Supplementary information

https://doi.org/10.1631/jzus.B2100502

Materials and Methods

Study Population

This retrospective study was conducted at Women's Hospital, Zhejiang University School of Medicine. Risk factors of endometriosis recurrence post-surgery were determined by analysis of the 90 patients in the study group without receiving postoperative hormonal therapy.

Detailed basal data were obtained from medical records, such as menstrual and obstetric history, cancer antigen (CA)-125 value, and revised American Society for Reproductive Medicine (rASRM) score.

Pelvic ultrasound was conducted on the subjects 1 month after surgery to check whether there is residual endometrioma. Afterwards, routine pelvic ultrasound was conducted every half to one year. The following up of all subjects lasted for more than 2 years, and recurrence was defined as a reoccurrence of endometrioma with a diameter >2.5 cm on ultrasound.

Ethics Approval

The study protocol was approved by the ethics committees of Women's Hospital, Zhejiang University School of Medicine. All procedures were performed in accordance with the relevant guidelines and regulations.

Immunohistochemistry

Archived, formalin-fixed, paraffin-embedded tissue blocks were retrieved from these subjects. Serial 4-mm sections were obtained from the selected paraffin blocks and placed on polylysine-coated slides. To further confirm pathologic diagnosis, the first resultant slide was stained for hematoxylin and eosin and the subsequent slides were stained for TERT and KI-67. Routine deparaffinization, rehydration, and immunohistochemistry procedures were performed(Taylor and Levenson, 2006). The tissue sections were immersed in 10 mM citrate buffer solution for the anti-hTERT antibody and 1 mM ethylenediaminetetraacetic acid (EDTA) solution for anti-Ki-67 antibody respectively, and heated in a water bath. Then, the tissue sections were incubated with 10% normal goat serum for blocking non-specific binding. Next, the tissue sections were incubated overnight at 4 °C with mouse anti-hTERT monoclonal antibody (clone 2C4, dilution 1:40; GeneTex, Inc., Irvine, CA, USA) or mouse anti-Ki-67 monoclonal antibody (clone MIB-1, dilution 1:100; Dako Denmark A/S, Glostrup, Denmark), respectively. A peroxidase-labeled anti-mouse antibody (Histofine Simple stain MAX-PO (MULTI); Nichirei Bioscience, Tokyo, Japan) were used to detect the immunoreactivity with 3,3'-diaminobenzidine as the chromogenic substrate. Finally, the nuclei were counterstained with hematoxylin.

The immunohistological hTERT or Ki-67 positive cells were evaluated by brown nucleus staining. The nuclear positivity index was determined as the ratio of immunostaining-positive cells to the total number of cells counted in each section analyzed by a light microscope.

Statistical analysis

All data were analyzed by SPSS17 (IBM, Armonk, NY, USA). Quantitative variables were analyzed using Student's t-test or Mann-Whitney U test for comparison between two groups. Results were presented as mean ± standard deviation. Qualitative data were compared with a chi-square test or Fisher's exact test. Correlation analysis was performed to evaluate the relationships between

serum levels of CA125 and endometrial hTERT expression index. The general linear regression model was performed with correlation coefficient (r) and R2 to analyze the correlations of serum levels of CA125 and hTERT protein expression index in ectopic endometrium from patients with endometriosis. $P<05$ was considered statistically significant.

Reference

Taylor, C.R. and Levenson, R.M., 2006. Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment II. *Histopathology*, 49(4):411-24. https://doi.org/10.1111/j.1365-2559.2006.02513.x