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ORIGINAL ARTICLE

# *BHLHE22* Expression as a Potential Diagnostic and Prognostic Biomarker in Cervical Cancer

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## Abstract

**Background/Aim:** Cervical cancer is a prevalent and deadly form of gynaecological malignancy, particularly among developing countries. *BHLHE22* is a transcription factor that functions as a transcriptional inhibitor and is significant in cancer-related regulatory mechanisms. In cervical cancer, epigenetic alterations transpire, notably in the *BHLHE22* gene, which influences gene and protein expression. The objective of this research was to examine the protein and gene expression of *BHLHE22* in tissue from cervix cancer in comparison to those found in normal cervix, to investigate its association with various clinical profiles and prognoses and to assess the association of *BHLHE22* gene expression with tumour-infiltrating immune cells.

**Methods:** This research employed a cross-sectional analytical study and retrospective cohort analyses. The expression of *BHLHE22* protein in tissue from cervix cancer and normal cervix was analysed using immunohistochemistry (IHC) examinations. The level of *BHLHE22* gene expression, clinical profiles and prognosis were collected from the GTEx and TCGA studies. The association of *BHLHE22* expression and tumour-infiltrating immune cells has been investigated using the Pearson correlation test or Spearman's rank correlation test from the TIMER database.

**Results:** *BHLHE22* protein and mRNA expression in cervical cancer decreased considerably compared to the normal cervix, with protein expression reduced by 1.5-fold and mRNA expression reduced by 3.6-fold, respectively. High levels of *BHLHE22* expression were determined to be linked with favourable disease-specific survival. Furthermore, *BHLHE22* expression positively correlated with tumour-infiltrating immune cells with antitumour immunity.

**Conclusion:** *BHLHE22* expression as a biomarker for early detection a prognostic biomarker for disease-specific survival and a modulator of the immune microenvironment in cervical cancer might be suggested.

**Key word:** *BHLHE22* protein; *BHLHE22* gene; Uterine cervical neoplasms; Immunity; Cells; Prognosis; Biomarkers.

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## Introduction

Cervical cancer emerges as one of the four most prevalent neoplasms among women. The Global Cancer Observatory (GLOBOCAN) of the WHO estimates that there were 662,301 new cases globally in 2022, with a fatality rate of 52 %. Asia exhibits the most significant incidence and mor-

tality rates. Cervical cancer counts among the two most commonly diagnosed malignancies in women in Indonesia, with 36,964 new cases reported and a fatality rate of 56 %.[1, 2] This highlights the urgency for improved prevention, diagnosis and treatment strategies.

The accessibility of the cervix allows for practical clinical assessment, enabling prevention and early diagnosis of cervical cancer.[3] A significant tumour size in stage III or higher cervical cancer might shorten life expectancy.[4] Cervical cancer screening has evolved from traditional cytology to include *Human papillomavirus* (HPV) testing, which offers improved sensitivity but lacks specificity.[5] To address this, the methylation of DNA biomarkers is starting to appear as a potential triage tool for women who are HPV-positive, offering comparable performance to cytology with the added benefits of convenience and cost-effectiveness.[6]

Despite advancements in screening and therapeutic intervention for cervical cancer, the management of advanced-stage cervical cancer continues to pose challenges due to medication resistance and the lack of precise and dependable prognostic indicators. Resistance to chemotherapy, radiation and chemoradiotherapy can lead to treatment failure, diminishing the survival rate at one year to below 30 % in individuals with advanced or recurrent illness.[7] The challenges of therapy at this advanced stage highlight the necessity to the discovery of new predictive biomarkers for cervical cancer to anticipate patient response to therapy and outcomes.

A number of studies indicate that cancer is now both a genetic and an epigenetic condition. The methylation of DNA is a form of epigenetic control that governs gene expression.[8] *Basic Helix-Loop-Helix E22 (BHLHE22)* is a transcription factor belonging to the BHLH family. *BHLHE22* acts as a transcriptional inhibitor and has a role in neuronal differentiation throughout neurodevelopment. *BHLHE22* is classified as an intron-less gene, essential for regulating processes associated with specific nerve functions and cancer, neuropathy and disease progression. Several BHLH family genes, such as the *ASCL1* gene (eg, in gliomas and neuroblastomas), the *Olig2* gene (eg, in astrocytomas) and the *ATOH1* gene (eg, in medulloblastomas), have been recognised as cancer biomarkers.[9] Based on this finding, *BHLHE22* may hold a similar potential.

