Patterns of Different Cervical Cytokine Expression in High-Risk Human Papillomavirus–Infected Patients With Cervical Cancer and Its Precancerous Lesions

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ABSTRACT

BACKGROUND: Cervical cancer is the second most common cancer in Bangladesh and is primarily caused by persistent high-risk human papillomavirus (HR-HPV) infection. Several risk factors, including immunological, genetic, environmental, and viral factors, may contribute to the development of cervical cancer. Moreover, a disruption in an otherwise delicate balance between immune response and cytokine production may lead to diseased states. Henceforth, this study aimed to determine and compare selected cytokines, including interleukin-6 (IL-6), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), interleukin-10 (IL-10), GM-CSF, interleukin-8 (IL-8), and MCP-1 among HR-HPV-infected patients with cervical cancer, precancer individuals, and healthy participants to test the propensity of these cytokines to serve as predictive biomarkers for the detection of cervical cancer during its early stages.

METHODS: A cross-sectional study was conducted on female patients visiting two referral hospitals in Bangladesh from September to November 2022. Among them, 80 women were enrolled in the study as patients with cervical cancer and precancerous lesions along with HPV DNA–negative healthy individuals. The selected cytokines in the cervical swab were estimated by flow cytometry.

RESULT: Cervical cancer and precancer were primarily detected in patients aged above 40 years (73.3% and 46.7% of the patients in the respective groups). Other significant risk factors, including poor educational, socioeconomic status and nutritional conditions, age of first coitus, multiparity, and tobacco and betel nut consumption, were found significant for the development of cervical cancer and precancer ($P < .05$). The levels of IL-6, IL-1β, IL-10, IL-8, and MCP-1 were substantially elevated in patients with cancer than in patients with precancer and healthy individuals ($P < .001$). Moreover, the levels of IL-6, IL-1β, IL-10, and IL-8 were also significantly increased in patients with precancer than in healthy individuals ($P < .05$).

CONCLUSIONS: Thus, IL-6, IL-1β, IL-10, IL-8, and MCP-1 can be used as potential biomarkers for diagnostic and prognostic purposes in HPV-induced cervical cancer and precancer.

KEYWORDS: Cervical cancer, cervical precancer, HPV, IL-6, IL-1β, TNF-α, IFN-γ, IL-10, GM-CSF, MCP-1, Bangladesh

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Data Availability Statement included at the end of the article

Introduction

Cervical cancer is primarily caused by persistent human papillomavirus (HPV) infection (more than 95% of cases) and is characterized by the expeditious growth of abnormal cells in the cervix. It is the fourth leading malignancy and the fourth main cause of cancer-related deaths among women, worldwide.[1,2] GLOBOCAN, 2020 reports that cervical cancer incidence and mortality rates were 10.6 and 6.7 per 100 000 population in Bangladesh, respectively.[3] Human papillomavirus is an oncogenic, nonenveloped, DNA virus that causes approximately 12% of sexually transmitted infections in the world's population.[4] Approximately, 40 of the 200 genotypes of HPVs affect the genital tract, including high-risk HPV (HR-HPV) 16 and 18, which cause 70% of cervical cancer and precancer.[1] Most low-grade cervical intraepithelial lesions (LSILs) spontaneously resolve, whereas approximately 10% proceed to high-grade cervical intraepithelial lesions (HSILs) or invasive malignant lesions. Poor immune response of the host may lead to cancer from persistent infection.[5-7] There are different factors such as genetic, environmental, immunological, and viral factors that have a significant influence on the dysplastic changes of the cervix. It has been observed that early sexual exposure, sexual promiscuity, early age of marriage, high parity, usage of hormonal contraceptives, and tobacco chewing as well as nutritional status are key risk factors for developing cervical cancer.[8,9] In the case of cancer, the immune response is responsible for regulating the production and

modulation of different cytokines. Persistent high-risk HPV infections cause evasion of the host immune response, which may be responsible for cervical cancer. Usually, CD4+ and CD8+ T cells play an essential role in the clearance of HPV through host cellular and humoral immunity.[10] However, immune escape in cervical cancer is facilitated by several mechanisms, such as the downregulation of Major Histocompatibility complex (MHC) class I antigen presentation and upregulation of Natural killer (NK) cell receptors due to tumor-infiltrating lymphocytes (TILs) which may be responsible for the suppression of cytotoxic T lymphocytes (CTLs) activities.[11,12] Furthermore, the immune defense is also compromised by an alteration of T-helper cell responses, which is characterized by an imbalance of Th1 and Th2 cytokines. Effective immune recognition is further inhibited by HPV-specific immune evasion proteins, which progress chronic cervical infection to cancer.[10] Cytokines are produced by T cells, other immune cells, and other accessory cells such as keratinocytes, as well as the subepithelial cervical tissue, which influences host immunological responses and the progression or regression of systemic or local inflammation and malignancy.[7,13,14]

