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EFFECTOR MECHANISMS OF THE SITE SPECIFIC GRAFT REJECTION AFTER INTRAVAGINAL MESH IMPLANTATION－A LONG-TERM PROSPECTIVE CASE-CONTROLLED STUDY.

Hypothesis / aims of study
Quantification of interferon-producing cells (IPCs) enables the identification of acute rejection, which occurs within the first 12 weeks after polypropylene mesh implantation.
Study design, materials and methods
In a period of 5 years, women presented with vaginal graft-related healing abnormalities after they had undergone vaginal mesh augmentation and/or mid-urethral mesh sling (MUS) operations for both/either pelvic organ prolapse (POP) and/or urinary stress incontinence (USI) in a period of 12 weeks time were recruited. Power and sample size calculation based on previous reports that the incidence of vaginal mesh extrusion (erosion) was 0.7 – 17 % were done. To test with 80 % power, a p value of 0.05, at least 41 subjects were required for either the study or control group.
Exclusion criteria were a fasting blood sugar ≥ 180 mg/dl, a postprandil sugar ≥ 230 mg/dl and a vaginal infection.
For the study group, part or all of 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, mesh margin and vaganal tissue also took from those patients without mesh extrusion but they had developed urine retention or voiding/defecation difficulty or recurrent vaginal prolapse after the tension-free vaginal mesh (TVM) or MUS procedures. Those women needed to takedown the implanted mesh or repair the prolapsed vagina. All specimens were sent for electron microscopy for the bacteria and biofilm studies, immunohistochemistry (IHC) and INF-γ analyses. Post-operactive visits were arranged at 1-, 3-, 6- month and annually. The follow-up period was 29 months (22 - 43).
Results:
209 patients with a diagnosis of POP undergoing TVM, 25 of whom underwent vaginal revision because of mesh extrusion. Of the 209 patients, another 17 without mesh extrusion (control group) underwent vaginal repair/taking down of mesh for the reasons mentioned above. Meanwhile, 50 patients with a diagnosis of POP combined with USI undergoing mesh augmentation (TVM) and concurrent MUS operation. 16 of whom underwent vaginal revision because of mesh extrusion. Of the 50 patients, 13 without erosion (control group) underwent vaginal repair/ taking down of mesh for the reasons mentioned earlier. Moreover, 60 women with USI underwent MUS during the study period. Of the 60 women 5 underwent vaginal revision for healing abnormalities and 9 underwent taking down of the mesh sling due to voiding difficulty. Vaginal repair for recurrent prolapse or taking down of mesh sling for the control group were performed one to 30 months after the initial procedures. 10 patients with mesh extrusion were referred to us from other hospitals. Mesh extrusion rate was 10% (31/309).
A comparison of the IHC and INF- γ studies between the study and control groups revealed a significantly higher levels of $CD68^+$macrophages, CD20 B cells, $CD4^+$ T cells and $CD8^+$ T cells (all p<0.03, 2-smaple t tests) in the study group (Table 1)
Table 2 presents a significantly higher level of IPCs (≥median of IPCs) in patients who are at the increased risk of mesh rejection (p = 0.024, Chi-Square test). Table 3 presents the T/B ratios in the control group; the IPCs marker identified as being potent antigen presentation in this group revealed non-significant difference (p = 0.731, Chi-Square test), thus, these patients possibly have a decline risk of mesh rejection.
Of the 32 patients with T/B ratio< 10, 28 needed a second time of vaginal revision for the recurrent mesh extrusion, 14 needed total explantation of the mesh because of intractable healing abnormalities of the vaginal wound. Of the 9 patients with T/B ratio≥ 10, 3 needed vaginal revision on one occasion and 6 needed a second time of the procedure. In the study group, the positive rate of bacteria which were located by EM was 66 % (27/41) and that for the control group was 20% (8/39) (p< 0.0001,Chi-Square test).

Table 1 Comparison of immunohistochemistry and INF-γ studies in 2 groups.

