CHAPTER III

RESEARCH METHODOLOGY

3.1 Research questions

3.1.1 Primary research question

Can the pilocarpine in carboxymethylcellulose saliva substitute reduce the xerostomia sign and symptom scores in post irradiation patients with head and neck cancer compared with carboxymethylcellulose solution alone?

3.1.2 Secondary research questions

3.1.2.1 Does this solution have adverse events compared with carboxymethylcellulose solution alone?

3.1.2.2 Is the xerostomia symptoms scores correlated with physical examination in oral dryness?

3.2 Objectives

3.2.1 Primary Objectives

To compare the effectiveness of topical pilocarpine in carboxymethylcellulose saliva substitute with carboxymethylcellulose solution alone in post irradiation xerostomia patient

3.2.2 Secondary Objectives

3.2.2.1.1 To evaluate the adverse effect of both regimens

3.2.2.1.2 To assess the correlation between symptoms of xerostomia and the oral physical examination

3.3 Hypotheses

3.3.1 Research hypotheses

The mean change from baseline in xerostomia symptom visual analogue scores at 3 weeks in patient receiving the topical pilocarpine is different from those who receiving control regimen.

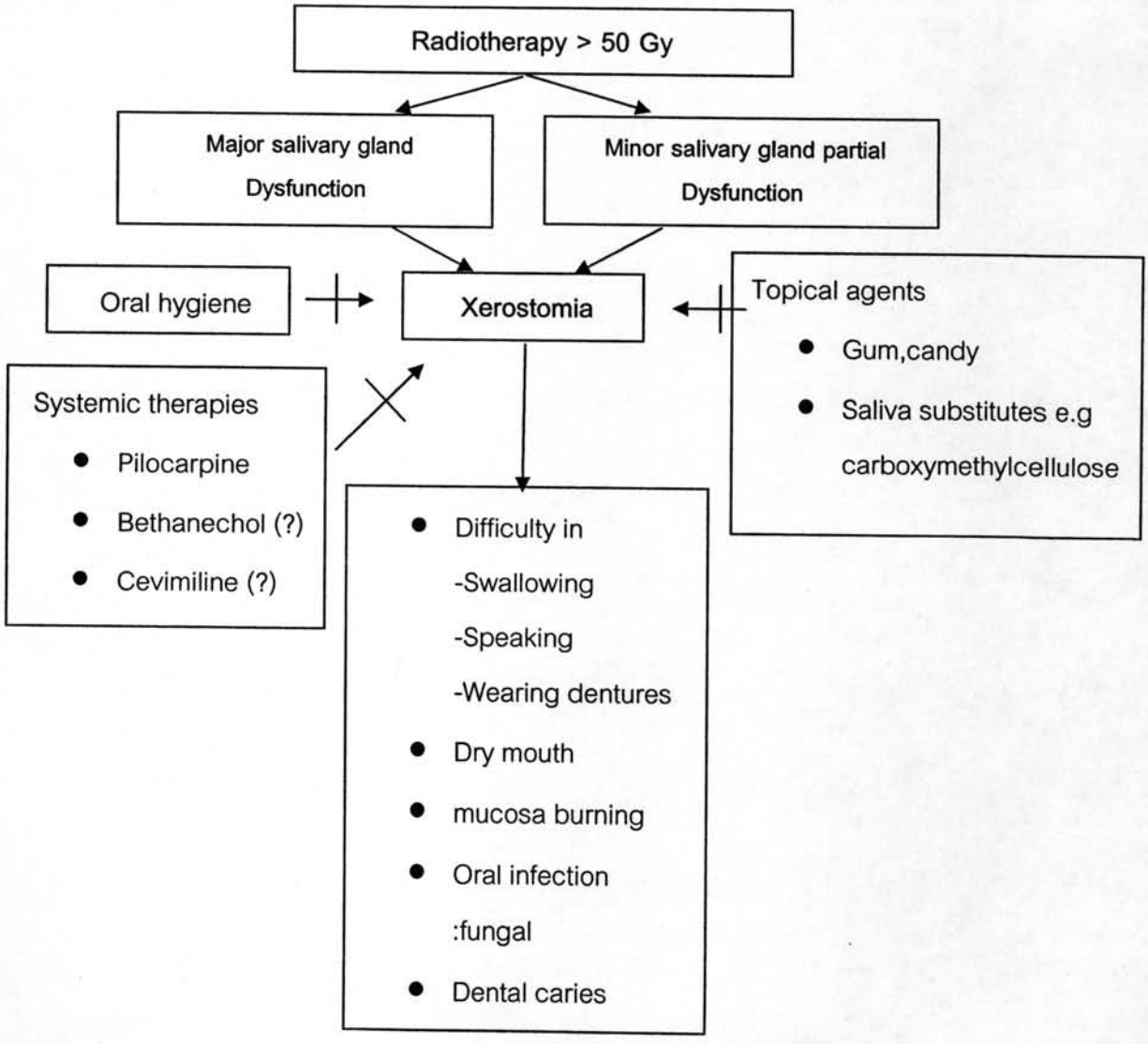
3.3.2 Statistical hypotheses

Null hypothesis: $\mu_1 - \mu_2 = 0$

Alternative hypothesis: $\mu_1 - \mu_2 \neq 0$

where μ_1, μ_2 = Mean change from baseline at 3 weeks of xerostomia VAS in patients receiving topical pilocarpine suspension and carboxymethylcellulose solution (control regimen) respectively

3.4 Conceptual framework



3.5 Key words:

xerostomia, irradiation, pilocarpine, saliva substitute

3.6 Operation definitions

3.6.1 Xerostomia

- Xerostomia is an abnormal reduction of saliva, and it can be a symptom of certain diseases or an adverse effect of certain medications or therapies. [33]
- The diagnosis of xerotomia is based on subjective impressions by the patients and the clinicians.

3.6.2 Topical Pilocarpine

- Topical Pilocarpine is prepared by adding pilocarpine hydrocholide powder (Boehringer Ingelheim KG, D-55216 Ingelheim) into carboxymethylcellulose artificial saliva until the concentration of 1 mg/ml is obtained.
- The solution is filled in the special designed- pumping 15 ml bottle spray and each pump will deliver 0.2 ml of the solution. The total dose which the patients received per day is 15 mg. according the optimum dose of oral piloarpine. [34]