Levels of different cytokines, such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-10 (IL-10), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-8 (IL-8), and MPC-1, are altered by the inflammatory process caused by HPV infection. Interleukin-6 (IL-6) and IL-1β activate multiple inflammatory pathways, damage cervical epithelial DNA, upregulate other inflammatory mediators and chemokines, suppress cancer cell death, and accelerate disease progression.[13] Furthermore, sustained release of TNF-α aggravates inflammatory reaction by activating the NF-κB signaling pathway and preventing cancer cell apoptosis, perhaps these processes enhance the cancer development process.[15] The IFN-γ interacts with IFN-γ-producing cells and promotes tumor immunoevasion by suppressing cytotoxic T-lymphocytes and NK cells recruiting T-regulatory cell (T_{TEG}), and MDSC cells and inhibiting antitumor immunity.[16]

Interleukin-10 induces peripheral tolerance and reduces antigen-specific T-cell proliferation, as well as cytotoxic T-cell responses. These indirect inhibitory effects of IL-10 may promote the progression of cervical cancer.[17] Granulocyte-macrophage colony-stimulating factor also produced by cervical cancer cells influences local inflammatory pathways by cyclooxygenase-2 (COX-2) and iNOS expression in immunomodulatory dendritic cells and is associated with cervical cancer metastasis.[18]

In addition, IL-8 and MCP-1 promote angiogenesis, and the synthesis of growth factors leads to the accumulation of immunosuppressive and other pro-tumorigenic immune cells, and may also be associated with lymphocyte infiltration in the tumor microenvironment and tumor development. This may be a possible process of the progression of cancer.[19,20]

Higher local concentrations of certain cervical cytokines are associated with the development of Cervical Intra epithelial neoplasia (CIN) to invasive cervical cancer and metastasis.[6] The alteration of the levels of different local cytokines associated with HPV infection, cervical precancer, and cervical cancer may enhance, reduce, or even suppress the immune response. Several cytokines with elevated levels were intimately correlated to cervical precancer and cancer severity, whereas some cytokines with low levels were not associated with HPV-induced cervical infection.

Therefore, the purpose of this research was to identify cervical cytokines that could have diagnostic implications in the detection of cervical cancer. As such, after assessing the cytokine levels in patients with cervical cancer and precancer, individuals with healthy participants, we found that the levels of IL-6, IL-1β, IL-10, IL-8, and MCP-1 were significantly altered in the former 2 groups. These findings could potentially highlight their propensity to serve as predictive biomarkers for different stages of HPV-induced cervical precancer and cancer.

Methods

Study population

This cross-sectional study was conducted at the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Study participants were recruited from the Department of Gynaecological Oncology, BSMMU, and the National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh. After taking informed written consent from September to November 2022, 105 patients who tested positive for VIA and colposcopy and 45 healthy participants were initially enrolled in this study. According to the histopathological report, 31 patients had cervical cancer and 55 suffered from cervical precancer (CIN I, CIN II, CIN III). Among them, from their cervical swab samples, 30 cervical cancer and 30 precancer women were diagnosed with HPV infection via Hybrid Capture 2 assay and were subsequently recruited in this study. Cervical swabs were additionally collected from 45 healthy individuals who came to the colposcopy clinic of BSMMU for regular screening, of whom, 20 participants were VIA, colposcopically, and HPV DNA-negative and enrolled in the study as healthy participants. A total of 80 study participants including 30 patients with cervical cancer, 30 patients with cervical precancer, and 20 healthy controls were finally enrolled in this study and included in the statistical analysis. Patients were excluded from the study if any of the following criteria were met: (1) pregnancy, (2) a previous confirmatory diagnosis of any sexually transmitted diseases (STDs) other than HPV, (3) history of hysterectomy or invasive cervical treatment (eg, LEEP and thermocoagulation), (4) received of vaginal and cervical medication within 1 week, (5) any chronic diseases (diabetes mellitus, chronic renal failure, autoimmune diseases), other malignancies (ovarian cancer, endometrial cancer, vaginal cancer, vulval cancer, and other), and any kind of immunosuppressive

therapy or cancer chemotherapy. Patients' relevant histories have been recorded on a predesigned questionnaire.

Sample collection

Cervical swab from the women participating in the study was collected into 2 tubes using a Digene HC2 (hybrid capture 2) DNA collection device (Qiagen). One tube was for the detection of HPV DNA using the Hybrid Capture 2 test, whereas the other was analyzed for selected cytokines.

