

Elements survey in endometriosis applying the electron probe X-ray microanalyser to scanning electron microscopy

Takashi HAYATA, MD PhD

Kagoshima Women's Junior College
1-59-1 Murasakibaru, Kagoshima, 890-8565, Japan

Abstract.

Object; To investigate the endometriotic lesion through applying the electron probe X-ray microanalyser to scanning electron microscopy (SEM).

Plan; Endometriotic lesions were extirpated after operations from 5 endometriosis cases and examined on SEM by electron probe x-ray microanalysis.

Results; By this method endometriosis showed some data on the nuclear cytoplasmic ratios.

Conclusion; This would be the first that endometriosis was surveyed for elements through the electron probe microanalysis on SEM.

Key words: endometriosis, SEM, electron probe X-ray microanalysis

Histogenesis of endometriosis has been obscure even in today's world wide literatures¹⁾. So the author tried the element analysis in endometriosis with some hope that would be hidden in elemental analyses. I have studied or attacked to this magnificently enigmatic lesion in this reason that endometriosis is not cancer but invade to the normal tissue, such as the musculature of the whole body. An elemental analysis in human endometrium for the first step of further endometriosis investigation has been done by author for this decade²⁾³⁾. And now you can invade or penetrate to the endometriosis by this weapon or X-ray microanalysis.

Material and method

1) Patients

Five cases of endometriotic tissues were treated on SEM and processed for the dual photon electron microanalysis.

2) Tissue fixation and embedding

They were fixed with glutaraldehyde and osmium tetroxide, hydrated by gaded alcohol and lastly embedded in epon 812 resin (TAAB, USA).

3) Cutting and stain

Epon blocks were sectioned into 5 μ thickness, toluidine-blue stained for the orientation of the endometriotic gland. For SEM those were stained again by uranyl acetate and lead hydroxide.

4) Electron probe X-ray microanalysis on SEM

Tissues were put on the grid made of copper that was formerly covered with collodion film and coated by carbon. Hitachi electron microscope (JEM 200EX, JEOL, JAPAN) connected to energy dispersive X-ray microanalyser detected elements. Counting time was 300 seconds and evoking voltage was 80 kV. Tracor Northern NS 880 computer was used to find out the peak value of each element.

Results

Computer tied up with SEM revealed 3-4 digits on amount of area which was shown in Table 1. Peaks of values were shown on lead (Pb), chloride (Cl), uranium (U) and Copper (Cu). Nuclear cytoplasmic (N/C) ratios of those elements were calculated. The reason why I picked up the ratios was proposed in previous bulletin²⁾.

Table 1 shows you the electron probe microanalytical data on SEM in these five cases of endometriosis. You can see the ratio of each element as 0.89 ± 0.21 on Pb, 1.01 ± 0.22 on Cl, 1.02 ± 0.20 on U and 0.95 ± 0.11 on Cu.

Table 1 Qualitative element assessment in lesion by X-ray microanalysis

Element Case #	Pb	Cl	U	Cu
1	237	565	598	2997
	369	641	797	3417
N/C ratio	0.64	0.88	0.75	0.87
2	823	398	883	3130
	1060	403	887	3606
N/C ratio	0.77	0.98	1.00	0.86
3	770	620	1066	3804
	752	445	798	3706
N/C ratio	1.02	1.39	1.33	1.02
4	412	747	951	2963
	474	736	971	3308
N/C ratio	0.86	1.01	0.97	0.89
5	1407	346	1085	6253
	1173	414	1007	5576
N/C ratio	1.19	0.83	1.07	1.12
Mean \pm SD of N/C ratio	0.89 ± 0.21	1.01 ± 0.22	1.02 ± 0.20	0.95 ± 0.11

N/C, nuclear cytoplasmic

Upper, middle, and lower lines of each case show the data in the nucleus, the cytoplasm and nuclear cytoplasmic ratio, respectively.

Comment

Why am I eager to know the histogenesis of endometriosis? The answer is that it is not only for endometriosis patients but in one sense furthermore important cancer ones because endometriosis has an ability to invade to the normal tissues mimicking cancer. So endometriosis investigation should or supposed to give us not only some hints for diagnosis but for treatment in endometriosis and further to cancer I hope.

Secondly what makes me so stick to SEM? Because SEM is the extreme of morphology that sees the object on its surface appearances or structures. Yes morphology is most important of all scientific activities in this type of research, because endometriosis search needs to be done by normal appearing or endometrium like glandular structures in other places not supposed to exist in such as musculature, that is, invasiveness that should be revealed by light microscopy in toluidine blue stained epon block sections for SEM. After this process is over you can observe this ectopic gland on its surface precisely. I am convinced that this method will be helpful also in cancer investigation and make it much easier.

Anyhow data of element on endometriosis was shown in 5 cases. In future referring to the normal endometrium as described before²⁾³⁾ comparison of data in these 2 categories, that is to say, eutopic endometrium and ectopic one would be important, starting to say only about data of 5 cases of endometriosis in this report.

By the way here is an previous article⁴⁾ that I passed through because it is not an endometriosis although he used elemental X ray analysis on SEM. For all I know this is a case of peritoneal melanosis due to ruptured ovarian dermoid cyst which has been usual happenings in routine Ob/Gyn clinic resembling endometriosis in a way that I had said for these decades that endometriosis would be endometrial shedding into pelvic cavity forming peritoneal endometriosis. In other words this is a melanoma case who had an peritoneal shedding on its surface that I recognized this is supposed to be a model of endometriosis, such as peritoneal endometriosis. So I am going to compare these data on 5 cases to not only normal endometrium but peritoneum in the near future.

Finally here again is one of my favorite reports⁵⁾ that would support implantation theory of myself in endometriosis⁶⁾. Former says endometrial epithelium plays a key role via inflammatory factors between endometrium and embryo by the technique of SEM. This is supposed to be strongly supportive to the implantation theory of endometriosis from the standpoint of so to speak factors such as cytokines.

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