*BHLHE22* may influence the composition of the tumour's immune landscape. Tumour-infiltrating immune cells (TIICs) are pivotal in the tumour microenvironment, influencing the development of malignancy and patient outcomes. TIIC exhibits heterogeneity and flexibility, which can either inhibit or promote tumour development.[10] Understanding the make-up and density of TIIC, along with tumour mutation load and immune checkpoint inhibitor scores, helps reveal the immunological landscape of cancer, which makes it easier to design personalised treatments.[11]

In a study by Huang et al in 2017, DNA methylation was examined in cervical scrapings to identify endometrial cancer. The study revealed that out of the 14 genes that showed increased methylation in endometrial cancer, the *BHLHE22* gene was among the top three genes with the most effective performance.[12] In their 2019 study, Liew et al utilised cervical scrapings to identify endometrial cancer. They employed a mix of methylated DNA of genes and genetic changes as biomarkers. The findings showed that the methylation profile of the *BHLHE22* gene successfully recognised both subtypes of endometrial cancer, type I and type II.[13] Darmawi et al conducted a study employing immunohistochemistry (IHC) to examine the *BHLHE22* gene expression in 54 normal endometrial tissues and cases of endometrial cancer. The findings indicated a significant decline in *BHLHE22* gene expression in endometrial cancer tissue relative to normal tissue.[9]

Multiple studies have used cervical scrapings to analyse DNA methylation on the *BHLHE22* gene for the purpose of detecting endometrial cancer. These studies suggest that examining *BHLHE22* protein and gene expression in normal and cervical cancer tissue may also be helpful in detecting cervical cancer. Furthermore, it can help determine the association between *BHLHE22* protein and gene expression in cervical cancer and various clinical characteristics, such as stage, grading and prognosis of patients diagnosed with cervical cancer. This research also investigated the *BHLHE22* function within the immunological microenvironment of cervical cancer.

## Methods

This research employed a cross-sectional analytical study to examine the protein and gene ex-

pression of *BHLHE22* in normal cervical tissues in comparison to those found in cervical cancer tissues, the association of the *BHLHE22* gene expression with cancer stage and grading, as well as TIIC. A retrospective cohort approach was utilised to examine the correlation between *BHLHE22* gene expression and cervical cancer prognosis.

## Clinical samples

IHC examinations were performed using formalin-fixed paraffin-embedded (FFPE) tissue to compare the protein expression of *BHLHE22* in cervical cancer and normal cervical tissue. Thirty-eight samples (each cancer tissue and normal cervix) from formalin-fixed paraffin-embedded tissues from patients diagnosed with cervical cancer, confirmed through histopathological examination, as well as normal cervical FFPE tissues, were collected from the Laboratory of Pathological Anatomy Arifin Achmad General Hospital in Riau Province, Indonesia, during the 2023-2024 period, based on a confidence level of 90 %, a precision of 10 % and a proportion of 16.8 %.[1] Ethical Review Board for Medicine and Health Research, Faculty of Medicine, Universitas Riau, has granted ethical approval for this research. All subjects provided informed consent.

## Data source

Clinical profiles and *BHLHE22* expressions from patients with cervical cancer were gathered from *The Cancer Genome Atlas* (TCGA), a platform that hosts a comprehensive genomic database. The data was obtained by analysing the gene expression of *BHLHE22* and examined with the Illumina HiSeq 2000 RNA Sequencing platform. The analysis was conducted through the website *https://xenabrowser.net*. The TCGA target GTEx database provided the expression data of the *BHLHE22* gene in both normal cervical tissue and cervical tumour tissue. The patient clinical information and the *BHLHE22* gene expression were collected from the TCGA Cervical Cancer (CESC) database.[14] The research data was retrieved on September 2024.

## Exploring the TIMER database

The infiltration of immune cells associated with *BHLHE22* expression was obtained from the Tumour Immune Estimation Resource (TIMER), accessed September 2024. These immune cells include B cells, CD4+, CD8+, macrophage polarisation, neutrophils and myeloid dendritic cells. The estimation component on TIMER was utilised, which estimated the number of TIICs compared to the expression pattern of the genes employing the cellular composition estimation method.[15]

## Statistical analysis

A total of 101 clinical samples of cervical tissue was obtained and 314 samples from the CESC and GTEx database. The numerical data of the protein and gene expression of *BHLHE22* were analysed using the Mann-Whitney test to find the difference in *BHLHE22* protein and gene expression between normal and cervical cancer tissue. We also obtained 303 samples from the TCGA CESC to find the association of the *BHLHE22* gene expression with clinical characteristics. All clinical characteristics were transformed into categorical data and the *BHLHE22* gene expression was categorised into high levels and low levels according to the mean value. The chi-square test was then applied to evaluate the data and performed a Kaplan-Meier survival analysis. The correlation among *BHLHE22* gene expression and TIICs was analysed in the TIMER datasets utilising Pearson's rank correlation test for data distributed normally and Spearman's correlation test for data distributed abnormally. The data were subjected to statistical analysis using SPSS 25.0 and a p-value < 0.05 was considered statistically significant.