Detection of HPV DNA

Digene HC2 High-Risk HPV DNA Test (QIAGEN, REF-5197-1330, Germantown) was used for the detection of HR-HPV DNA (types 16/18/31/33/35/39/45/51/52/56/58/59/68).

Cytokine measurement

The concentrations of selected cytokines, IL-6, IL-1β, TNF-α, IFN-γ, IL-10, GM-CSF, IL-8, and MCP-1, in cervical swab samples were estimated by cytometric bead array (CBA) method using the BD CBA Human IL-6 (Cat no: 558276), IL-1β (Cat no: 558279), TNF-α (Cat no: 558273), IFN-γ (Cat no: 558269), IL-10 (Cat no: 558274), GM-CSF (Cat no: 558335), IL-8 (Cat no: 558277), MCP-1 (Cat no: 558287) Flex sets, and BD CBA human soluble protein master buffer kit (Cat no: 558264; BD Biosciences, Becton Dickinson, San Diego) through BD Accuri C6 plus flow cytometer.

Statistical analysis

Statistical analysis was done using SPSS/PC 25.0 (IBM, New York City, New York) software. Association of sociodemographic characteristics and risk factors status with the study participants (cervical cancer, precancer, and healthy participants) were analyzed by chi-square test with or without continuity correction as appropriate, but the mean age of study participants among these groups was compared by the analysis of variance (ANOVA) test and the results of the comparison of cytokine among these groups were analyzed by the Kruskal-Wallis test. Results were interpreted based on *P* values, where $P < .05$ indicated statistically significant. Association between 2 groups was determined by the Bonferroni post hoc pair-wise test and identified significant associations between specific groups based on Bonferroni threshold levels. The Bonferroni threshold was set at 0.006 ($P = .006$) and the *P* values were adjusted in the Bonferroni post hoc analysis. The Bonferroni post hoc pair-wise test was used to analyze the mean cytokine levels of 2 age groups with the study participants (cervical cancer, precancer, and healthy participants) and the Bonferroni threshold ($P = .013$) was used to determine the significant associations between specific groups.

Result

A total of 80 participants made up the final study population. The mean age (year) of the patients with cervical cancer, patients with cervical precancer, and HPV-DNA-negative healthy participants were 49.4 ± 9.9, 41.3 ± 7.2, and 37.5 ± 7.2, respectively, which differs significantly among the groups ($P < .05$) (Table 1). As summarized in Table 1, 73.3% of patients with cancer and 46.7% of patients with precancer were >40 years of age. In addition, 76.7% of patients with cervical cancer and 26.7% of patients with cervical precancer were illiterate, whereas the family income of 76.7% of patients with cancer and 50.0% of patients with precancer was ⩽20 000 BDT. About 60.0% of patients with cervical cancer and 10.0% of patients with precancer were with poor nutritional status (body mass index $< 18.5\ kg/m^2$). Moreover, it was observed that 73.3% and 50.0% of patients with cervical cancer and precancer, respectively, experienced their first coitus before 18 years of age. However, 65.0% of healthy participants had a history of first coitus after the age of 18 years. About 66.7% of patients with cervical cancer and 50.0% of patients with cervical precancer had more than 2 children. Furthermore, 80.0% of patients with cervical cancer and 40.0% of patients with precancer were habituated to tobacco leaf and betel nuts, or both, respectively. Importantly, the women who were not on regular screening had developed cervical precancer and cancer. The majority (76.7%) of cervical cancer and (60.0%) of cervical precancer patients' husbands had multiple sexual partners ($P < .05$).

Among the 8 cytokines, levels of 5 cytokines, IL-6, IL-1β, IL-10, IL-8, and MCP-I, varied significantly among the groups (Figures 1 to 3). The median values of IL-6 were 2640.7 (1347.17-3912.86), 530.6 (455.66-669.10), and 143.5 (50.2-262.5) (pg/mL) in cervical cancer, precancer, and healthy participants, respectively. Thus, IL-6 levels were shown to differ significantly among the groups ($P = .000$, Kruskal-Wallis test). In addition, the median level of IL-1β (in pg/mL) was 7.1 (4.79-8.27) in patients with cervical cancer, 0.5 (0.21-0.70) in patients with cervical precancer, and 0 (0.02-0.04) in healthy participants, and as such, the result also differed significantly among 3 groups ($P = .000$, Kruskal-Wallis test). In the cervical cancer group, the median IL-10 level (in pg/mL) was 4.3 (2.76-4.82), whereas the median was 0.9 (0.57-1.25) and 0.1 (0.06-0.23)in the cervical precancer and healthy participant group, respectively, and this was statistically significant in 3 groups ($P = .000$, Kruskal-Wallis test). Furthermore, the median IL-8 levels (in pg/mL) were 6369.1 (4687.09-7736.47), 1126.9 (923.95-1220.10), and 592.0 (475.22-658.45) in cervical cancer, precancer, and the healthy participant groups, respectively, and this result was statistically significant as well ($P = .000$, Kruskal-Wallis test).

