



Dynamic expression of ITGB1 in peripheral blood: a potential biomarker for noninvasive diagnosis of squamous intraepithelial lesion and cervical cancer

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ABSTRACT

Objective: Integrin $\beta 1$ (ITGB1) plays a role in cancer progression; however, its potential as a blood-based biomarker in cervical carcinogenesis remains unclear. This study investigated the expression level of ITGB1 in the peripheral blood of patients with cervical cancer and precancerous lesions to evaluate its potential as a non-invasive biomarker.

Methods: Multi-platform analyses, including bioinformatic screening (GEO/TCGA) and experimental validation, were conducted using clinical samples from the control, squamous intraepithelial lesion (SIL), and cervical cancer (CC) groups. Upregulation of ITGB1 expression across different sample types was thoroughly examined using several analytical methods.

Results: The results showed that ITGB1 expression was significantly increased during progression of cervical diseases in all sample types (all $P < 0.05$). High ITGB1 levels were associated with poor prognosis, with its expression predominantly localized to the membrane of peripheral blood mononuclear cells (PBMCs). Significant differences in human papillomavirus (HPV) status and inflammatory markers (Percentage of neutrophils, %NEUT; Percentage of lymphocytes, %LYMPH) were also observed among the groups.

Conclusions: Overall, ITGB1 has potential as a reliable non-invasive biomarker for the early detection and monitoring of cervical cancer and its precancerous stages, showing consistent overexpression and strong clinical significance.

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Introduction

Cervical cancer is a highly aggressive malignancy and ranks as the fourth most common cancer among women worldwide, with an estimated 660,000 new cases reported in 2022 according to the latest report by the WHO [1]. It remains one of the major causes of cancer-related deaths in women globally [2]. Cervical cancer development generally follows a multistep process that begins with normal cervical epithelial tissue and progresses to squamous intraepithelial lesion (SIL), which may eventually progress to invasive cervical cancer. Cervical cancer is one of the few human malignancies with a clearly defined etiology, namely persistent infection with oncogenic human papillomavirus (HPV) genotypes, making it largely preventable through effective primary and

secondary preventive measures [3]. SIL is a well-recognized precancerous stage that is closely associated with the onset of cervical carcinoma [4]. Therefore, early detection and appropriate clinical management of SIL are essential for preventing progression to invasive cervical cancer, improving patient outcomes, and reducing the overall disease burden.

Cervical precancerous lesions involve abnormal growth and differentiation of the cervical epithelium and carry a risk of progressing to invasive cervical cancer. SIL is classified into low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL), which differ notably in their cytological characteristics, underlying causes, clinical significance, and recommended treatment approaches (Table S1).

Histopathological analysis of biopsy specimens remains the gold standard for diagnosing SIL. Cervical tissue samples are typically examined using hematoxylin and eosin (H&E) staining to assess morphological features. However, relying solely on H&E staining can be challenging due to factors such as inter-observer variability among pathologists, differences in section thickness, and suboptimal tissue processing or preservation. Peripheral blood has emerged as an easily accessible and minimally invasive source for molecular diagnostics. Unlike tissue biopsies, which provide only a single, static view and may not capture tumor heterogeneity or dynamic microenvironmental changes, peripheral blood enables repeated sampling over time. This allows for longitudinal monitoring of disease progression or treatment response, improves patient compliance, and highlights the potential of liquid biopsy as a non-invasive diagnostic approach [5]. Therefore, integrating highly specific biomarkers alongside histopathological evaluation is essential for improving the accuracy, reproducibility, and consistency of SIL diagnoses.

Integrins are a superfamily of transmembrane glycoprotein receptors that play a key role in cell adhesion. They consist of 18 distinct α -subunits and 8 β -subunits, which combine to form 24 unique heterodimeric receptors [6]. Integrins, particularly Integrin $\beta 1$ (ITGB1), are broadly expressed on the surface of most leukocytes, including T lymphocytes, monocytes, and granulocytes. ITGB1 interacts with extracellular matrix proteins, such as fibronectin, enabling leukocytes to recognize and adhere to vascular endothelial cells, which supports their transmigration through the endothelium and movement from the bloodstream into tissues [7]. ITGB1 is a key regulator of epithelial-mesenchymal transition (EMT) and influences essential cellular processes such as adhesion, proliferation, and apoptosis. It also contributes to tumor development and metastatic spread. Due to its diverse roles in cancer progression, ITGB1 has gained attention as a potential prognostic biomarker and a therapeutic target in multiple types of malignancies [8, 9]. The potential of ITGB1 as a biomarker for the early detection of SIL and cervical cancer has not been fully investigated. This study aimed to examine the relationship between ITGB1 expression levels in peripheral blood and the severity of cervical lesions, ranging from SIL to invasive cancer. By quantitatively measuring ITGB1 in patient blood samples, this study seeks to evaluate its clinical value as a novel non-invasive biomarker and to provide a basis for

early detection and precision medicine in cervical carcinogenesis.

