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CHANGES OF TOTAL COLLAGEN IN FEMALE PATIENTS WITH GENUINE STRESS INCONTINENCE.

Aims of Study

Collagen is a very important constituent of pubocervical fascia that supports the urethra and bladder neck (1). Changes in the quantity and quality of collagen could cause weakening of pubocervical fascia with subsequent defective support of bladder neck and inadequate transmission of intraabdominal pressure to urethra, which could lead to development of Genuine Stress Incontinence (GSI)(2).It has been reported that total collagen reduction of the pubocervical fascia is associated with the development of G.S.I (3,4), but the relevant literature is limited. Objective of this study was to determine possible changes in the quantity of total collagen in the pubocervical fascia of women with G.S.I.

Methods

Thirty-five women participated in the study and they were divided in to two groups as follow: Twenty-one patients had Genuine stress incontinence (GSI) and pelvic relaxation (group 1) and fourteen patients had pelvic relaxation without GSI (group 2). All patients had anterior vaginal wall prolapse stage I or less and the severity of prolapse was determined according to International Continence Society classification. All women underwent a complete preoperative urodynamic workup. Patients with previous operation in the pelvic floor, urge incontinence or mixed incontinence were excluded from the study. Biopsies were obtained during surgery for benign disease from pubocervical fascia and were maintained at -80^{o} C until final elaboration. The Student's t-test was used for the statistical analysis and a $p< 0.05$ was considered statistically significant. The amount of total collagen in tissue specimens was determined with the use of the following protocol :

Hydroxyproline determination in tissue samples (5)

The collagen content of the tissue samples is estimated by measuring hydroxyproline concentration, as the later is found almost exclusively in collagen. This has been done by using the method of Woessner (1961), as follows. Briefly, tissue samples are weighted and hydrolyzed with HCl 6N for 3h at 130^{o}C in sealed tubes. Consequently, the samples are neutralized with NaOH 2.5N in the presence of a few drops of bromothymol blue indicator. Colour change from red to light blue is observed at desired pH. Then, the volume of all samples is adjusted to 2ml. To all samples and to standard solutions of L-hydroxyproline (1-5 μg/2ml), 1ml of chloramine-T (0.05M in cellosolve-containing citrate buffer, pH 6.0) is added to initiate hydroxyproline oxidation. After 20min, 1ml of perchloric acid (3.15M) is added in order to destroy excess chloramine-T. After 5min, 1ml of p-dimethylaminobenzaldehyde (20% in methyl cellosolve) is added. Tubes are capped and placed in a 60^{o}C water bath for 20mins, and then cooled under running tap water for 5min. Finally, the absorbance of each sample at 557nm is measured. The absorbance of the standards vs. the hydroxyproline concentration (μg/ml) of each is plotted and the linear fit obtained is used in order to determine the hydroxyproline concentration of the tissue samples. From the volume obtained after neutralizing, and the original weight of the tissue sample, the % hydroxyproline content of the tissue is found.

Results

Both groups of patients were comparable in respect to age, parity, body mass index and stage of anterior vaginal wall prolapse. The amount of total collagen in specimens from patients of group I was **mean** 1.95 $\pm$ 0.365 μg HP/ 100 μg of tissue (range 1.3-2.59 μg HP/ 100 μg of tissue) and in specimens from patients of group II was **mean** 2.67 $\pm$ 0.80 μg HP/ 100 μg of tissue (range 1.07 – 3.74 μg HP/ 100 μg of tissue). The amount of total collagen was significantly reduced ($p<0.001$) in patients with G.S.I and pelvic relaxation (group 1), compared to patients of group 2. The power of the study for $p<0.05$ was 94%, for $p<0.01$ was 79.8% and for $p<0.001$ was 50.7%. Also, there is a 27% reduction in the total collagen content in the pubocervical fascia of patients of group 1 compared to patients of group 2.

Conclusions

In this study, we found that women with G.S.I had a reduced amount of total collagen in the pubocervical fascia, regardless of the presence of anterior vaginal wall prolapse. It appears that collagen content of the pubocervical fascia has a significant role in the maintenance of urinary continence but the mechanism by which collagen metabolism is altered remains unknown.

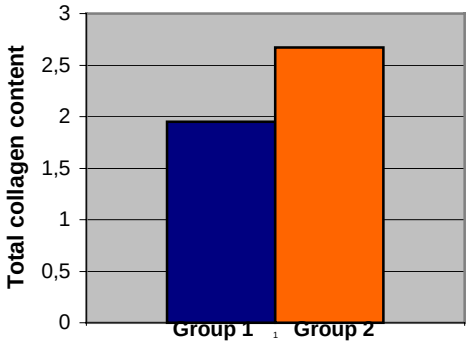

Table 1. Mean of total amount of collagen in patients of group1 and 2 (µg of Hydroxyproline / 100 µg of tissue).

References.

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