Moreover, the IL-6, IL-1β, IL-10, and IL-8 levels in patients with cervical cancer were significantly higher than the patients with precancer and the healthy participants ($P = .000$)

Table 1. Sociodemographic characteristics and risk factors associated with the study participants (N = 80).

VARIABLES	CERVICAL CANCER (%) N = 30	CERVICAL PRECANCER (%) N = 30	HEALTHY PARTICIPANTS (%) N = 20	*P* VALUE
Age				
Mean age, y	49.4 ± 9.9	41.3 ± 7.2	37.5 ± 7.2	.000[a]
⩽40	8 (26.7)	16 (53.3)	16 (80.0)	.001
>40	22 (73.3)	14 (46.7)	4 (20.0)	
Educational status of participants				
Illiterate	23 (76.7)	8 (26.7)	2 (10.0)	
Up to primary	6 (20.0)	8 (26.7)	4 (20.0)	.000
Above primary	1 (3.3)	14(46.7)	14 (70.0)	
Socioeconomic status (in BDT)				
<12 000	15 (50.0)	7 (23.3)	1 (5.0)	
12 001 to 20 000	8 (26.7)	8 (26.7)	2 (10.0)	.000
20 001 to 40 000	7 (23.3)	8 (26.7)	7 (35.0)	
>40 000	0(-)	7 (23.3)	10 (50.0)	
Nutritional status, kg/m^2				
<18.5	18(60.0)	3(10.0)	0 (–)	.000
⩾18.5	12(40.0)	27(90.0)	20 (100.0)	
Residence				
Rural	16 (53.3)	17 (56.7)	8 (40.0)	.402
Urban	14 (46.7)	13 (43.3)	12 (60.0)	
Age of first coitus of the participants, y				
<18	22 (73.3)	15 (50.0)	7 (35.0)	.022
⩾18	8 (26.7)	15 (50.0)	13 (65.0)	
Multiple sexual exposure of participants				
Yes	4 (13.3)	5 (16.7)	0 (–)	.170
No	26 (86.7)	25 (83.8)	20(100.0)	
Number of children				
⩽2	10 (33.3)	15 (50.0)	16 (80.0)	.012
>2	20 (66.7)	15 (50.0)	4 (20.0)	
Contraceptive use				
No	12 (40.0)	7 (23.3)	9 (45.0)	.223
Yes	18 (60.0)	23 (76.7)	11 (55.0)	
Habituated to				
Tobacco leaf	15 (50.0)	9 (30.0)	1 (5.0)	
Betel nut	3 (10.0)	2 (6.7)	1 (5.0)	.000

(Continued)

Table 1. (Continued)

VARIABLES	CERVICAL CANCER (%) N = 30	CERVICAL PRECANCER (%) N = 30	HEALTHY PARTICIPANTS (%) N = 20	*P* VALUE
Both	6 (20.0)	1 (3.3)	0 (–)	
None	6 (20.0)	18 (60.0)	18 (90.0)	
Regular screening				
Yes	0 (-)	1 (3.3)	23 (76.7)	.000
No	30 (100.0)	29 (96.7)	7 (23.3)	
Multiple sexual exposure of husband				
Yes	23 (76.7)	18 (60.0)	4 (20.0)	.000
No	7 (23.3)	12 (40.0)	16 (80.0)	
Husband occupation				
Unemployed	3 (10.0)	0 (-)	0 (–)	
Service	5 (16.7)	12 (40.0)	11 (55.0)	
Business	3 (10.0)	3 (10.0)	4 (20.0)	.02
Others[b]	13 (43.3)	13 (43.3)	5 (25.0)	
Deceased	6 (20.0)	2 (6.7)	0 (–)	

P value was determined by the chi-square test with or without continuity correction as appropriate.
[a]*P* value was determined by the ANOVA test.
P < .05 is considered significant.
[b]Driver, garments worker, rickshaw puller, or farmer.

