I. INTRODUCTION

1.1 Background

Cervical cancer (CC) is the fourth most common malignancy that affects females worldwide. In 2018, there were around 600 thousand new cases of CCs globally with a high mortality rate of approximately 300 thousand deaths per year (Cohen et al., 2019). The human papillomavirus type 16 (HPV16) is included amongst the high-risk HPV (hrHPV) in both cases with and without cervical abnormalities, in which it accounts for approximately 70% of CC cases (Cerasuolo et al., 2017). HPV16 is a non-enveloped double-stranded DNA virus which primarily infects mucosal and epithelial cells. The HPV16 genome is made up of several types of early (E) and late (L) genes, including E1, E2, E4, E5, E6, E7, L1, and L2 genes (Yu et al., 2022). It has been studied that these translated proteins are associated with the viral replication and cervical carcinogenesis, as well as the interaction between the viral and host cells (Yajid et al., 2017).

In cervical carcinogenesis, there are five different cytological stages for determining the rate of the precancerous lesions which include negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASCH), and high-grade squamous intraepithelial lesion (HSIL) (Çakmak &Köseoğlu, 2014). The absence of abnormal epithelial cells during HPV infection indicates the NILM stage, in which it may progress to ASCUS that shows signs of atypical squamous cells suggestive to LSIL (Cubie, 2013). LSIL is defined as the presence of abnormal cells in the basal layers which are usually around one-third of the squamous epithelium (Ye et al., 2017). Then, ASCH is the presence of abnormal squamous cells suggestive to HSIL. The accumulation of abnormal squamous cells throughout the epithelium layers is called HSIL, in which it may progress to invasive cancer if it remains untreated (Darragh et al., 2013). Moreover, HPV detection can be done in any of the precancerous stages; however, the

HPV16-related gene expressions may vary in different stages which can be a proposed biomarker (Yajid et al., 2017).

The current cervical cancer screening can be done through: (1) Pap smear test observes the presence of abnormal cells which has variable and low sensitivity (Khan, 2017), and (2) HPV test detects the viral genetic materials, such as L1 DNA which could not indicate the definite cytology stage (van den Heuvel et al., 2020) and E6/E7 mRNA which had an increased risk of false-positive results (Swid & Monaco, 2022). Therefore, it is necessary to develop a new potential marker for defining the stage of cervical carcinogenesis during HPV16 infection. One of the HPV early proteins, called E4, is known to be associated with viral release and transmission. This protein induces the disintegration of the cellular membranes by disrupting the desmosomal contacts and protects the released viral particles from desiccation (Shiraz et al., 2020). Several studies had mentioned the utilization of E4 protein as a potential biomarker in detection of productive HPV infection which could lead to the early precancerous stages of CC, that is the growth of low-grade neoplasia or LSIL (van Zummeren et al., 2018). Furthermore, E4 expression may be associated with the degree of incorporated HPV genome into the host cells that induce cell cycle arrest which lead to the progression of invasive cervical carcinoma (Griffin et al., 2015). However, there are still limited studies on the significance of HPV16E4 mRNA expression in different cytological stages as a marker for viral carcinogenesis.

1.2 Objective

The objective of the research was to quantify the E4 mRNA expression in different cytology stages of HPV16-positive cervical swab samples through qRT-PCR for utilization as a potential biomarker of cervical carcinogenesis.

1.3 Research Scope

The scope of the research encompassed sample collection from the local hospital, RNA extraction of the clinical samples, cDNA synthesis of the extracted RNA, and determination of the HPV16E4 mRNA expression using qPCR analysis as summarized in **Figure 1**.

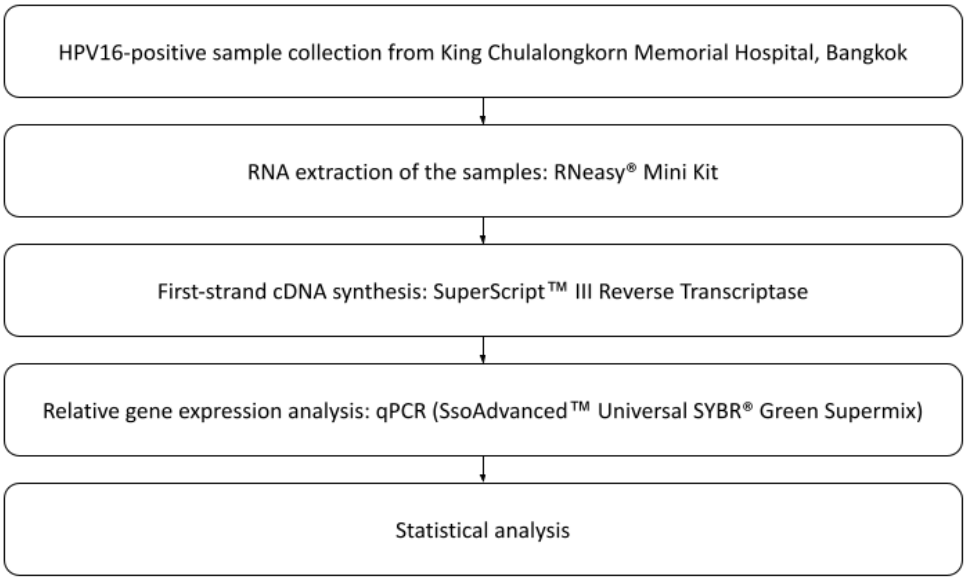

Figure 1. Overview of the research project. The flow of the research was done by sample collection, two-step qRT-PC, and data analysis.

1.4 Hypothesis

The hypothesis of the research was that HPV16E4 mRNA expression correlates with productive HPV infection which is a contraindication of disease severity.