# Results

## The expression of the *BHLHE22* in cervical cancer showed a significant reduction compared to normal tissue

The expression of the *BHLHE22* protein was investigated in 53 tissues from cervix cancer and 48 normal cervixes using IHC. The expression of the *BHLHE22* protein was measured employing the IHC H-score. The H-score was obtained by combining the proportion of stained cells by the strength values of the staining, which range from 0 for "negative" to 3 for "high", with a maximum achievable value of 300. The statistical analysis indicated a significant reduction (1.5-fold) in *BHLHE22* protein expression in tissue from cervix cancer relative to normal cervix (Figure 1a-c).

In line with laboratory investigation findings, the

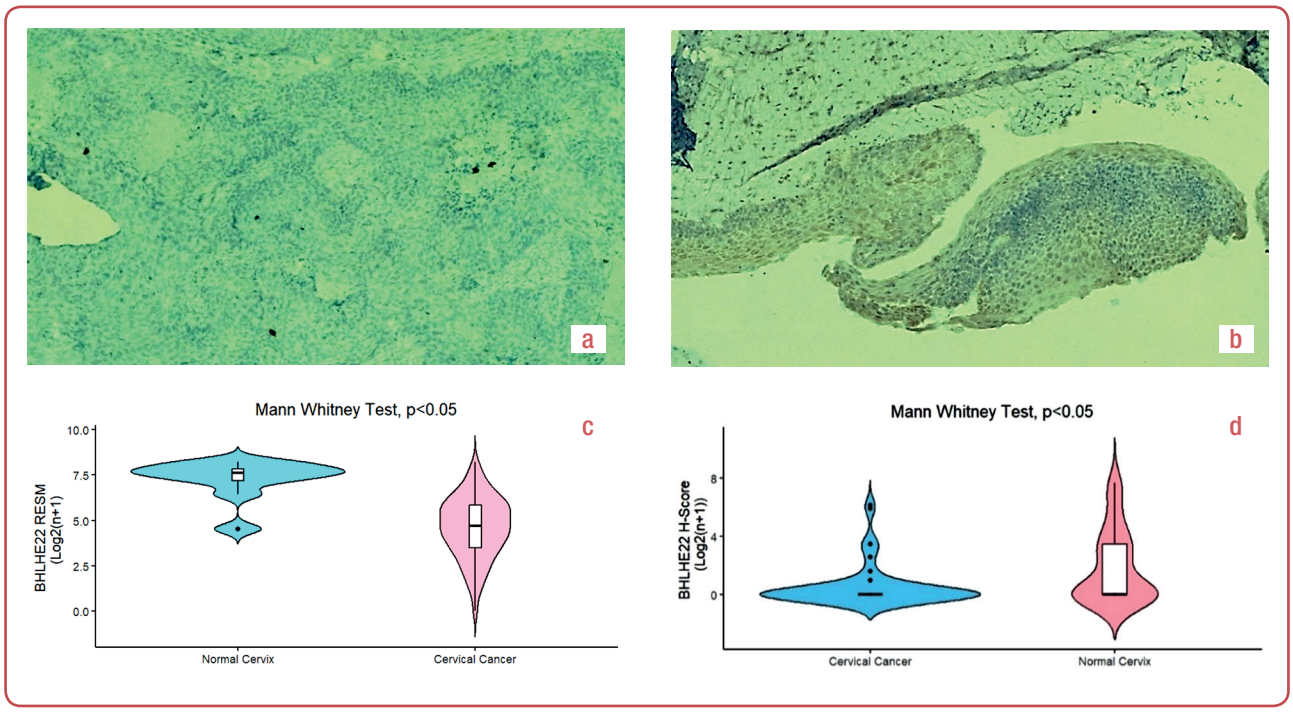


*Figure 1: The expression of the Basic Helix-Loop-Helix E22 (BHLHE22) in tissue from cervix cancer shows a significant reduction compared to normal cervix.*

*(a, b): The protein expressions of BHLHE22 in tissue from cervix cancer (b) and normal cervix (a) were assessed through immunohistochemistry (IHC) analysis; (c): The protein expression of BHLHE22 between tissue from cervix cancer and normal cervix is shown and the differences were tested using the Mann-Whitney test; (d): The BHLHE22 gene expression of tissue from cervix cancer (TCGA) compared to normal cervix (GTEx) was analysed, with differences assessed using the Mann-Whitney test;*

statistical analysis of *BHLHE22* gene expression data derived from RNA-seq acquired from the TCGA CESC and TGEX databases indicated a significant reduction (3.6-fold) in *BHLHE22* gene expression in tissues from cervix cancer in comparison to normal tissues (Figure 1d).

