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THE ROLE OF FOXP3, IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPY IN THE INVESTIGATION OF MESH REJECTION AFTER POLYPROPYLENE MESH AUGMENTATION PROCEDURES- PRELIMINARY RESULTS OF A CASE CONTROLLED STUDY.

Hypothesis / aims of study

To explore the etiology and natural history of prosthesis rejection after polypropylene mesh augmentation procedures for pelvic organ prolapse (POP)

Study design, materials and methods

This is a prospective case controlled study. In a period of 3 years, 221 subjects with advanced or recurrent POP underwent either ant. or post. compartment TVM (tension free vaginal mesh) procedures. Power and sample size calculation based on previous reports that the incidence of vaginal mesh extrusion/erosion was 0.7-17% [1, 2]. To test with 80% power, a P value of 0.05, at least 41 subjects were required for each group (study and control). Exclusion criteria were a fasting blood sugar ≥180 mg/dl, a postprandial sugar≥230mg/dl and a vaginal infection.

For the study group, the exposed mesh and surrounding vaginal tissue were excised and vagina was closed when the vaginal lesion was not responded to local conservative treatments. Moreover, those patients without mesh extrusion but who had developed urine retention or voiding/defecation difficulty or recurrent vaginal prolapse after the TVM procedures needed to takedown the implanted mesh or revision for the recurrent prolapse. All specimens were sent for electron microscopy for bacteria and biofilm , immunohistochemistry (IHC) and Foxp3 via RT-PCR studies [3]. Informed consents were collected from all of the patients recruited.

Results

Table 1

	Study group(n=25)	Control group(n=9)	p-value
CD4	572.84 (17.01, 2496.02)	680.93 (30.73, 1443.62)	0.878
CD8	275.99 (0.55, 2578.33)	186.56 (22.50, 762.14)	0.355
CD20	234.84 (0, 1089.71)	137.72 (2.19, 954.73)	0.645
CD25	35.12 (0, 1445.82)	70.23 (0.55, 654.60)	0.878
CD40	43.90 (0.55, 914.13)	61.45 (0.55, 1156.65)	0.848
CD68	690.26 (0.55, 2372.02)	567.35 (66.39, 1670.78)	0.591
(CD4+CD8)/CD25	20.96 (2.18, 398.50)	16.25 (2.00, 140.00)	0.890
(CD20+CD68)/CD25	30.14 (1.52, 374.14)	12.79 (2.57, 265.00)	0.872
(CD4+CD8+CD20)/CD25	33.85 (2.87, 436.75)	16.72 (2.38, 171.85)	0.984
IFN/GAPDH	1.25 (0.90, 1.70)	1.19 (1.10, 1.30)	0.316
FOXP3/GAPDH	1.47 (1.20, 1.68)	1.43 (1.30, 1.49)	0.216

Using the Wilcoxon rank sum test
Data are present as median (min, max)
IFN/GAPDH: interferon/gamma glyceraldhyde3-phosphate dehydrogenase
Foxp3：a forkhead transcription factor

221 patients with a diagnosis of POP undergoing mesh augmentation surgery, 25 of whom underwent vaginal revision because of the cause mentioned earlier. Of the 221 patients, another 9 without mesh extrusion underwent vaginal repair/taking down of mesh for the reasons mentioned earlier. The followup period was 13 months (6-36). Mesh extrusion rate was 11%(25/221).

For all the parameters there are no statistically significant differences noted (Table 1). However, based on the existence or not of local vaginal symptoms/bacteria, the individual differences in $CD25^+$ and $CD40^+$of 34 subjects, have clinical significance.

Table 2

	Bacteria -	Bacteria +	P
Symptom free(n=11)	8	3	0.024
Symptom present(n=22)	6	16	

Local vaginal symptoms including any of the follows: increased vaginal discharge, pain, bleeding from vaginal wound, dyspareunia were present in 22 subjects, while another 11 subjects were symptom free.

In comparison to the 11 women without local vaginal symptoms, the 22 with symptoms had statistically significant higher proportion of positive bacteria exam by EM (16/22 vs. 3/11, P=0.024,Fisher's exact test).

Table 3

	$CD25^+$	$CD40^+$	P
Bacteria – (n=14)	10	4	0.035
Bacteria + (n=20)	6	14	

20 subjects with positive bacteria and biofilm formation and 14 without the above findings were noted.
In comparison to the positive $CD25^+$ cell density the proportion of patients with positive bacteria in that of $CD40^+$was significantly higher (14/18 vs. 6/16, P=0.035 , Fisher's exact test.)
Additionally, the tissue presented with $CD25^+$in 10 patients without bacteria outweighed the 4 presented with $CD40^+$. However the 14 subjects presented with $CD40^+$ and positive bacteria outweighed the 6 presented with $CD25^+$.

Interpretation of results

$CD25^+$ T cells presented to shift to TH2 (tolerance) pathway, therefore, $CD40^+$ T cells favored to shift to Th1 (activation) pathway and elicited rapid rejection of vaginal mesh. Interferon- producing cells triggered antigen- presenting cells such as B cells, macrophages, dendritic cells to skew tolerance immune cells (type II cells) or to activate immune cells or cytotoxic CD8 T cells (type I cells) and finally graft rejection response were generated.
Among the immunity-relative cells, CD40+APCs play an important role in initiating bacteria to naïve T cells, B cells, CD8 and CD68 and led those cells to their activation.
The bacteria inhabited in the implanted mesh, elicited individual immune dysfunction and up-regulation of cellular surface marker (CD20,CD40,CD68APCs) to trigger the adaptive cells(CD4,CD8) to be an immune network.

Concluding message

1) Implanted mesh without bacteria inhabitant appears to be more tolerable than its counterpart.
2) The process of mesh erosion/rejection in bacteria positive patients shifts to numerous CD40+APCs which link to the innate and/or adaptive immune surveillance system.
3) CD40+APCs(antigen presentation cells) which can play an active roll in phagocytosis of the infected tissue after mesh augmentation procedures seem to be immature dendritic cells.
4) Our current study provides local cellular immunity biomarker for predicting the outcome of vaginal mesh implantation.

References

1. Wang AC, Lee LY, Lin CT, Chen JR. A histologic and immunohistochemical analysis of defective vaginal healing after continence taping procedures: a prospective case-controlled pilot study. Am J Obstet Gynecol 2004;191(6):1868-74
2. Siegel AL, Kim M, Goldstein M, Levey S, Ilbeigi P. High incidence of vaginal mesh extrusion using the intravaginal slingplasty sling. J Urol 2005;174:1308-11
3. Zheng Ye, Rudensky AY. Foxp3 in control of the regulatory T cell lineage. Nature immunology 2007;8(5):457-462

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