	Study group(n=41)	**Control group(n=39)**	**P-value**
CD4	847.44 ± 776.86	463.50 ± 460.53	0.01
CD8	412.46 ± 589.86	190.72 ± 211.14	0.03
CD20	408.16 ± 408.95	233.66 ± 254.45	0.02
CD25	268.73 ± 469.49	219.57 ± 259.49	0.56
CD40	218.59 ± 254.29	286.46 ± 388.22	0.36
CD68	1502.98 ± 727.36	391.77 ± 452.09	<0.001
(CD4+CD8)/CD20	486.08 ± 2986.60	11.97 ± 33.47	0.32
IFN/GAPDH	1.33 (0.93, 10.11)	1.40 (1.10, 9.84)	0.85[a]

Data presented as mean± SD or median(min., max.). Two-sample t and Wilcoxon rank sum[a] tests.
INF/GAPDH : interferon-γ glyceraldehydes 3- phosphate dehydrogenase

Table 2 Correlation between T/B ratio and IPC level in individual subjects of the study group

	≥ median of IPCs (1.33)	**< median of IPCs (1.33)**	**P = 0.024**
< 10 (N=32)	14	18	
≥ 10 (N=9)	8	1	

T/B ratio= (CD4+CD8)/CD20, Chi-Square test

Table 3 Correlation between T/B ratio and IPC level in individual subjects of the control group

	≥ median of IPCs (1.40)	**＜median of IPCs (1.40)**	**P ＝ 0.731**
< 10 (N=11)	5	6	
≥ 10 (N=28)	15	13	Chi-Square test

Interpretation of results

In this study, we used IHC stain for CD68, as an antigen-presenting cells and electron microscopy for locating the bacteria and biofilm in the explanted mesh and vaginal tissue. The IHC analysis revealed significantly higher level of CD68 in the study group. A primary impact of surgical trauma is the establishment of inflammatory environment including production of the proinflammatory cytokines TNF alpha and IL-1 and up-regulation of adhesion molecule expression on the vascular endothelium. The production of chemokines, cytokines has chemoattractant properties for leukocytes, especially macrophages which play the dominant role. This evidence indicates that vaginal mesh implant and bacteria elicit inflammatory and/or infective events during and after the procedures.

Zheng et al [1] reported that the T/B ratio, ie; the ratio between CD4 + CD8 and CD20 seems critical for the induction of the activation of immunity (rejection or extrusion). The ratio is less than 10 when the mesh is extruded /rejected while the mesh is tolerated when the ratio is more than 10.

In our study, Table 1 shows the T/B ratio is higher in study group, but there is no statistical significant difference between the 2 groups. This is possibly due to the effect of pooling of the matched and mismatched data. However, the difference in IPC level was significant when we analysed the matched/mismatched T/B ratio and IPC level in individual patients of the study group (p= 0.024, Table 2).

In a graft versus host disease model, polarization toward a Th1 or Th2 phenotype following T cell receptor /co-stimulatory factor (antigen such as foreign body; bacteria foci in mesh) stimulation is determined by cytokine expression [2]. INF-γ producing cells recruit Th1/Th2 Cd4/CD8 lymphocytes to mediate cell-cell interaction between CD40 and CD40 ligand (CD154) and generate vaginal mesh rejection through FAS (CD95) pathway [3]. Thus, of the 41 women with mesh extrusion, 32 with T/B ratios less than 10 can be ascribed to acute mesh rejection (78%,32/41) The other 9 with T/B ratios more than 10 have potential to develop rejection at a later date.

Concluding message

These findings indicate that quantification of IPCs for patient with vaginal mesh implantation is valuable for the observation of progression and prediction of abnormal graft-tissue interaction. IPCs represent a novel marker for identifying the patients with acute mesh rejection. The acute rejection rate of patients presented with mesh extrusion within 12 weeks of implantation is 78%.

References

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Specify source of funding or grant	**Ethics approval from the appropriate institutional review board was obtained before initiating this study. Informed consents were collected from all of the patients recruited.** **Funding:96-2314-B-182-015-MY2**
Is this a clinical trial?	**Yes**
Is this study registered in a public clinical trials registry?	**Yes**
Specify Name of Public Registry, Registration Number	**Clinical Trails. gov Identifier:** **NCT 00564044**
Is this a Randomised Controlled Trial (RCT)?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**CGMH IRB No. 95-1055B** **Institutional Review Board** **Chang Gung Memorial Hospital**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**