CHAPTER VI

DISCUSSION

HPV is the main etiological factor of benign and malignant genital neoplasia. Cervical cancer worldwide are infected with specific types of HPV DNA mainly HPV-16 and HPV-18 (10, 22). In Thailand, the incidence of cervical cancer ranks top among cancers in women and was reported that HPV-16 causes 55.9% of cervical cancer (23). Cervical cancer screening is currently based on cytology and HPV detection.

In this study, an optical detection assay of HPV type 16 antigens using gold nanoparticle based on immunoagglutination was developed. The assay used AuNPs conjugated with either HPV-16L1 or HPV-16E6 polyclonal antibodies for detecting HPV-16 L1 or E6 proteins directly from clinical specimens. And the results could be visibly detected by an agglutinate of the reaction.

In the present study, the purified HPV-16 antigens, L1 and E6 proteins were purified for used as positive control to detection HPV-16 by immunogoldagglutination assay. Two recombinant fusion proteins, GST-HPV16L1 and GST-HPV16E6, were successfully prepared from $E.coli$. Several groups have previously reported methods for preparation of these 2 proteins from different systems, i.e., mammalian cells, yeast, baculovirus, transgenic plants, and bacteria (76-79). The bacterial strain $E.coli\ BL-21$ used in this study is the only strain able to express fusion protein. Compared to other systems, the bacterial system has the advantage of the ease of expression and purification and provides large amount of protein, which can be used as antigen for antibody detection and vaccine production. In addition, the glutathione-S-transferase (GST) gene fusion system provides an integrated system for the expression, purification and detection of glutathione-S-transferase fusion proteins using $E.\ coli$. Slightly modification of purification procedure was performed such as temperature condition, for which most studies used 37^0C instead of 25^0C for GST-HPV16E6. The localizations of GST-HPV16L1 proteins in inclusion bodies and of GST-HPV-16 E6 in soluble fraction

were in accordance with previous reports (9-11). Although 8 M urea was used as solubilizer in extraction of GST-HPV16L1 proteins, the yield of purified protein was very low. The expressed GST-HPV16E6 proteins was easily purified by GST affinity chromatography and obtained in large amount.

Immunogoldagglutination assay with HPV-16 fusion proteins, the results indicated the assay could detect HPV-16 fusion proteins (Fig.16) and is sensitive to concentrations of L1 or E6 fusion protein as low as 2.5 μg (Fig.20-21). Moreover, several control experiments were carried out to validate selectivity of this assay. The results indicated only polyclonal antibody coated AuNPs could form agglutinates in this assay because monoclonal antibody lacked of multiple binding sites that enable to form agglutinates among a large numbers of particle (Fig.22-23).

Immunogoldagglutination assay with control cell lysate as CaSki cell that is a human cervical carcinoma cell with about 600 copies of HPV-16 indicated that the assay can detect HPV-16 E6 while HPV-16L1 can not be detected (Fig.24) because the cervical carcinoma cells have E6, oncoprotein overexpression in cell transformation (8). The sensitivity of the assay with CaSki cell lysate prepared from at least 10^5 cells could be agglutinated. The results of specificity of the assay indicated that no nonspecific agglutination and no cross-reactivity of the antigens occurred (Table 12).

Among 40 samples obtained from patients with normal-pathology, LSIL, HSIL and CaCx, HPV DNA was able to be detected in 40 samples (100%). The prevalence of HPV-16 infection in normal-pathology, LSIL, HSIL and CaCx patients were 10%, 70%, 100% and 70%, respectively (Table13). This finding was slightly higher than previous report in Thailand (normal: 2.5%, LSIL: 10.4%, HSIL: 22.2%, CaCx: 55.9%) (11).

The results of immunogoldagglutination assay in clinical samples were shown in table15. The results showed that 100% of normal-pathology, LSIL (CIN1) and CIN2 were negative for E6 while some samples were positive for L1 (normal: 10%, CIN1: 14%, CIN2: 75%). Because after infection the viral genome is replicated, the late gene L1, L2

and E4 are expressed. L1 and L2 encapsidate the viral genome form progeny virions in the nucleus and shed to initiate a new infection. Low grade intraepithelial lesions (LSIL) supported productive viral replication (8). Conversely, in high grade CIN3 and CaCx samples were positive for E6 67% and 71%, respectively and were positive for L1 (CIN3: 83%, CaCx: 71%). All 25 HPV-16 samples were positive for L1 (60%) and E6 (36%). Therefore, the sensitivity of the assay was 60% (15/25) for HPV-16L1 detection and 36% (9/25) for HPV-16E6 detection which is sensitive less than detection by PCR. But, in high grade CIN3 and CaCx samples sensitivity of the assay for detection HPV-16E6 was reached to 69.23% (9/13). The results from this assay demonstrated a correlation of E6 oncoprotein positivity with both severity of cervical histopathology and risk for progression. Therefore, E6 protein could be an appropriate biomarker of HPV mediated oncogenic activity.

In conclusion, the newly immunogoldagglutination assays exhibit analytical sensitivity for detection HPV-16 infection although less than the type-specific PCR tests. However, detection of HPV-16E6 by the newly immunogoldagglutination assay correlated well to the severity of cervical histopathology. The agglutination of this assay was observed within 24 hr and high specificity. The assay requires no instrumentation and the detection results can also be read by naked-eye. These results indicated that the immunoagglutination assay for detecting HPV-16E6 might be an appropriate tool for screening cervical pre-cancerous and cancerous patients.