## Association between *BHLHE22* expression and the clinical characteristics of cervical cancer

There were 303 samples from patients diagnosed with cervical cancer, derived from the TCGA CESC database and Table 1 shows the findings of the descriptive analysis. Cervical cancer most commonly affects the reproductive age group, specifically individuals under the age of 49, accounting for 180 patients (59.4 %). Out of the total number of patients, 181 (59.7 %) had early-stage cancer, which was a higher proportion compared to those with late-stage cancer. The grading system classified the majority of patients as G2, which consists of 134 individuals, or 44.2 % of the population. The majority of cervical cancer patients survived during the study period, reflected by an overall survival (OS) probability of 76.2 % and a disease-specific survival (DSS) probability of 80.5 %. The investigation's disease-specific survival data revealed that 54 patients, representing 17.8 % of the sample, succumbed to cervical cancer. The majority of patients did not undergo any stage progression. Specifically, 232 patients (76.6 %) and 148 patients (48.8 %) did not encounter a recurrence of cancer during the study period.

The expression of the *BHLHE22* gene showed no significant association with the age, stage, or grade of patients suffering from cervical cancer. The expression of the *BHLHE22* gene did not show any statistically significant association with the age, stage, or grade of cervical cancer patients. OS and DSS prognosis showed that more patients with high *BHLHE22* gene expression were alive, respectively, 123 (53.2 %) and 134 (54.9 %) patients. According to the prognosis of the progression-free interval, patients who did not experience progression had a higher frequency of high *BHLHE22* gene expression, namely 124 (53.4 %) patients. The disease-free interval prognosis reveals that a more significant proportion of patients (52.0 %) with low *BHLHE22* gene expression continue to be cancer-free.

*Table 1: Clinical profile and distribution of Basic Helix-Loop-Helix E22 (BHLHE22) gene expression in cervical cancer*

| Clinical profile | n (%) | *BHLHE22* gene expression | | p-value |
|---|---|---|---|---|
| | | High n (%) | Low n (%) | |
| **Age (years)** | | | | |
| ≤ 49 | 180 (59.4) | 90 (50.0) | 90 (50.0) | 0.855 |
| 50-60 | 64 (21.1) | 34 (53.1) | 30 (46.9) | |
| ≥ 61 | 58 (19.1) | 31 (53.4) | 27 (46.6) | |
| **Stage** | | | | |
| Early stage | 181 (59.7) | 89 (49.2) | 92 (50.8) | 0.308 |
| Late stage | 114 (37.6) | 63 (55.3) | 51 (44.7) | |
| **Grading** | | | | |
| G1 | 18 (5.9) | 10 (55.6) | 8 (44.4) | 0.337 |
| G2 | 134 (44.2) | 73 (54.5) | 61 (45.5) | |
| G3 | 118 (38.9) | 50 (49.2) | 60 (50.8) | |
| GX | 23 (7.6) | 8 (34.8) | 15 (65.2) | |
| **Overall survival (OS)** | | | | |
| Alive | 231 (76.2) | 123 (53.2) | 108 (46.8) | 0.212 |
| Not alive | 71 (23.4) | 32 (45.1) | 39 (54.9) | |
| **Disease specific survival (DSS)** | | | | |
| Alive | 244 (80.5) | 134 (54.9 ) | 110 (45.1) | 0.024 |
| Not alive | 54 (17.8) | 20 (37.1) | 34 (62.9 ) | |
| **Progression free interval (PFI)** | | | | |
| Not progress | 232 (76.6) | 124 (53.4 ) | 108 (46.56) | 0.248 |
| Progress | 70 (23.1) | 31 (44.3) | 39 (55.7) | |
| **Disease free interval (DFI)** | | | | |
| Not recurrence | 148 (48.8) | 77 (52.0) | 71 (48.0) | 0.495 |
| Recurrence | 26 (8.6) | 15 (57.7) | 11 (42.3) | |

