CHAPTER V
DISCUSSION AND CONCLUSION

The persistence of high-risk HPVs infection in basal layers of cervix and getting other cofactors are important cause for progression to cervical cancer, the most common cause of cancer death among women in developing countries. Although the Papanicolaou (Pap) test is mainstay of cervical cancer screening and has relatively good specificity, the varied highly false-negative results from sampling error and an expert cytologists to justify the results are limitation of the test. After that, visual inspection with acetic acid (VIA) was explored as an adjunct to the Pap smear to decrease the false-negative rate of cytology. However, VIA is at least as sensitive as Pap smear in detecting high-grade lesions, but that its specificity is lower (2). Therefore, molecular techniques such as DNA hybridization or nucleic acid amplification have been developed for the detection of HPV, and evaluations of the commercial test kits are now appearing.

Nucleic acid amplification tests by using PCR-based assays have generally been more sensitive and specific than conventional cytology. However, there are several conditions, such as differences in sampling methods, sample transport/storage, DNA extraction procedures, primer sets, size of PCR product, reaction conditions, performance of DNA polymerase used in the reaction, spectrum of HPV DNA amplified, and ability to detect multiple types that can affect test performance (3, 7).

At present, most cervical samples are available preserved in fixative solution of commercial sampling kit of LBC, originally intended for cytology (*e.g. Liqui-*PREP™; LGM international, Inc., FL, USA) adequately preserve nucleic acids for molecular diagnosis. This fixative solution may effect the extraction of HPV DNA that have a variety of methods are available. Most of them are proteinase K digestion, freezing and boiling, and commercial kit (95, 105, 107). In this study, lysis buffer and proteinase K digestion that are simple and unnecessary to use a costly ready-to-use test kit were chosen for cervical cell extraction. Several reports have been used in varied concentrations and incubation times (107-109). After optimization, we reported the concentration of proteinase K was 200 μg/ml and 30 minutes incubation

time at 60°C was optimum. It is cost saving and less time consuming as compared to the commercial test kits. We used this protocol DNA extraction in cervical samples for further PCR analysis.

Several PCR methods for HPV detection involve an amplification step followed by any of a number of methods for distinguishing different HPV types have been developed to detect a broad spectrum of mucotropic HPV types using consensus primers for instance PGMY09/11, GP5+/6+, and SPF_{10} that are specific to a highly conserved region of the L1 gene (1, 3). Different PCR systems including commercial and in-house PCR assays have been evaluated by many groups of study. Most of them have been shown to be highly sensitive for the detection of HPV infection in cervical specimens. However, hindering factors like cost and specialized infrastructure have precluded using of PCR for diagnostic purposes especially in developing countries. Although most commercial test kits for diagnosis of HPV have been developed based on the convenient assay formats and have been available in many countries. However, the major drawback of their applications has been the budgeted spending for the assays.

We reported here the development of an inexpensive TaqMan-based real-time PCR for the detection of HPV in cervical specimen that was preserved in fixative solution. A significant improvement introduced by real-time PCR is the increased speed with that the results be obtained. This is largely due to reducing cycle times, removal of separate post-PCR detection procedures, minimizing of the potential for carry-over contamination, and using of sensitive fluorescence detection equipment, allowing earlier amplicon detection. In addition, this method has proven cost effectively when implementing in a high throughput laboratory. The assay developed in this study is a qualitative analysis and has lower detection limit at 5 fg for HPV DNA.

Fifty-two out of 367 (14.17%) samples were positive for HPV DNA. Among those positive samples, 36 (18.65%) were from the positive VIA and 16 (9.20%) negative VIA. Eighty-nine of 453 samples were negative for β–globin gene and excluded from analysis. It was possible that there was some PCR inhibitor or no DNA in the extracts due to long term storage of samples over 1 year.

The HPV positive samples were studied further for genotype distribution by using the REA technique. HPV16 was found most frequently in 22 of 43 (51.16%) samples followed by HPV18 (18.60%). HPV51, 58, and 59 were detected in 2 samples each (4.65%) and HPV6, 31, 35, 39, 52, 66, and 72 were detected 1 each (2.33%). HPV16 was also found most frequently among positive and negative VIA samples, 16 out of 36 (44.44%) and 6 out of 16 (37.50%), respectively. The three most common HPV types in HPV positive women from this study is similar to the prevalence of HPV types among women with normal cytology in Southeastern Asia (110).

Concerning with the VIA testing, we found only 18.60% that positive with both HPV and VIA, it would be suggested that women with VIA positive should be follow up closely with PAP smear or HPV DNA testing as the lesions might be regress spontaneously.