- Essential minerals e.g. calcium phosphate and fluoride will be added, including mint flavor

3.6.3 Carboxymethylcellulose solution (control regimen)

- Carboxymethylcellulose is the cellulose derivative (Beta- D- glucopyranose polymer) which is the water binding molecules used in many cosmetic compounds and in saliva substitution.
- Carboxymethylcellulose artificial saliva in this study has the same characteristic and taste of the pilocarpine group except for not adding of active ingredients (pilocarpine).
- All the medications are manufactured by Pharmacy department, Phramongkutklao hospital .According to the procedures, they are approved by the Drug Formulary Committee of the Medical College Center

3.6.4 LENT SOMA scales (Appendix C)

- LENT = Late Effect of Normal Tissues

  SOMA = Subjective, Objective, Management and Analysis [41]

- Initiate by EORTC from Europe and RTOG Radiotherapy Oncology group from North America to standardize the recording system in reporting toxicity of radiotherapy or chemotherapy to normal tissue.

- In objective part, the statuses of each organ are described by the ordinal scales ranging from 1-4 range for normal to the most toxicity.

- For objective xerostomia scales, the correlation with subjective sensation is in clinical studies.

3.7 Research design

Randomized (1:1) double-blinded controlled trial

3.8 Research Methodology

3.8.1 Population and sample

Target population

Head and neck cancer patients who had post-irradiation xerostomia symptoms

Sample population

Head and neck cancer patients who have post-irradiation xerostomia and meet eligible criteria at National Cancer Institute of Thailand and Department of Otolaryngology, Phramongkutklao Hospital

3.8.2 Inclusion criteria

- Patients with symptoms of xerostomia treated for head and neck cancer of nasopharynx, oral cavity, and oropharynx or lymphoma
- Age more than 18 years old
- Minimum radiotherapy dose more than 50 Gy including part of major salivary glands
- Radiotherapy completed at least 1 month before the inclusion
- Xerostomia symptoms reported for at least 1 month

3.8.3 Exclusion criteria

- Life-time expectancy of less than 6 months.
- Previous history of multiple sclerosis, asthma, diabetes mellitus, ophthalmic pathologies (acute iritis, glaucoma and ophthalmic surgery, ophthalmic prescription of pilocarpine)
- Pregnancy
- Hypersensitivity to pilocarpine