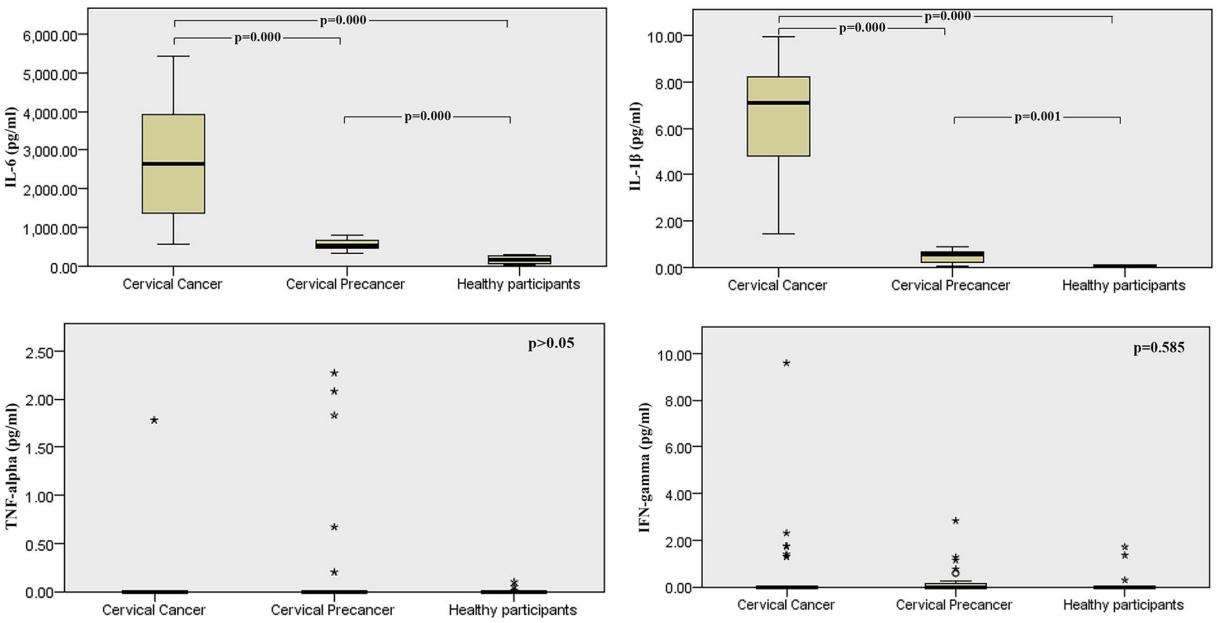

Figure 1. Box-plot representation of proinflammatory cytokine IL-6, IL-1β, TNF-αlpha, and IFN-gamma.

as well as in the patients with precancer were also significantly higher than healthy participants (IL-6, *P* = .000; IL-1β, *P* = .001; IL-10, *P* = .000; and IL-8, *P* = .000; Bonferroni post hoc test).

However, TNF-α, IFN-γ, and GM-CSF levels were not statistically significant among all groups. The median MCP-1 levels (in pg/mL) were 0 (0.0-2.3) and 0.42 (0.17-0.76) in cervical cancer and healthy participant groups, respectively, and

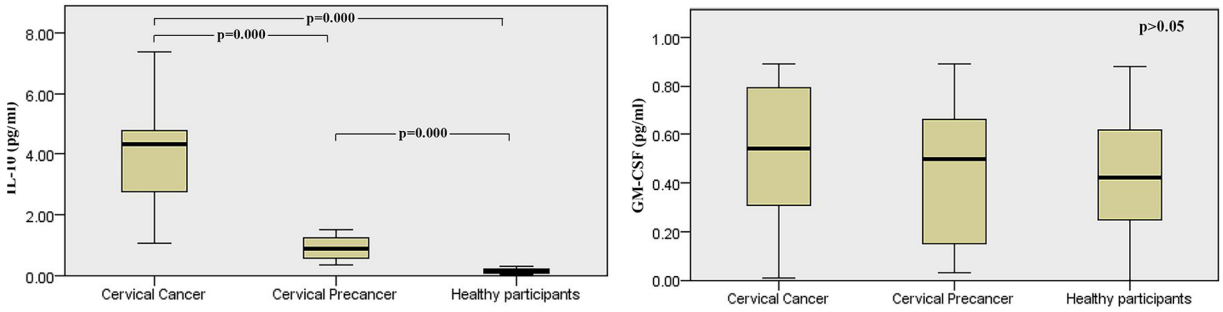

Figure 2. Box-plot representation of anti-inflammatory cytokine IL-10 and growth factor GM-CSF.