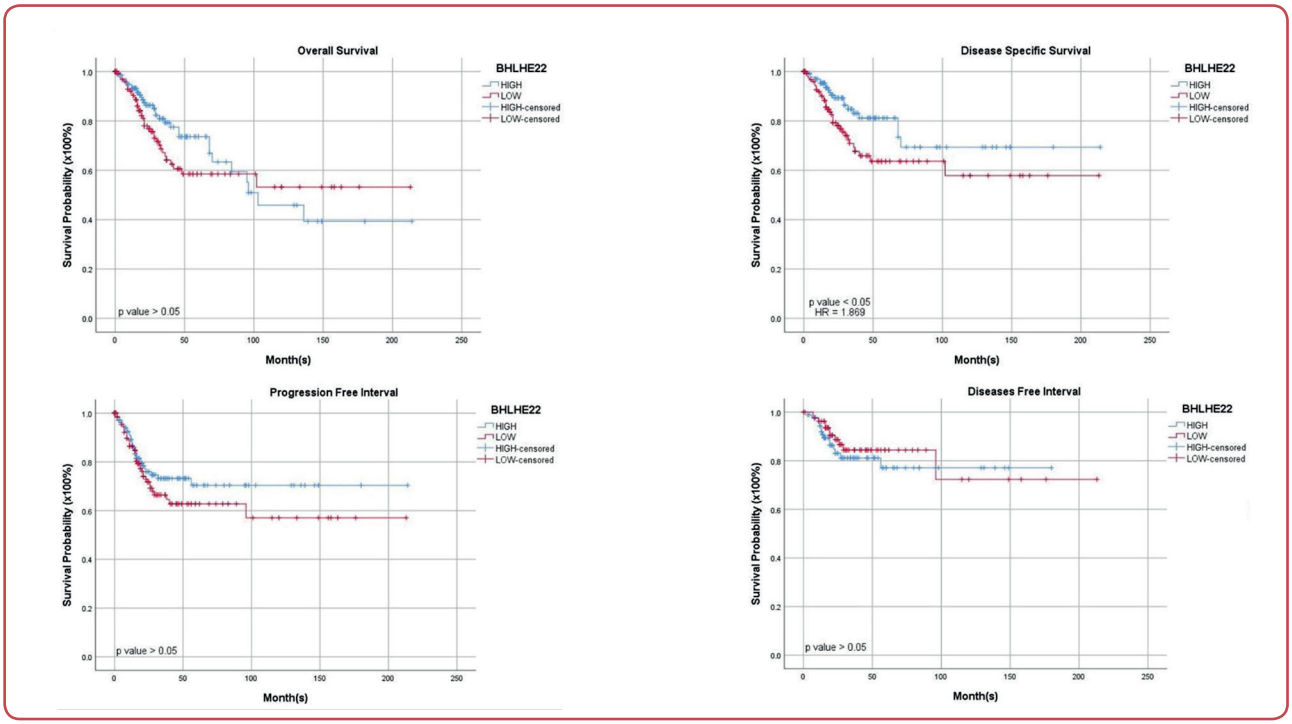


*Figure 2: Kaplan Meier survival analysis.*

*The high Basic Helix-Loop-Helix E22 (BHLHE22) patients (red line) showed higher disease-specific survival probability than low BHLHE22 patients. The median expression of BHLHE22 was used as the threshold. the Mann-Whitney test;*

