CHAPTER III

RESEARCH METHODOLOGY

3.1 Research questions

3.1.1 Primary research question. Whether estrogen cream (E) can reduce early vaginal shortening (difference in mean change of vaginal length), in cervical cancer patients after complete radiotherapy?

3.1.2 Secondary research questions.

3.1.2.1. What is the incidence of early vaginal shortening after radiotherapy?

3.1.2.2. Can vaginal cream improve vaginal epithelium in terms of maturation index (MI)?

3.1.2.3. Is there any systemic estrogenic absorption?

3.1.2.4. Do the patients satisfy with using vaginal cream?

3.1.2.5. What is the patient's compliance in using vaginal cream?

3.1.2.6. Is there any effect of tumor aggravation after using vaginal cream?

3.2 Objectives

3.2.1 To compare the mean change of vaginal length, between using vaginal estrogen cream and placebo, in cervical cancer patients after radiotherapy.

3.2.2 To compare the improvement of vagina epithelium after radiotherapy, between using vaginal estrogen cream and placebo.

3.2.3 To evaluate systemic absorption of estrogen cream from radiated vagina.

3.2.4 To evaluate patient's satisfaction in using vaginal cream after radiotherapy.

3.2.5 To evaluate the tumor response after using vaginal cream.

3.3 Hypothesis

To test the hypothesis that vaginal estrogen cream directly prevents vaginal shortening and improves general health of vagina after radiotherapy.

3.3.1 Null hypothesis. There is no difference in mean change of vaginal length in radiated cervical cancer patients, using estrogen cream and placebo.

3.3.2 Alternative hypothesis. There is a difference in mean change of vaginal length in radiated cervical cancer patients, using estrogen cream and placebo.