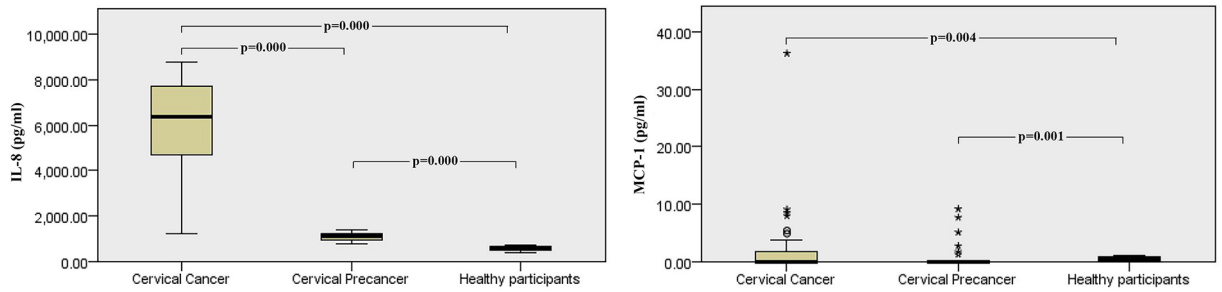

Figure 3. Box-plot representation of chemokine IL-8 and MCP-1.
[One MCP value in the precancer group was >300 000 pg/mL, which was considered an outlier. So, the total number of patients is 29 in the precancer group].

were statistically significant among 3 groups ($P = .000$, Kruskal-Wallis test). The levels of MCP-1, therefore, differed significantly in cancer ($P = .004$) and precancer participants ($P = .001$) than healthy individuals.

Figures 4 and 5 show that patients with cervical cancer exhibited elevated levels of IL-6, IL-8, IL-10, and IL-1β in both age categories, with individuals above the age of 40 years demonstrating even higher levels. In comparison with healthy participants and patients with cervical precancer, these cytokine levels were significantly higher in patients with cancer throughout both age groups ($P < .013$). The levels of IL-10 in patients with precancer aged 40 years or younger were significantly higher than those in healthy participants ($P < .013$). However, the rest of the cytokine levels being investigated were not significantly raised in the precancer group compared with the healthy group, irrespective of age. The healthy participants consistently demonstrated the lowest cytokine levels, which suggests a normal immune response.

Discussion

During an HPV infection, the local immune response plays a pivotal role through the release of different cytokines.[21] Approximately 73.3% of cervical cancer and 46.7% of patients with cervical precancer were aged 40 years or above. Jahan et al[22] found that there was less chance of developing cervical cancer below the age of 30 years in their study. Hence, the incidence of cervical cancer has been observed to steadily increase with age, demonstrating the conversion of high-grade lesions to cancer in the aged population.[8]

This study found that women with negligible educational background, low socioeconomic conditions, and poor nutritional status were more likely to develop cervical cancer and precancers (Table 1). Other studies have also shown that cancer was much more frequent among women with poor educational and socioeconomic status.[22,23] These observations support the hypothesis that educated women are more conscious of their health status, maintain cleanliness, avoid risk factors, and undergo regular check-ups. As such, precancer and cancer prevalence is lower among educated women.

Findings also demonstrated that 73.3% and 50.0% of patients with cervical cancer and precancer experienced their first coitus before 18 years of age with this feature being significantly associated with cervical cancer (Table 1). Another study from Bangladesh observed that 100% of women in their study had a history of first coitus before 20 years of age and 94.0% of multiparous women developed cervical cancer.[22] Tobacco and/or betel nut consumption and the usage of oral contraceptives are among the major risk factors for the development of cervical intraepithelial neoplasia and cervical cancer.[21] Accordingly, the current findings also observed that multiparity and tobacco leaf and/or betel nut consumption were associated with cervical cancer and precancers.

This study did not find a substantial relationship between contraceptive usage and cervical cancer. This non-association may be due to the small number of patients with cervical cancer. In contrast, Jahan et al[22] found an association between the use of oral contraceptives with cervical cancer. In addition, it was further observed that multiple sexual exposures of

