CHAPTER III

MATERIALS AND METHODS

This study was approved by the Research Ethic Committee of Medical Faculty at Chiang Mai University.

Participants

In this study, cervical cytology specimens were collected from 1,211 women, enrolled from February to December 2010.

ThinPrep™ procedure and interpretation

The specimens were collected using the Cervex-Brush™. Then, the broom was pushed into the PreservCyt® solution and submitted to the laboratory. At the laboratory, the sample vial was placed into the ThinPrep 2000. A gentle dispersion step broke up blood, mucus, and non-diagnostic debris, and then thoroughly mixed the sample. A series of negative pressure pulses were generated, which drew fluid through a ThinPrep2000 filter to collect a thin, even layer of diagnostic cellular material.

The cellular material was transferred to a glass slide using computer-controlled mechanical positioning and positive air pressure. The slide was then ejected into a cell fixative bath, ready for staining and evaluation. The cytologic results were reported using the Bethesda system classification 2001.

The ThinPrep Pap smears were separately evaluated by two pathologists (JS and KS). The final cytological interpretation was obtained by consensus agreement. The results were firstly classified as negative, atypical squamous epithelial cells of undetermined significance (ASC-US), atypical squamous cell-cannot exclude high grade squamous intraepithelial lesion (ASC-H), atypical glandular epithelial cell (AGC), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), and squamous cell carcinoma (SCCA). And, then, they were grouped in three categories: 1) Negative, 2) ASC or AGC, and 3) definite epithelial cell abnormality (LSIL or HISL or SCCA).

Hybrid Capture2 High-Risk HPV DNA test

Prior to HPV DNA testing, cervical specimens were collected for cytology with PreservCyt^{R} solution and conversed to Digene cervical sampler. HPV testing was performed using the Hybrid Capture2 (HC2) hybridization assay (Qiagen, Hilden, Germany). Thirteen types of high risk HPV were tested, including HPV types 16,18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The results were measured by comparing the light intensity with that of control (relative light units; RLUs). The positive test was defined by a RLU value equal to or greater than positive control, which corresponds to 1 pg of HPV DNA/μl (5,000 HPV viral copies).

Data and statistics

The prevalences of HR-HRV in each cytological category were compared using Chi-squared test. The The significant level was set as 0.05.

The interobserver reproducibility was tested by weighted kappa statistics. The weighted were 1.00 for data cells on the diagonal (i.e. exact agreement), 0.5 for cell adjacent to the diagonal, and 0 for cells 2 units from the diagonal. Disagreement was arranged in major and minor groups. Major discrepancy referred to a discrepancy between categories 1 and 3. Minor discrepancy referred to a discrepancy between categories 1 and 2; or categories 2 and 3.