

## Original article

# Cervical IL-6 Level: A Potential Diagnostic Indicator for Cervical Cancer

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**Background:** Chronic infection by high-risk human papillomavirus (HR-HPV) causes more than 95% of cervical cancer and its precursor lesions. Along with infection, some immunomodulatory components like cytokine are also responsible for the progression of the disease. Interleukin 6 (IL-6) regulates other cytokine production and secretion and induces the proliferation of normal and neoplastic cervical epithelial cells. The levels of IL-6 were estimated in both HPV-infected cancer and healthy cervical fluids for the establishment of this cytokine as a diagnostic indicator for the detection of disease severity. **Methodology:** A cross-sectional study was conducted based on histopathology and HPV DNA status from the Gynaecological Oncology department of BSMMU, 30 cervical cancer patients and 20 HPV DNA-negative healthy individuals women were enrolled for this study and the levels of IL-6 were estimated in cervical smear by Flowcytometry. **Result:** The mean level of IL-6 was found to be considerably elevated in individuals diagnosed with cancer compared to healthy women ( $p < 0.05$ ). **Conclusion:** Early cervical cancer diagnosis by measuring cervical cytokine levels might halt the disease progression in HPV-infected women and allow them to use anti-inflammatory medications to prevent the disease progression.

**Keywords:** HR-HPV-High-risk Human Papillomavirus; IL-Interleukin; TNF-Tumor Necrosis Factor

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### Introduction:

Cervical cancer (Ca cervix) is mainly caused by *Human papillomavirus* (HPV), an oncogenic non-enveloped DNA virus and member of the papillomaviridae family. In 2021, about 82.8% of invasive cervical cancer were due to HPVs 16 or 18.<sup>1</sup> It is the most common sexually transmitted infection (STI), affecting approximately 12% of the world's population.<sup>2</sup> Approximately 200 varieties of HPVs are divided into high-risk, intermediate-risk and low-risk categories, around 40 infect the genital tract<sup>3</sup> primarily the

cutaneous and muco-cutaneous epithelia of vertebrates and the high-risk varieties cause cervical cancer mainly.<sup>4</sup> About 10% of individuals with HPV infection of the female genital tract failed viral clearance and remain infected though it is believed that the host's immunological response to HPV infection is a critical role in the elimination of the virus.<sup>5</sup> Numerous genetic variants in immune-associated genes, such as interleukins (ILs) are a class of cytokines that have a role in immunological and inflammatory responses

as well as tumor growth and progression.<sup>6</sup> After infection, the tumor microenvironment secretes different polarizing cytokines such as IL-2, IL-4, IL-6, IL-8, IL-21, TNF- $\alpha$  and IL-10 which also progress the disease condition.<sup>7</sup>

Interleukin-6(IL-6) is one such inflammatory cytokine, involved in the proliferation and differentiation of malignant cells and has been detected in high concentrations in the serum and tumor tissues majority of malignancies. Tumor cells, as well as tumor-associated macrophages (TAMs) and CD4+T cells are the principal producers of IL-6 in the tumor microenvironment. In the tumor microenvironment, IL-6 promotes carcinogenesis by modulating the intrinsic and extrinsic activities of cancer cells and influencing stromal cells that indirectly promote tumorigenesis.<sup>8</sup> IL-6 has a major role in promoting the proliferation of cervical tumor cells. E6 oncoprotein with cancer-associated fibroblasts (CAF) releases more IL-6 and causing the aging of other normal fibroblasts (NF) and promoting cervical carcinoma.<sup>9</sup> This pleiotropic multifunctional interleukin might be the most therapeutically useful and investigated cytokine, with applications in chronic inflammation and cancers, including cervical cancer. The relationship between the levels of cytokine in the cervical fluid in HPV-induced cervical precancer and cancer is an important area of research. Moreover, the mechanisms of HR-HPV-induced chronic infection and progression of cervical cancer were analyzed to provide an immunity theory for preventing HR-HPV infection and disease progression. Thus this study is designed to determine and compare the level of IL-6 in cervical cancer patients and healthy women.