3.8.4 Sample size calculation

Sample size calculation was based on the ability to detect a clinically important difference of mean of xerostomia scores between two groups. The sample size formulae for testing the difference in two independent means was as follows:

$$\text{n/group} = 2\left(\frac{\acute{o}^2 [Z_{\alpha/2} + Z_{\beta}]^2}{(\mu_1 - \mu_2)^2}\right)$$

where α = Probability of type 1 error = 0.05 (2-tailed)

β = Probability of type II error = 0.10

$Z_{\alpha/2}$ = 1.96

Z_{β} = 1.28

From the literature review, many articles studied the effect of the pilocapine in post-irradiated xerostomia patients, but only one study showed the descriptive statistics. [30] It was a study of the effect of systemic concomitant pilocarpine with head and neck irradiation with the following results.

Pilocarpine (n=18): Mean ± SD of VAS xerostomia score post Rx= 40.3 ± 22.0

Control group (n=21): Mean ± SD of VAS xerostomia score post Rx= 57 ± 21.5

Thus,

$$\acute{o}^2 = \frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{(n_1-1)+(n_2-1)} = 472.24$$

$$\text{n/ Group} = \frac{2(472.24)[1.96+1.28]^2}{(40.3-57)^2} = 36.73$$

With an expected 10% drop out ,

n/Group = 40

3.8.5 Randomization and allocation concealment

Post irradiaton xerostomia patients was randomized in a 1: 1 ratio of two treatment groups (control and topical pilocarpine group) using block randomization of size 4. Each treatment code was concealed in opaque envelope.

The allocation was concealed and blinded to physicians, patients and personnel involved in the study.

3.8.6 Intervention

- Study group: topical pilocarpine spray in carboxymethylcellulose saliva substitute

- Control group: carboxymethylcellulose saliva substitute alone

1. Visual analogue scales (VAS) used in the study were the questionnaire developed from standardizes visual analog scales for xerostomia symptoms [42] which included 6 aspects as follows.

a. general xerostomia symptoms
b. oral discomfort
c. sleeping problems
d. speaking difficulty
e. eating difficulty
f. oral mucosa pain

The original version of the questionnaire was first translated into Thai, then back into English. It was finally tested for validity and reliability.

2. Content validity of the questionnaire was evaluated by 5 content experts (M.K, M.P, J.S, P.A and N.S). The structures of questions were re-assessed to ensure the completeness of the content, and the final consensus was made by the conference. The Item Objective Congruence (IOC) was also calculated and showed good congruence of the questions with the score ranging from 0.8-1 (Appendix E) and meant the good congruence of the questions. The unclear wordings were rewritten.

3. The reliability (internal consistency) of the Thai VAS xerostomia symptoms questionnaire was assessed in 30 subjects and reported by Cronbach's alpha.

4. The subjects, after signing an informed consent, had to complete a baseline evaluation form and rated the visual analogue scales (VAS) for xerostomia symptoms from 0-100 mm.(Appendix D)

5. The patients were taken the physical examination, including the oral cavities. Objective grading of xerostomia was done according to objective grades of Late Effect of Normal tissues Subjective, Objective Management and Analysis (LENT SOMA scales). [42] The examination was scored by one blinded senior otolaryngologist in each institute (PMK and NCI). The inter-observer reliability between 2 clinicians was analyzed using kappa statistic.

6. Randomization was performed in doubled-blinded fashion to either control group or topical pilocarpine group.

7. Each subject was received one 330 ml artificial saliva bottle (with no label of either pilocarpine or control solution) and one small empty 15 ml mouth spray bottle to be filled the solution for daily uses.

8. The 15 ml mouth spray bottle for the intervention group contained 15mg. pilocarpine dissolved in carboxymethylcellulos artificial saliva. In control group, the solution had the same general characteristics except for no pilocarpine.

9. The subjects were encouraged to use all the 15 ml artificial saliva for a day (should not over or under use) and refilled it everyday for daily usages. Following this regimen, the 330 ml. solution was consumed by at the end of 3rd week.

10. The subjects were instructed not to take any additional sialogogues or medication that may interfere with salivation.