Materials and Methods

GEO database analysis

The gene expression dataset GSE63514 was obtained from the Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/>) by searching with the keyword “cervical cancer” and filtering the results for relevant datasets. This dataset was selected because it included histologically confirmed samples of normal cervical epithelium ($n = 24$), cervical intraepithelial neoplasia ($n = 76$), and cervical squamous cell carcinoma ($n = 28$); contained complete clinical annotations; and showed no significant batch effects. Differential gene expression analysis was performed using the GEO2R online tool, which applies the Limma package in R, with statistical significance defined as a $P < 0.05$ and an $|\text{FC}| > 1.2$. Data visualization was performed using the ggplot2 and pheatmap packages in R. Volcano plots were created to illustrate global transcriptomic changes, and heatmaps with hierarchical clustering were used to display the expression patterns of significantly dysregulated genes. This study used only publicly available, pre-deidentified datasets; therefore, no additional ethical approval was required.

GEPIA2 database analysis

The Gene Expression Profiling Interactive Analysis 2.0 (GEPIA2) platform (<http://gepia2.cancer-pku.cn/>) was employed to analyze RNA sequencing data obtained from the Cancer Genome Atlas Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (TCGA-CESC) project ($n = 306$) and normal cervical tissue controls from the Genotype-Tissue Expression (GTEx) database ($n = 13$). Differential expression analysis focused on ITGB1 and related family members, including ITGB2, ITGB5, ITGB6, and ITGB8. For the survival analysis, patients were divided into high- and low-expression groups based on the median expression level (50% cutoff). Overall survival (OS) and disease-free survival (DFS) outcomes were subsequently analyzed. Cox proportional hazards models were used to calculate hazard ratios (HRs) and corresponding 95% confidence intervals, with survival duration measured in months. All statistical analyses were conducted using a two-sided significance threshold of $\alpha = 0.05$. To visualize the results, Kaplan–Meier curves and survival maps were generated.

Study cohort and grouping criteria

This study enrolled patients who were pathologically diagnosed at the Second Affiliated Hospital of Shandong First Medical University, including individuals with cervicitis, SIL, and cervical cancer. The control group (CON) comprised patients with cervicitis but no evidence of SIL or cervical cancer. The SIL group (SIL) included cases of both low-grade and high-grade SIL, whereas the cervical cancer group (CC) consisted of patients diagnosed with clinically staged I–IIA cervical squamous cell carcinoma. All pathological diagnoses were verified by certified and experienced pathologists. This study was approved by the Institutional Ethics Committee of the Second Affiliated Hospital of Shandong First Medical University (approval No. 2022-016). All participants provided written informed consent before enrollment.

Inclusion Criteria: Women aged 25–75 years who provided written informed consent; had complete clinical and pathological records; were diagnosed with cervicitis (without SIL or cervical cancer), SIL, or stage I–IIA cervical cancer; had not received chemotherapy or radiotherapy within three months before diagnosis; had no history of other malignancies such as ovarian or breast cancer; and possessed basic literacy, full consciousness, and effective communication ability.

Exclusion Criteria: Participants were excluded if they had systemic or autoimmune disorders; a history of other malignancies; were pregnant or breastfeeding; had incomplete clinical data; or were unable or unwilling to comply with the study requirements.

Immunohistochemistry (IHC) staining

Cervical epithelial tissue samples from the control, SIL, and cervical cancer groups were fixed in 4% neutral-buffered formalin for 24 h, followed by dehydration through graded ethanol, clearing in xylene, and paraffin embedding. Serial sections (4 μ m thick) were prepared, mounted on slides, deparaffinized, and rehydrated. Antigen retrieval was conducted, and endogenous peroxidase activity was quenched using 3% H₂O₂. The sections were then blocked with 1% Bovine Serum Albumin (BSA) and incubated overnight at 4°C with an anti-ITGB1 primary antibody (1:50 dilution, Proteintech, 12594-1-AP, USA). After washing, a horseradish peroxidase-conjugated secondary antibody (1:100, CWBIO, CW0103, China) was applied, and antigen-antibody binding was visualized using 3,3'-Diaminobenzidine (DAB). Finally, the sections were counterstained with hematoxylin, de-

hydrated, and mounted for examination. Tumor and adjacent normal regions were identified on H&E-stained serial sections by two board-certified pathologists. Tumor cells were defined by typical malignant squamous features, including invasive nests, marked nuclear atypia, hyperchromasia, pleomorphism, and keratin pearl formation. Adjacent normal tissues were collected at least 2 cm from the tumor margin and were histologically free of tumor. The corresponding regions on ITGB1 Immunohistochemistry sections were then selected for imaging and quantification.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum samples from the control, SIL, and cervical cancer groups were assessed for ITGB1 levels using a human ITGB1 ELISA kit (Bioswamp, HM11454, China). The standards were serially diluted following the manufacturer's instructions, and both samples and standards were added to the appropriate wells. The detection antibody and enzyme conjugate were applied after incubation and washing. The color development was achieved using a chromogenic substrate, and the reaction was stopped. The absorbance was measured at 450 nm, using the blank well as a reference. A standard curve was constructed using four-parameter logistic regression ($R^2 > 0.98$), and the concentrations of ITGB1 in the samples were determined by interpolation from the curve.