### Methodology

In this cross-sectional study 30 patients who were diagnosed as cervical cancer by histopathology with HR-HPV infection by Hybrid Capture II (HC II) in the department of Gynecological Oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, with no history of pregnancy or postpartum in last 6 months, additional sexually transmitted diseases infection (STIs), hysterectomy or invasive cervical treatment, other any cancer chemotherapy or malignancies, chronic infection, or vaginal medication in the last 2 weeks were enrolled by purposive sampling. About 20 normal women who came for regular cervical screening also enrolled in this study with negative Hybrid Capture II reports. All patients and healthy participants were informed and gave their written consent and data on epidemiology were collected by survey. Cervical smear samples were collected by cytobrush of cervical sampler (Digene® HC-2 DNA Collection Device) and transferred into microcentrifuge tube and centrifuged (14,000 RPM X 10 min) to separate the supernatant and cellular components. The level of IL-6 was measured by using cervical swab samples were estimated by cytometric

bead array (CBA) method using the kit BD cytometric bead array (CBA) human soluble protein master buffer kit (Cat no: 558264) through BD Accuri™ C6 plus flowcytometer by IL-6 reagent (Cat no: 558276). The concentration was determined in pg/ml. Statistical analysis was done using SPSS/PC 25.0 software and Microsoft Excel. P-value of <0.05 was considered significant.

### Result

Cytokine IL-6 levels were measured in samples collected from 30 cervical cancer women and 20 apparently healthy women. The mean age of the cervical cancer patients was 49.4 $\pm$ 9.9 years (Mean  $\pm$  SD), while the mean age of the HPV-DNA negative healthy participants was 37.5 $\pm$ 7.2 years (Mean  $\pm$  SD). The difference in mean age between the two groups was found to be statistically significant. In this study, there were about 40% healthy women in the age range of 31 to 40 years and 33.3% cancer women in the age group of 41 to 50 years (Figure 1). The study aimed to measure the levels of IL-6 in the collected samples. The results revealed that the mean level of IL-6 was significantly higher in cervical cancer patients compared to healthy individuals. In this study, IL 6 levels were significantly different among the age 31 to 60 years of cervical cancer and healthy women (Figure 2).

Table: Levels of IL-6 in pg/ml among cervical cancer and healthy women

Cytokine (Mean in pg/ml)	Cervical cancer (n=30)	Healthy participants (n=20)	p-value*
IL -6	2747.7	152.8	<0.05

\*p-value was determined by independent T test.p< 0.05 considered as significant

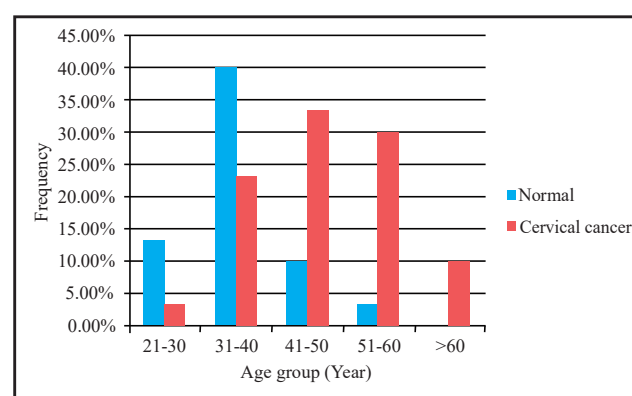
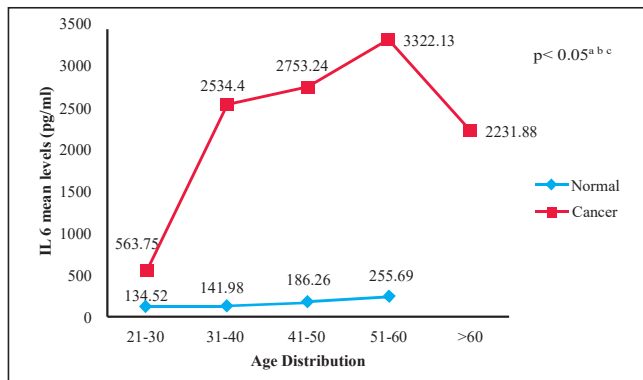