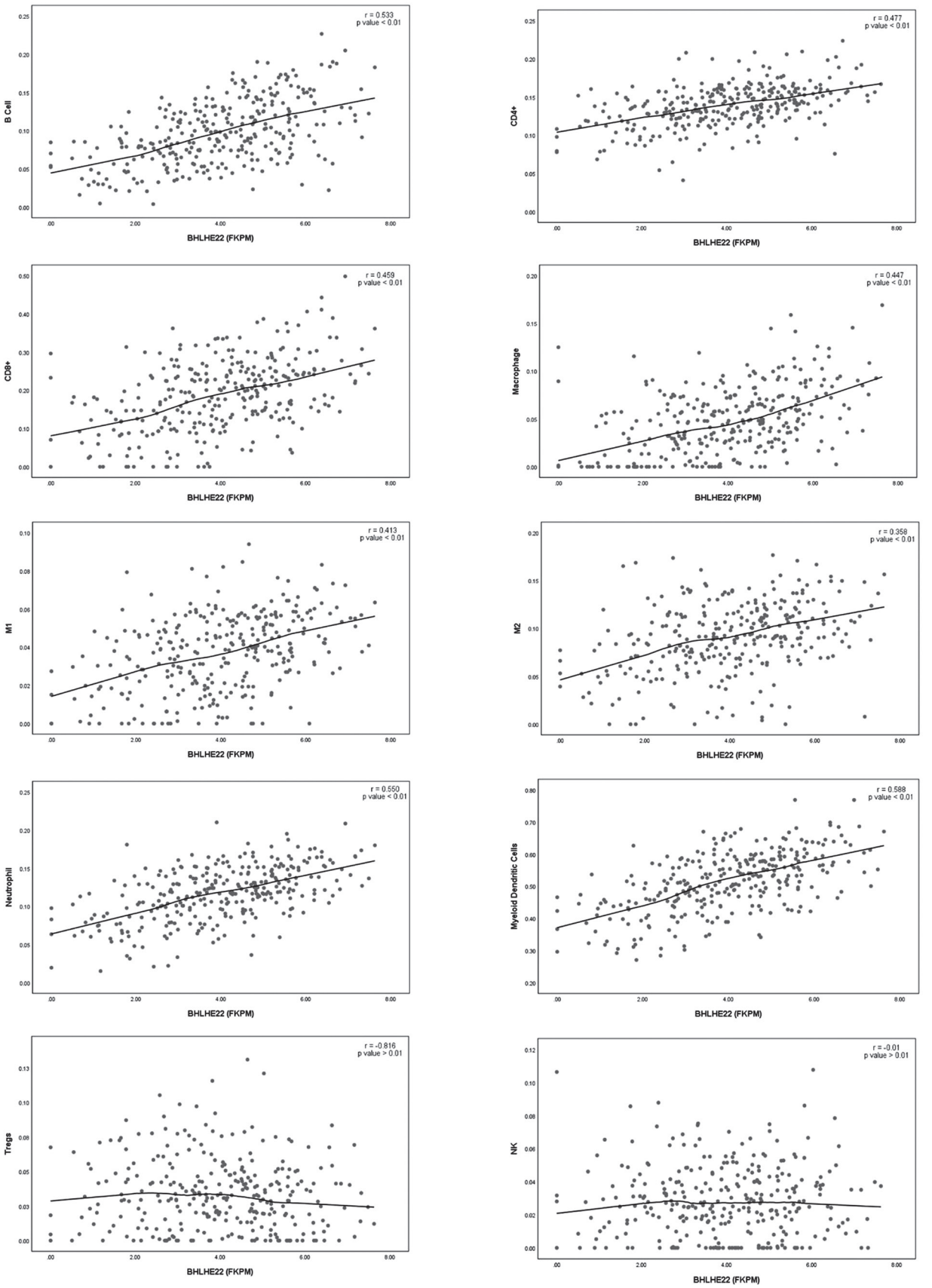


*Figure 3: The expression level of Basic Helix-Loop-Helix E22 (BHLHE22) is linked with tumour-infiltrating immune cells (TIICs) that possess antitumour immunity in tissue from cervix cancer.*

### Association between *BHLHE22* gene expression and prognosis of cervical cancer patients

The results of the analysis regarding patients' prognosis based on *BHLHE22* gene expression are shown in Figure 2. According to the chart, there is no significant difference between the OS, PFI and DFI among cervical cancer patients with varying levels of *BHLHE22* gene expression. In contrast, high expression of *BHLHE22* conferred significant favourable DSS with a hazard ratio of 1.869, suggesting that patients with low gene expression had disease-specific survival that is 0.869 times shorter than patients with high gene expression. The five-year DSS probabilities were 81 % for patients with high *BHLHE22* levels and 64 % for those with low levels.

### Correlation between *BHLHE22* gene expression and TIICs

TIICs correlation with the level of expressions of the *BHLHE22* gene was analysed in the TCGA CESC database using TIMER. This tool predicts and quantifies the amount of TIIC based on gene expression patterns. The expression of *BHLHE22* demonstrated a markedly favourable link with immune cells infiltrated in the tumour microenvironment of cervical cancers, encompassing B cells, CD8+, CD4+, myeloid dendritic cells, macrophages, M1 macrophages, M2 macrophages and neutrophils (Figure 3).