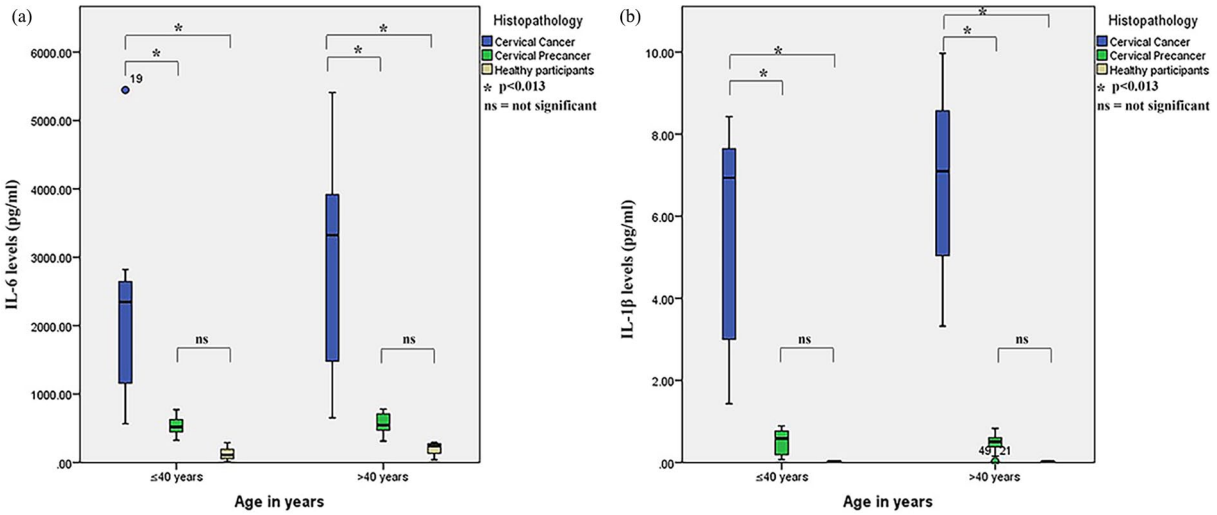

Figure 4. Box-plot representation of the IL-6 (a) and IL-1β (b) levels in different age groups.
(a) The IL-6 levels in different age groups. (b) The IL-1β levels in different age groups.

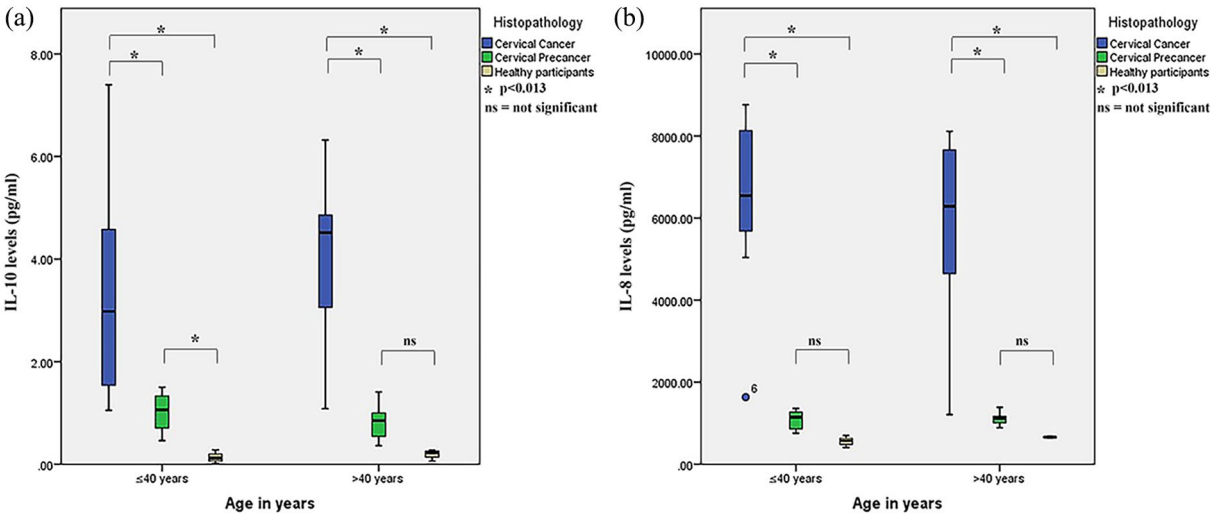

Figure 5. Box-plot representation of the IL-10 (a) and IL-8 (b) levels in different age groups.
(a) The mean IL-10 levels in different age groups. (b) The mean IL-8 levels in different age groups.

participant's husbands, as well as certain jobs (such as driver, garments worker, rickshaw puller, or farmer) of husbands, where these men were likely to have multiple sexual encounters alongside the participants, were associated with cervical cancer and precancer (Table 1). Previous studies in Beijing and Nigeria also revealed that HPV-induced cervical cancer and precancer were associated with the occupation of husbands and multiple sexual exposures of both husband and participants.[24,25] In this study, it was found that, unlike healthy individuals, patients with cervical cancer and precancer failed to undergo regular screening procedures. This indicates that patients who did not undergo regular screening had a higher risk of developing cancer.