Figure 1: Cervical cancer and healthy women among different age groups



\*p-value was determined by independent T test.  $p < 0.05$  considered as significant

a= p value for 31-40 years age group

b= p value for 41-50 years age group

c= p value for 51-60 years age group

Figure 2: Line chart of the mean IL-6 levels in different age groups

## Discussion

HPV causes cervical cancer which is the second most common cancer in Bangladeshi 15–44 years women.<sup>10</sup> Globally, there were an estimated 6, 04,000 new cases and 3, 42,000 deaths from cervical cancer in 2020. More than 95% of cervical cancer is due to persistent high-risk HPV infection.<sup>11</sup> During HPV infection, the local immune response plays a significant role and releases different cytokines. Cytokines were considered to be immune system messengers that guided leucocytes to the areas of inflammation.<sup>12</sup> However, it has been established that dysregulation of cytokine is associated with most neoplastic tissues and may play a role in malignant transformation, proliferation, survival, angiogenesis, invasion and metastasis.<sup>13</sup> The pro-inflammatory cytokine IL-6 was tested for its ability to regulate epithelial cervical cell cytokine production and secretion and to induce the proliferation of normal and neoplastic epithelial cervical cells.<sup>14</sup> The incidence of cervical cancer steadily increased with age, about 33.3% in 41 to 50 years (Figure 1). Nessa et al.<sup>15</sup> demonstrated that the conversion of high-grade lesions to cancer occurred usually in the elderly population. There is an inverse relationship between immune function and the development of several types of cancer; as immune function declines with age, the incidence of cancer rises.<sup>16</sup> The result of this study showed that the level of the pro-inflammatory cytokine, IL-6 in cervical cancer patients increased significantly compared to the HPV DNA-negative healthy participants ( $p < 0.05$ ). Some authors also found the levels of IL-6 were significantly raised in HPV DNA-positive patients as well as cervical cancer and precancer patients.<sup>5,17</sup> During the development

and progression of HPV infection, IL-6 levels rise dramatically without any auto-inhibition. Therefore, infected cells continue to produce huge amounts of IL-6 and prevent tumor cell death as well as cause uncontrolled atypical cell proliferation.<sup>18</sup> However, according to Mhatre et al.<sup>19</sup> the levels of IL-6 were not significantly elevated in HR-HPV-induced different stages of cervical neoplasia. A persistent inflammatory condition, precancerous lesions and cervical cancer stimulate the immune system and release several cytokines. As such the current study observed that the mean level of IL-6 was increased significantly in cervical cancer patients than in healthy individuals with increasing age (Figure 2).

## Limitation

Despite the significance of our findings regarding the elevated IL-6 levels in cervical secretions of cervical cancer patients, it is essential to acknowledge certain limitations inherent in our study. A more comprehensive understanding could have been achieved if all patients were of the same age or if we could observe the same cytokine levels over a specified period. Unfortunately, due to constraints in time and resources, we were unable to design the study in such a controlled manner. Prospective studies using a more uniform sample and a longitudinal methodology might provide more understanding the correlation between IL-6 levels and the progression of cervical cancer.

## Conclusion

The mean level of cervical IL-6 is more in cervical cancer patients than in healthy individuals. Detection of IL-6 early in cervical cancer by measuring the levels of cervical cytokine may pave the way for the use of anti-inflammatory medications such as steroids or monoclonal antibodies against the raised cytokine as a treatment protocol and halt the disease progression.

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## Conflict of interest

The authors thereby declare no conflict of interest exists.

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