecting tendons, muscles, joints, ligaments peripheral nerves, and blood vessels. These conditions frequently produce functional impairment and pain, commonly affecting the upper extremities and the neck (Buckle & Devereux, 2002; Punnett & Wegman, 2004). The strain produced by repetitive mechanical loading of the tendons stimulates the expression of selected inflammatory genes, including CRPs, cytokines IL-6, and IL-1β and PTGS2, that may potentially initiate a sub-clinical inflammatory response. Therefore, examining the expression of selected inflammatory genes after performing repetitive work cycles with the upper extremities can provide information about associations between mechanical tendon load and early signs of tendon inflammation, which have been associated with the development of WMSD.

1.1 Reverse Transcription Quantitative Polymerase Chain Reaction

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is a laboratory technique to quantify messenger ribonucleic acid (mRNA) expression, which corresponds to the production of proteins, including those associated with tendon inflammation (Bustin, Benes, Nolan, & Pfaffl, 2005). This technique involves the extraction of mRNA from a biological sample that is synthesized into complementary deoxyribonucleic acid (cDNA) to be used as a template for a quantitative polymerase chain reaction (qPCR). The resulting PCR product is an indicator of the expression of a specific gene in the sample.

The RT-qPCR technique has been used as a tool to measure changes in gene expression after a clinical intervention, to monitor drug therapy or to diagnose genetic diseases. In a study by Fujiwara et al. (2017), RT-qPCR was used to monitor the progression of muscle deterioration by examining changes in genes associated with muscular athropy at different time points.

2. Methods.

A total of 17 participants were recruited, and 16 were included in the final analysis. All participants were part of a college-age student convenience sample from the University of Texas at El Paso. All participants were considered as part of a cohort, and each participant served as their own control.

2.1 Repetitive Manual Task

The participants were asked to complete a simple manual task for 5 consecutive days using only the dominant hand. The task consisted of moving a 0.5 lb. ball up and down three platform levels positioned at varying heights: level 1 was positioned, so the shoulder was flexed at a 45° angle, level 2 at a 68° angle, and level 3 at a 90° angle. The participants first grasped the ball with the hand in a neutral position and the wrist at a 0° angle. The participants completed 1,800 cycles—moving the ball up or down to a different level was considered one cycle. A digital metronome was used to set the pace at 72 beats per minute (BPM), where each beat of the metronome signaled the participant to move the ball up or down one level. Additionally, the 1,800 cycles were divided into three stages: two stages of 576 cycles (8 minutes) each and the third of 648 cycles (nine minutes). The participants were given 10 minutes of rest between stages.

2.2 Electromyography (EMG)

Non-invasive surface electromyography (SEMG) data from the upper limbs were obtained by placing Noraxon dual EMG electrodes (Noraxon USA) in the anterior deltoid and the *extensor carpi radialis* muscles of the dominant arm. The anterior deltoid of the non-dominant arm was collected as a control. Surface EMG data were used to monitor muscular activity while the participants performed the manual task. Muscular activation was maintained below 15% of the maximal voluntary contraction (MVC) throughout the activity to simulate the muscular activity of manual workers and to reduce the risk of injury. SEMG recordings were obtained simultaneously in three different channels and were sampled at 1,500 Hertz (Hz). Recorded data were rectified using a bandpass with a high frequency of 100 Hz and a low frequency of 15 Hz (Merletti & Di Torino, 1999).

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2.3 Blood Samples

Venous blood samples were collected from the antecubital area of the arm before the beginning of the task (baseline), on days three and five after completion of the task, and one week following the end of the task assignment. Each blood donation consisted of an approximate 10 milliliter (mL) sample of whole blood collected in one acid citrate dextrose (ACD) vacutainer tube (Becton, Dickinson, and Company). Tubes were centrifuged at 3,200 RPM for ten minutes to separate plasma, white and red blood cells using the Adams Compact II centrifuge (Clay Adams). The interphase containing white blood cells was collected and washed with tris ammonium chloride (TAC) to remove red blood cells and then centrifuged at 3,200 RPM to form a pellet. The washed white blood pellet was resuspended in freezing media and frozen at -80^{o}C until analysis (Thavasu, Longhurst, Joel, Slevin, & Balkwill, 1992).

2.4 Reverse Transcription Qualitative Polymerase Chain Reaction

White blood cell samples were thaw, and 1 mL of TRIzol (Thermo Fisher) was added to dissociate nucleic acids, and the TRIzol Plus RNA Purification System (Thermo Fisher) was used to purify total RNA. cDNA was synthesized from total RNA using the high capacity cDNA Reverse Transcription Kit (Applied Biosystems). TaqMan gene primers (Life Technologies) for CRP, IL-6, IL-1β, and PTGS2 genes were added to the master mix (Applied Biosystems) containing the cDNA. Expression of inflammatory genes was assessed using the Step One System (Applied Biosystems) and reported as changes compared to the housekeeping gene Beta Actin.

3. Results

After analysis, we found an association between the expression of inflammatory genes with the mechanical tendon. There was a higher expression of all the inflammatory genes compared to the housekeeping gene at baseline, CRP (0.75-fold), IL-6 (0.66-fold), IL-1β (0.51-fold) and PTGS2 (0.43-fold). Also, there was an increase in all the inflammatory genes after five days of completing a repetitive manual task, CRP (0.07-fold), IL-6 (0.04-fold), IL-1β (0.06) and PTGS2 (0.04-fold), followed by a decrease below the baseline levels after one week of rest CRP (-0.13-fold), IL-6 (-0.19-fold), IL-1β (-0.15-fold) and PTGS2 (-0.16-fold). The average values of all results were used to quantify the RNA. Since RNA degrades rapidly once outside the organism, several samples were under the detection threshold limit or missing a time point for some genes.

4. Concluding Remarks

In conclusion, we showed a feasible method for monitoring the changes in the expression of an inflammatory gene associated with mechanical tendon load. Currently, no other studies are investigating the effects of the repetitive motion of the upper arms on the expression of inflammatory genes associated with mechanical tendon load. These preliminary results of this study show the possibility for the analysis of inflammatory genes to assess the development or progression of WMSDs associated with mechanical tendon load. Even though these preliminary results show an increase in all the inflammatory genes after 5 days of activity, followed by a decrease after one week of rest, the gaps in the data should be considered. Future studies should consider the immediate extraction of RNA and synthesis into cDNA to avoid sample degradation.

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