## Discussion

This study demonstrated that the *BHLHE22* protein and gene expression in cervical cancer tissue was significantly reduced compared to normal cervical tissue. The decreased expression of the *BHLHE22* in cervical cancer tissue is associated with gene methylation, which leads to a decrease in its expression. The function of the *BHLHE22* gene functions is a transcriptional inhibitor. It has multiple target genes and the essential domain is essential in the positive and negative regulation of these genes.[16] Liew et al conducted a study to investigate the effectiveness of gene methylation status in diagnosing endometrial cancer using cervical scraping. The study revealed that the *BHLHE22* gene significantly exhibited hypermethylation in endometrial cancer, in contrast to normal endometrial tissue.[13]

According to the descriptive analysis presented in Table 1, it showed that the age group that was most commonly affected by cervical cancer was women of reproductive age, namely those under the age of 49. HPV is a prevalent virus that is transmitted via sexual contact and can result in multiple malignancies, particularly cervical cancer. Women of reproductive age who participate in frequent sexual activity face an increased risk of being infected with HPV.[17]

Screening and early diagnosis are highly effective in preventing cervical cancer, making it one of the most preventable malignancies in women. An extensive understanding of cervical cancer and a conscious effort to conduct screening tests can significantly decrease mortality rates associated with cervical cancer. Patients diagnosed at an early stage will have a more favourable prognosis compared to patients diagnosed at a late stage.[18] Consistent with the findings of this study, the majority of patients were at an early stage and based on OS and DSS probability, the majority of participants were still alive during the study period.

Furthermore, presented study found there were no significant differences in *BHLHE22* gene expression associated with the histological grade of cervical cancer. Nevertheless, the data suggests that the majority of patients in G3 had lower *BHLHE22* gene expression levels. This is in contrast to a study by Darmawi et al on endometrial cancer, which found a significant decrease in *BHLHE22* expression and a significant association to the malignancy's grade.[9]

This study showed that the *BHLHE22* expression in tissues from cervix cancer did not correlate significantly with the patients' clinical stage. However, a pattern was observed indicating that the majority of patients in the late stages had higher *BHLHE22* gene expression. This may suggest fluctuations in *BHLHE22* expression at various stages; nevertheless, it lacks sufficient strength to establish a substantial association between the gene's expression and cancer stage. The results are different from what Yin et al found, which said that advanced metastatic prostate cancer had higher levels of *BHLHE22*.[19] The data indicate that *BHLHE22* expression may have diverse functions in various cancer types. More research is needed to understand the full link between *BHLHE22* expression and cancer stages.

The examination of *BHLHE22* expression and OS

probability in patients with cervical cancer revealed an insignificant disparity in OS probability between individuals with low and high levels of *BHLHE22* expression. In contrast to what Darmawi et al found in their study on endometrial cancer, it was shown that high levels of *BHLHE22* were linked to a significant enhancement in OS among endometrial cancer patients.[9] However, in presented study, the analysis of the 5-year survival probability of patients with cervical cancer reveals that those with high *BHLHE22* gene expression had better outcomes compared to those with low *BHLHE22* gene expression.

The DSS analysis of cervical cancer patients based on the level of the *BHLHE22* gene indicated a considerable disparity in the disease-specific survival rates between those who had low levels of the *BHLHE22* gene expression and those who had high levels. Cervical cancer patients with higher gene expression showed a superior 5-year survival rate compared to those with lower gene expression, in line with the study conducted by Darmawi et al, which focused on the *BHLHE22* gene in endometrial cancer and revealed its significant involvement in strengthening immune system signalling pathways. *BHLHE22* specifically enhances pathways, including IL2-STAT5 signalling, activation of the complement system, rejection reactions to allografts, responses to inflammation and IL-6 and JAKSTAT3 signalling. In addition, a linkage is present between immunological checkpoints such as cytotoxic T-lymphocyte antigen 4 and programmed death-ligand 1 with *BHLHE22* expression.[9] The research results suggest that immune-modulatory factors significantly influence endometrial cancer and other cancer forms, including cervical cancer. Specifically, the increased expression of the *BHLHE22* gene in cancer patients leads to enhanced immune system activity, which helps defend the cancer and potentially improves the patient's life expectancy.