The result of this study showed that the levels of IL-6 and IL-1β in patients with cervical cancer increased significantly compared with patients with cervical precancer and HPV DNA–negative healthy participants (P=.000) as well as these also significantly increased in patients with cervical precancer than healthy individuals (IL-6, P=.000; IL-1β, P=.001; Figure 1). Bonin-Jacob et al[7] and Vahedpoor et al[26] also found that the levels of IL-6 were also significantly raised in HPV DNA–positive patients as well as patients with cervical cancer and precancer. With the growth and development of HPV infection, IL-6 levels increase substantially without autoinhibition. Consequently, infected cells continue to release large quantities of IL-6, preventing tumor cell death and promoting uncontrolled atypical cell growth.[13] According to Long et al,[27] IL-1β was also found to be significantly elevated in different stages of cervical precancer. In CIN with HPV infection, increased levels of IL-1β expressing keratinocytes and HR-HPV stimulate the release of IL-1β from keratinocytes, which leads to the proliferation of immortal and cervical epithelial cells, damage to their DNA, and

eventually facilitates the integration of HPV DNA and the development of E6 and E7 oncoproteins, accelerating the rate of cervical cancer development.[28]

In the present study, IL-10 level was significantly raised in patients with cervical cancer and precancer in comparison with healthy participants ($P = .000$) (Figure 2). A study conducted by Ali et al[29] observed higher levels of IL-10 in cervical HPV infection. Other studies also observed increased IL-10 expression in HPV-induced CIN progression.[7,27] On the contrary, significant differences in IL-10 levels were not found between the groups in some studies.[30-32] Interleukin-10 is a cytokine produced by T-helper type-2 (Th-2) cells that inhibits cell-mediated immunity. Interleukin-10 inhibits the activation of CTLs and NK cells and hence reduces the immune response against malignancies. Its increased expression in cervical HPV-transformed tumor cells may suppress the generation of Th-1 cytokines, thus contributing to the development of cancer.[33]

The findings of this study show that IL-8 was significantly increased in patients with cervical cancer and precancer compared with healthy individuals and also significantly raised in patients with cancer than patients with precancer ($P = .000$) (Figure 3). Iwata et al[31] also found that significantly high levels of IL-8 were associated with neoplastic progression in different CIN stages. Furthermore, he emphasized that squamous epithelium and inflammatory cells produce more IL-8 when CIN progresses to cancer. This ultimately promotes malignancy via neovascularization, VEGF production, and prevention of cancer cell apoptosis. In this study, MCP-1 levels were raised significantly in patients with cancer more than in healthy participants ($P = .004$). The levels of MCP-1 were also significantly higher in patients with precancer than in healthy individuals ($P = .001$) (Figure 3). Otani et al[32] similarly observed that notably higher levels of MCP-1 were associated with disease severity. The CCL2/CCR2 axis stimulates E6 and E7 proteins, which enhances proteolysis of the P^{53} protein and inactivates the retinoblastoma gene, which causes cervical cancer.[34]

The present study did not observe any significant relationship between the levels of TNF-α, IFN-γ, and GM-CSF with disease severity. However, due to the study involving only a limited number of participants, it cannot be confidently stated that these cytokines do not contribute to disease.

A persistent inflammatory condition, precancerous lesion, and cervical cancer stimulate the immune system and release several cytokines. Consequently, the current study observed that the mean levels of IL-6, IL-1β, IL-10, and IL-8 were elevated in patients with cervical cancer with increasing age (Figures 4 and 5). It is essential to find out whether the local microenvironment is influenced by age and age-related alterations in the immune system, which may facilitate the development of cancer. The proinflammatory response of older patients with prostate cancer is more intensive, as observed by Begley et al.[35] This suggests that aging might affect both systemic and local immunological responses in a manner that promotes tumor development.

Conclusions

The levels of IL-6, IL-1β, IL-10, IL-8, and MCP-1 were all markedly increased with the severity of cervical infection by HR-HPV. Patients with cancer and precancer had considerably higher levels of IL-6, IL-1β, IL-10, IL-8, and MCP-1 than healthy participants. Moreover, IL-6, IL-1β, IL-10, and IL-8 levels were significantly higher in patients with cancer than in patients with precancer. Therefore, the outcome of this study suggests that any of these cytokines may serve as potential biomarkers for diagnostic purposes and may also be established as cervical cancer prognostic indicators, as well as enlightening the clinical and public health challenges posed by cervical cancer and introducing new insights into cytokine signaling in disease progression. This research, concentrating on the particular immune mediators associated with disease progression for the first time in Bangladesh, may lead to a path of immunomodulation.

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Data Availability Statement

The data supporting the findings of the study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participants

This study was approved by the Ethics Committee of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. In this study, precaution was taken to protect the confidentiality of the participants. This research protocol was approved by the Institutional Review Board (IRB) of BSMMU, Dhaka (BSMMU/2022/7159). All patients provided written informed consent before participating in the study, which was conducted in accordance with the principles of the Declaration of Helsinki. Obtaining written informed consent was a challenge in this study due to the prevailing culture where a majority of the subjects lacked literacy skills, as well as the sensitive nature of requesting thumbprints or

signatures. Participants were assured that data obtained from the study was used only for research purposes. Patients were not confronted with any physical, psychological, or social risks throughout this research. Participant's basic human rights were not violated in any way.

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