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IMMUNOLOGIC CHARACTERIZTION OF THE LEVATOR ANI MUSCLE IN WOMEN WITH PELVIC ORGAN PROLAPSE

Hypothesis / aims of study
To determine whether the mean pro-inflammatory and anti-inflammatory cytokine expression indices obtained from levator ani muscle (LAM) biopsies correlates with severity of stage of prolapse.

Study design, materials and methods
This was a cross-sectional study sampling women presenting for pelvic organ prolapse surgery with varying stages of prolapse from July 2013 to December 2014. A 3DUS was performed and two ultrasound guided LAM biopsies were obtained in the operating room, prior to undergoing their planned surgical procedure. Total protein from both the right and left LAM samples were prepared by homogenizing muscle samples in a phosphate buffered saline containing protease inhibitor cocktail and phenylmethanesulfonyl fluoride. The samples were then studied to ascertain inflammatory marker levels using markers known to be involved in wound healing. The inflammatory markers examined included: IL-1α, IL-1β, IL-1RA, IL-6, IL-8, IL-10, IL-15, IL-17α, VEGF-α, TGF-α, IP-10 (CXCL10), MCP-1 (CCL2), MIP-1α (CCL3), and MIP-1β (CCL4). Muscle expression of the above mentioned pro-inflammatory cytokines were determined by multiplex immunoassays and presented relative to the total protein as determined by the bicinchonic acid assay. The protocol mean values of each cytokine level along with standard deviations were calculated for each severity level of prolapse. Categorical variables were compared using chi-square or Fisher's exact test as appropriate. An ANOVA or non-parametric Kruskal-Wallis test was used to make comparisons for the continuous variables if there were more than two groups.

Results
Of the 39 patients in this study, over 84% were Caucasian. The mean age of the patients was 58 years (SD ± 16.5) with mean BMI of 29.5 kg/m^2 (SD ± 5.8). Seventy-four percent of participants were menopausal, and 21% were receiving some form of hormone replacement therapy. Nine patients had stage 0/1 prolapse and there were ten patients in each category of stage 2, 3, and 4 prolapse. The age and weight of the patients differed significantly by the stage of prolapse (p= 0.0004 and 0.04, respectively). Additionally, there was a significant difference noted between stage of prolapse and menopausal status (p=0.002) as well as stage of prolapse and a history of anal sphincter injury (p=0.005).
A total of 78 muscle samples were analyzed using the multiplex cytokine expression assays, with a LAM biopsy from the right and left from each patient. The level of IL-1α was the only cytokine that was significantly different by stage of prolapse, and this was true only in the right LA but not in the left (p= 0.04 vs 0.62). There were no noted differences between stage of prolapse and IL-1β, IL-1RA, IL-6, IL-8, IL-10, IL-15, IL-17α, VEGF-α, TGF-α, IP-10 (CXCL10), MCP-1 (CCL2), MIP-1α (CCL3), and MIP-1β (CCL4).
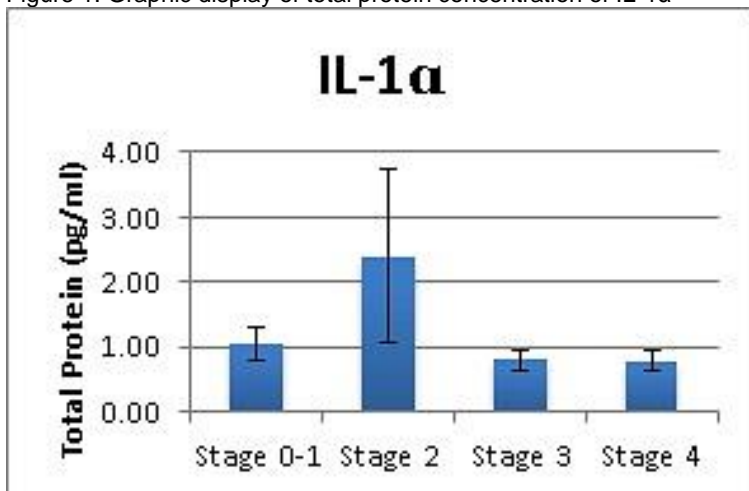
Interpretation of results
This preliminary data shows that there is a difference between stage of prolapse and IL-1α.

Concluding message
There is some evidence that certain cytokines may play a role in pelvic floor healing and repair. Larger, prospective studies are needed to further assess the role of different cytokines in pelvic organ prolapse at the time of the initial injury

Figure 1: Graphic display of total protein concentration of IL-1α