The PFI probability in cervical cancer patients, based on the level of the *BHLHE22* gene in this study, revealed no significant difference between patients with low and high levels of *BHLHE22* gene expression. Patients with cervical cancer who exhibit low gene expression within 5 years have a 63 % probability of not progressing. In contrast, those with high gene expression within 5 years have a 70 % probability of not progressing. Darmawi et al conducted a study on endometrial cancer and found that upregulating the expression of the *BHLHE22* gene significantly improves the patient's PFS probability. Patients with high *BHLHE22* gene expression have a 77 % 5-year progression-free survival (PFS) rate, compared to 69 % for those with low *BHLHE22* gene expression.[9] Moreover, the disease-free interval in cervical cancer patients, focusing on the *BHLHE22* gene level in this study, revealed no significant difference between patients with low and high levels of *BHLHE22* gene expression. According to the observed trend over 5 years, patients exhibiting low gene expression demonstrate a superior likelihood of remaining cancer-free compared to those with high gene expression. However, a decade of careful monitoring has determined that the probability of cervical cancer patients with low gene expression remaining cancer-free is lower compared to those with high gene expression. Several factors influence cervical cancer recurrence and prognosis. Deep stromal invasion correlates with higher recurrence rates and poorer survival outcomes.[20] Tumour size, lymphovascular space invasion and parametrial involvement are also significant prognostic factors.[21] Ramirez et al conducted a study that links the minimally invasive radical hysterectomy treatment to a reduced disease-free interval in patients with early-stage cervical cancer, compared to the abdominal radical hysterectomy.[22]

The positive and significant correlation between *BHLHE22* and B cells (Rho = 0.553), CD4+ (T cell helper) (Rho = 0.477) and CD8+ (cytotoxic T cells) (Rho = 0.459) suggests that *BHLHE22* may be involved in modulating responses of acquired immunity in patients with cervical cancer. The response of acquired immunity is vital in the development and prognosis of cervical cancer. Research indicates that infiltrating lymphocytes, specifically CD3+, T helper cells and cytotoxic T cells, decreased in lesions of cervix tissues but increased in invasive malignancies in comparison with normal epithelium.[23] Higher levels of TIICs are associated with favourable DSS in cervical squamous cell carcinoma.[24]

The relatively strong correlation between *BHLHE22* and myeloid dendritic cells (Rho = 0.588) also hints at the possibility that this gene influences antigen presentation and T cell activation, which are crucial components of the anti-tumour immune response. Dendritic cells (DCs) play a vital role in initiating and sustaining immunity of anti-tumour mediated by T-cells, particularly in cervical cancer, where immune evasion is

common. DCs are key sentinels linking innate and adaptive immunity and their presence and function within the tumour microenvironment can influence the efficacy of various cancer therapies.[25, 26]

The correlation with macrophages, particularly M1 macrophages (Rho = 0.413), supports the hypothesis that *BHLHE22* may contribute to pro-inflammatory immune activity, which is critical for tumour eradication. Previous research has shown that macrophage polarisation towards an M1 phenotype can enhance anti-tumour responses, while M2 macrophages are often associated with immunosuppression and tumour progression.[27, 28] In this study, the lower correlation with M2 macrophages (Rho = 0.358) further suggests that *BHLHE22* may be more involved in promoting inflammatory rather than suppressive immune mechanisms within the tumour microenvironment. An interesting lack of association between *BHLHE22* expression and either Tregs (p = 0.138, Rho = -0.86) or natural killer cells (p = 0.980, Rho = -0.01) was found, which suggests that the gene may not have a big effect on controlling immune-suppressing pathways in this setting.

Research has shown that *BHLHE22* influences tumour-associated immune cells by affecting their differentiation and function, particularly in tumours where immune evasion mechanisms are critical. *BHLHE22* contributes to an immunosuppressive bone tumour microenvironment in prostate cancer by facilitating the infiltration of immunosuppressive neutrophils and monocytes. In endometrial cancer, elevated *BHLHE22* expression correlates with a proinflammatory immune microenvironment and an improved prognosis.[9]

## Conclusion

Considering the results of this study, both protein and gene expression of the *BHLHE22* significantly decreased in tissue cervix cancer compared to normal cervix. Consequently, the expression of the *BHLHE22* can serve as a biomarker of early detection, as epigenetic alterations in cancer tend to occur at a faster rate than genetic changes. Furthermore, there were significant differences in DSS based on the *BHLHE22* expression. Hence, evaluating the expression of the *BHLHE22* gene could be a biomarker for predicting the outcome of cervical cancer patients. *BHLHE22* has positively correlated with tumour-infiltrating immune cells that possess anti-tumour immunity and modulate the immune microenvironment in cervical cancer. Furthermore, we can use it as a benchmark to improve cervical cancer treatment strategies.

## Ethics

Ethical approval for this study was obtained from the Ethical Review Board for Medicine and Health Research, Faculty of Medicine, Universitas Riau. Approval decision number: B / 080 / UN19.5.1.1.8/UEPKK/2024, dated 26 July 2024. Written informed consent was obtained from all participants where applicable.

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## Conflicts of interest

The authors declare that there is no conflict of interest.

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## Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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