11. At the end of the 1st week, the subjects were visited by phone regarding the compliances and adverse effects.

12. The amount of the solution used each week and all the adverse effects (sweating, headache, tearing, rhinitis, flushing and stomach cramps were recorded by the patients using the weekly-diary form (Appendix D). The diaries were reviewed by the physicians at 3rd week after initial therapy.

13. At 3rd week follow up assessment, all subjects completed post treatment xerostomia visual analogue scores, questionnaire of the frequency of fluid intake and the adverse effects of saliva substitute. Physical and oral examinations were also taken to evaluate the LENT SOMA scales after treatment.

14. Any subjects missing more than 45 ml or 3 consecutive mouth spray solution per week was considered as a non compliance.

15. If the adverse effects occurs and posing physical risk, the subjects would be removed from the study and be followed up by the physicians until the symptoms resolved.

3.8.7 Outcome measurement

Demographic variables (appendix A)

- Age (year)
- Gender (male/ female)
- RT dose (Gy)
- Radio-portal route
- Onset and duration of xerostomia
- Chemotherapy

Outcomes variables

1. **Primary outcome variable**
   - Visual analogue scales of xerostomia symptoms
2. **Secondary outcome variables**
   - Frequency of fluid intake to reduce xerostomia
   - Adverse effects of the intervention and placebo groups
   - LENT SOMA scales assessed by the physician

3.9 Data collection

The case record form (Appendix C) comprised the following information.

Table 1 : Summary of content record form

No.	Record sheets	Recorder
1	Patient consent form	Research assistant
2	Patient demographic data and characteristics	Research assistant
3	VAS of xerostomia	Patient
4	Frequency of fluid intake	Patient
5	Amount of medication used and adverse effects	Patient
6	LENT SOMA scales	Physician

3.10 Data analysis

- Thai questionnaires were validated by content validity method using 5 expert opinions. The internal consistency of the Thai VAS xerostomia symptoms questionnaire was evaluated in 30 subjects using Cronbach's alpha.

- The efficacy analysis was based on the intention-to-treat population (i.e., patients with at least one medication intake after randomization).

- Demographic and baseline characteristics of the patients in each treatment group was presented using descriptive statistics.

- The comparison of pre and post treatment xerostomia VAS at 3rd week between 2 treatments was performed using Mann-Whitney U test.

- The frequency of fluid intake (ordinal scale) to relieve xesostomia symptoms in this study and control group was compared using Fisher's exact test.

- Difference in pre and post treatment LENT SOMA scale in the study and control group was analyzed by Fisher's exact test

- The correlation between post treatment objective and subjective xerostomia summary scores was assessed by using Spearman's rank correlation.

- The inter-observer reliability in LENT SOMA scale between 2 clinicians was analyzed by weighted kappa using quadratic weight.

- Adverse events of both groups were summarized as numbers and percentages and compared using Fisher's exact test.

- A 2-sided p-value of less than 0.05 was considered a statistically significant difference. All statistical data analyses were performed by SPSS version 13.

Table 2: Summary of Statistic analysis

Outcome variables	Type of variable	Statistical analysis
Demographic data	Continuous and categorical scale	Descriptive statistics
VAS xerostomia	Continuous scale	Mann-Whitney U test
Amount of fluid used	Ordinal scale	Fisher's exact test
LENT SOMA	Ordinal scale	Fisher's exact test
Adverse effects	Categorical scale	Fisher's exact test
Correlation between subjective (VAS) and objective (LENT SOMA) xerostomia	VAS = continuous LENT SOMA = ordinal	Spearman's rank correlation
Validity of questionnaire	Continuous scale	Content validity
Reliability of questionnaire	Continuous scale	Cronbach's alpha
Inter-observer variation	Ordinal scale	Weighted Kappa with quadratic weight

3.11 Ethical considerations

- The research proposal was submitted to Cancer institute of Thailand and Phramongkutklao hospital Ethical Committee and was approved before starting the study.

- The information about the details of the interventions, potential adverse effects and treatment of the adverse effects were explained to the patients before signing the consent forms (Appendix B).

- The subjects had the right to withdraw from the protocol at any time without interfering with their medical care.

- Oral pilocarpine was registered by the U.S. and Thai FDA to be used for the indication of xerostomia patients. The adverse effects are well tolerated in recommendation doses. If the adverse effects occur, the subjects would be removed from the study and followed up by the physicians until the symptoms were resolved.