3.4 Conceptual framework

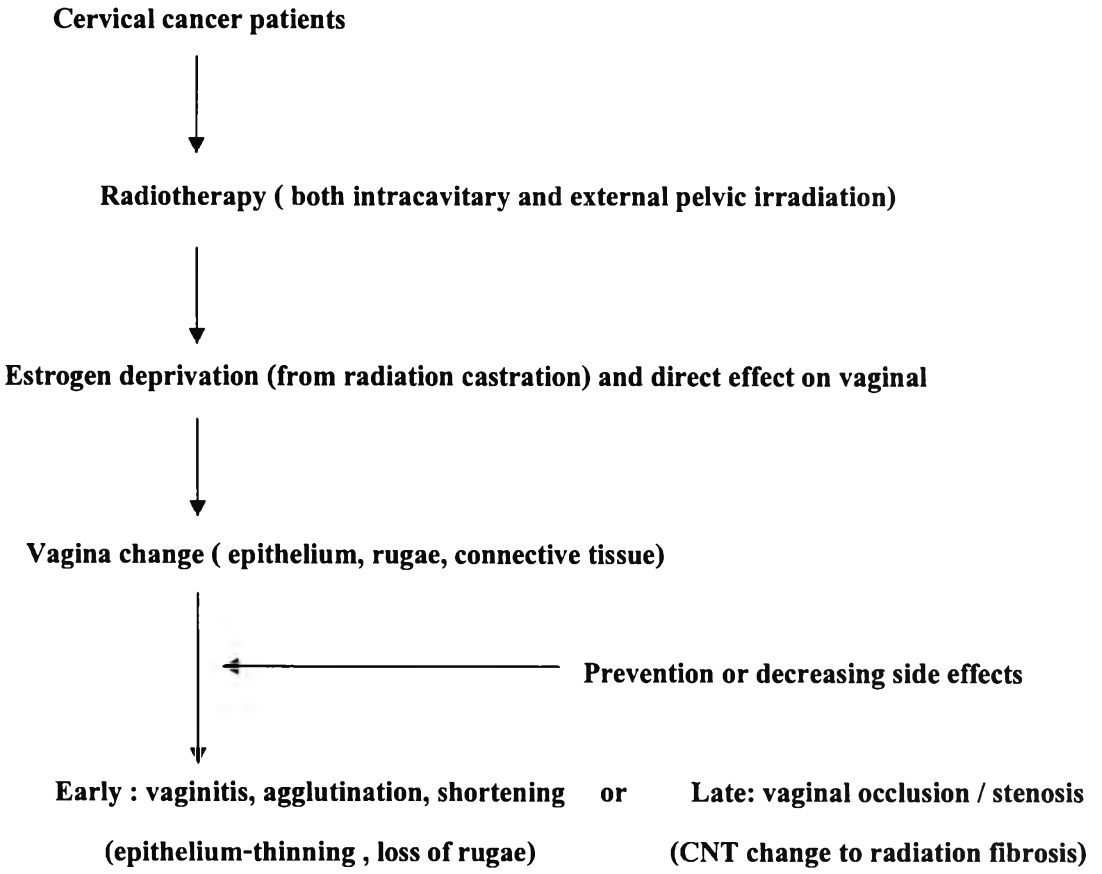

Figure 1 Conceptual framework

3.5 Keyword

Cervical cancer, Radiation vaginitis, Vaginal occlusion , Estrogen cream

3.6 Operational definitions

3.6.1 Cervical cancer. Squamous cell type, clinical stage IB2- IIIB.

3.6.2 Radiotherapy. Both intracavitary and pelvic irradiation, with or without concurrent chemotherapy.

3.6.3 Radiation vaginitis. Vaginal epithelial change after radiation: epithelial thinning, loss of rugae.

3.6.4 Vaginal occlusion. Degree of vaginal change, varying from shortening, agglutination, occlusion or stenosis.

3.6.5 Vaginal shortening. The irradiated patients who had decreased their vaginal lengths which were compared between before and after using cream.

3.6.6 Estrogen cream. Conjugated estrogen , each gram contains 0.625 mg. of conjugated equine estrogen(CEE).

3.6.7 Placebo cream. Cream- base without CEE prepared in the identical container by the pharmacist at Siriraj hospital. (Composition as shown in Appendix A)

3.7 Research design

A randomized (1:1) double-blinded, placebo controlled trial.

3.8 Research methodology

3.8.1 Target population. Primary cervical cancer patients.

3.8.2 Sample. The patients who were diagnosed with primary cervical cancer ,squamous cell type, with clinical stage IB2 -IIIB , at Siriraj Hospital and fulfilled the following eligibility criteria.

3.8.2.1 Inclusion criteria

3.8.2.1.1 Patients with primary squamous cell carcinoma of cervix, clinical stage IB2 to IIIB, who had clinical response, one month after complete radiotherapy or concurrent chemo-radiotherapy.

3.8.2.1.2 Age less than 55 years old.

3.8.2.1.3 Agree to participate in the study with informed consent.

3.8.2.2 Exclusion criteria

3.8.2.2.1 History of menopause before radiotherapy

3.8.2.2.2 History of vaginal operation

3.8.2.2.3 History of prior vaginal or pelvic irradiation

3.8.2.2.4 Previous estrogen use within 3 months before study

3.8.2.2.5 Prolapsed uterus

3.8.2.2.6 Vaginal fistula

3.8.2.2.7 Allergy to vaginal cream

3.8.2.2.8 Having severe infection in vagina or perineum

3.9 Sample size calculation

Sample size estimation was based on a comparison of two independent means using a 2-sided type I error of 5% , 90% power, and anticipated 10% drop-out. According to the following calculation, 32 patients in each group were recruited.

$$n = 2\left[\frac{(Z_\alpha + Z_\beta)\delta}{\triangle}\right]^2 ; \delta = 1.14, \triangle = 1$$

Where $\triangle$: Difference in mean change of vaginal length between 2 groups = 1

δ : Standard deviation of change in vaginal length in each group = 1.14

α : Probability of type I error = 0.05 (2-sided) , $Z_\alpha = 1.96$

β : Probability of type II error = 0.1 , $Z_\beta = 1.28$

Thus, $n = 2\,[(1.96+1.28)\,1.14]^2 = 27.38$

To compensate for 10% drop-out, n = 32 / group

3.10 Randomization

The patients who met the eligibility criteria were randomized into 2 groups; estrogen (E) and placebo (P) using a block randomization (block of size 4). Randomization codes were put in concealed envelops.

3.11 Intervention

After randomization , the patients were allocated and planned for :

3.11.1 History taking for general evaluation

3.11.2 History taking for vaginal symptoms for scoring : itching, irritation, and wetness.

3.11.3 Baseline serum estradiol (E_2) in picogram./milliliter

3.11.4 Physical and pelvic examination for baseline evaluation

3.11.5 Measurement of vaginal length , using a modified small vaginal dilator calibrated in millimeter (from posterior fornix to vaginal outlet at posterior commissure). The average values from two measurements in each patient was used.

3.11.6 Vaginal cytologic study for maturation index (percentage of parabasal / intermediate / superficial cells)

3.11.7 All patients were instructed to use digital application of 0.5 gm cream, nightly before sleep for 4 weeks as follow: pressed the cream on palmar surface of distal phalange of index, inserted the finger into the vulvar vestibule and vagina until she touched her cervix, then rotated the finger two times before pulling the finger out.

3.11.8 All patients were asked to record date of cream use and bring back all cream containers for evaluation of patient's compliance.

3.12 Outcome assessment

3.12.1Primary outcome. Absolute difference in vaginal length (mm) at baseline and 4 weeks after using cream.

3.12.2 Secondary outcomes

3.12.2.1 Maturation index (MI) as percentage of parabasal cell , before and after 4 weeks of cream use.

3.12.2.2 Proportion of the patients with parabasal cell > 25 %

3.12.2.3 Serum estradiol (picogram /ml) before and after 4 weeks of cream use.

3.12.2.4 Proportion of patients with serum estradiol > 50 pg/ml.

3.12.2.5Tumor response after 4 weeks: proportion of patients having clinical response, progression, and persistence.

3.12.2.6 Patient's symptoms scoring

3.12.2.7 Patient's compliance

3.13 Data collection

The following data were recorded.

3.13.1 Demographic data, baseline characteristics

Age in full year , Parity

History of Cesarean section (yes or no)

Tumor staging and tumor size (< 4 , ≥ 4 cm. in diameter)

Total radiation dose

Baseline vaginal length (millimeter)

Baseline maturation index (% of parabasal cells)

Baseline serum estradiol (pg/ml.)

Sexual practice (insertion sexual intercourse)

3.13.2 Outcomes

3.13.2.1 Vaginal length at baseline and 4 weeks after cream use: Measured by using a modified small vaginal spatula, calibrated in millimeter from posterior vaginal fornix to posterior commissure at level of vulvar vestibule. Vaginal calibration was perfored by only one researcher. The average value of two calibrations in each patient was computed.

3.13.2.2 Vaginal cytological evaluation for maturation index(MI): Percentage of parabasal / intermediate / superficial cells at baseline and 4 weeks after treatment. Cytological evaluation for maturation index (MI) was interpreted by only one cyto-technician.

3.13.2.3 Serum estradiol (pg / ml) at baseline and 4 weeks after treatment.

3.13.2.4 Summary patient's symptoms after 4 weeks of using cream : using scoring index as shown in the Table 1.

3.13.2.5 Patient's compliance: poor (cream use < 4 weeks) ; good (use cream completely 4 weeks).

3.13.2.6 Tumor response : clinical response or persistence and progression.

Table 1 Vaginal symptom scoring

Symptoms / severity	No (1)	Mild (2)	Moderate (3)	Severe (4)
Itching				
Irritation				
Wetness				
Total score(< 6 or ≥ 6)				

Note: Total score $\geq$ 6 defined as not satisfied ; total score < 6 as satisfied

3.14 Data analysis

Statistical data analysis was based on intention-to-treat principle. For patients who used the cream less than 4 weeks or dropped out , the reasons were recorded.

Demographics, baseline laboratory values and outcome values were reported using proportion, mean, and standard deviation as appropriate.

To compare categorical outcomes (e.g. incidence of vaginal shortening, parabasal cell > 25%, patient's satisfaction and patient's compliance) between two treatment groups, a Pearson's chi-square test or Fisher's exact test were employed.

To test the difference in change of vaginal length from baseline and week 4, between estrogen and placebo group , unpaired t-test was applied.

Unpaired t-test was also used to test the difference between two treatment groups in vaginal length at baseline and week 4, serum estradiol at baseline and week 4, age and body mass index (BMI).

Mann-Whitney U test was performed to test the difference in parity between estrogen and placebo group.

Pearson's correlation was used to assess the linear relationship between change from baseline at week 4 in vaginal length and change from baseline in serum estradiol.

All statistical data analyses were performed using SPSS version 11.0 . A 2-sided p-value of less than 0.05 was considered a statistical significance.

3.15 Ethical consideration

These was no strong evidence that estrogen aggravates cervical cancer and estrogen replacement therapy was not and absolute contraindication for cervical cancer patients , especially squamous carcinoma cell type. This study tried to clarify whether estrogen cream had benefit in vaginal restoration after radiation , which distinguished from effect of placebo. The placebo which was not composed of estrogen, could give the benefit to the patients as a vaginal lubricant. This study was done after an approval of ethics committee from the Faculty of Medicine, Chulalongkorn University and Faculty of Medicine, Siriraj Hospital, Mahidol University. And for patent safety , the rescue protocol was planned for patients with persistence or progression of disease , additional surgery or chemotherapy should be done as appropriate. In cases that the events occurred before 4 weeks , the patients would be withdrawn from the study and underwent for other treatments. If the result of this study showed that estrogen cream was promising , the patients in placebo cream should be informed to use as intervention group. And

for placebo group, the level of serum estradiol would help the physician to guide for prescription the estrogen cream in proper period .

3.16. Limitation

This study should be designed to look for long term outcomes in late effects such as ; at 6 month period for evaluation of sexual function , but because of time limitation.

And during digital application of vaginal cream , digital pressure from each patient might be uncontrolled , but we tried to instruct the patient for insertion of the digit deeply enough until she touched the cervix , with some pressure that she would not suffered from discomfort.