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood samples were collected in heparinized tubes and processed within 2 h of collection. Each sample was diluted 1:1 with Phosphate-Buffered Saline (PBS) and gently layered onto a human lymphocyte separation medium (tbdscience, LTS1077-1, China). Following centrifugation at 2000 rpm for 30 min with slow acceleration and deceleration, the mononuclear cell layer was carefully extracted, washed twice with PBS, and resuspended in serum-free Roswell Park Memorial Institute 1640 Medium. Cells were incubated at 37 °C with 5% CO₂ for 2 h to promote adhesion. Adherent PBMCs were then cultured in RPMI-1640 supplemented with 10% FBS and 1% penicillin–streptomycin under the same conditions.

Western blotting

PBMCs isolated from the control, SIL, and cervical cancer groups were cultured for 24 h. After incubation, the culture supernatant was removed, and the cells were gently washed with cold PBS to remove

residual medium. Radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, China) supplemented with phenylmethylsulfonyl fluoride (PMSF) (Beyotime, China) at a 1:100 ratio was used to extract total protein. Protein samples were separated on a 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel (ncmbio, P2011, China) and electrophoretically transferred to Polyvinylidene Fluoride (PVDF) membranes. Membranes were blocked with 5% non-fat milk for 1 h at room temperature, then incubated overnight at 4 °C with gentle shaking with primary antibodies against ITGB1 (1:2000, Proteintech, 12594-1-AP, USA) and beta-actin (1:5000, Proteintech, 66009-1-Ig, USA). After washing, the membranes were incubated with HRP-conjugated secondary antibodies: goat anti-mouse IgG (1:8000, CWBIO, CW0102, China) and goat anti-rabbit IgG (1:8000, CWBIO, CW0103, China) for 1 h at room temperature. Protein bands were detected using an enhanced chemiluminescence (ECL) kit (Beyotime, China) on a chemiluminescence imaging system (Tanon, China), and band intensities were quantified by densitometry using ImageJ software.

Immunofluorescence staining

PBMCs derived from the cervical cancer group were plated in pre-equilibrated confocal culture dishes and cultured until reaching 80% confluence. Cells were then fixed with 4% paraformaldehyde (Biotopped, Top0382, China) for 30 min, permeabilized with 0.3% Triton X-100 for 10 min at room temperature, and subsequently blocked with 5% BSA at 37 °C for 30 min. After blocking, the samples were incubated overnight at 4 °C with a primary anti-ITGB1 antibody (1:50, Proteintech, 12594-1-AP, USA), followed by incubation with an FITC-conjugated goat anti-rabbit secondary antibody (1:50, Solarbio, SF134, China) for 2 h at 37 °C in the dark. Nuclei were counterstained with 4',6-Diamidino-2-Phenylindole (DAPI). A 40× objective on an inverted fluorescence microscope was used to perform imaging. For each sample, three-channel images (DAPI, FITC, and merged) were captured from at least three independent fields.

Statistical analysis

All statistical analyses were conducted using Graph-Pad Prism (version 8.0) and SPSS Statistics (version 27.0). Continuous data are presented as mean ± standard deviation (SD). Comparisons between groups were performed using a paired two-tailed Student's *t*-test or one-way analysis of variance (ANOVA) as appropriate. Categorical data are reported as frequen-

cies and percentages, and group differences were assessed using the chi-square test. All experiments were performed in triplicate. A *P* value of less than 0.05 was considered statistically significant compared with the control group.

Result

Progressive overexpression of ITGB1 during cervical carcinogenesis

ITGB1 expression was significantly higher in the SIL and cervical cancer groups than in the control group (FC = 1.22, *P* < 0.001, **Figure 1A, 1D**; FC = 3.56, *P* < 0.01, **Figure 1C, 1F**), demonstrating a consistent increase from normal cervical epithelium to SIL and further to cervical cancer. Furthermore, *ITGB1* levels were higher in cervical cancer than in SIL (FC = 5.73, *P* < 0.001) (**Figure 1B, 1E**), indicating that *ITGB1* overexpression occurs early in malignant transformation and continues to increase with disease progression. This progressive upregulation indicates that *ITGB1* may play a crucial role in promoting tumor development.

High ITGB1 expression is associated with poor prognosis in cervical cancer

Survival analysis using TCGA data revealed that high *ITGB1* expression was significantly associated with poorer outcomes in patients with cervical cancer. In the DFS analysis, patients with high *ITGB1* expression had shorter survival times than those in the low-expression group (log-rank *p* = 0.027, HR = 1.9, *p*(HR) = 0.03) (**Figure 1G**). The impact was even more pronounced for OS, where high *ITGB1* expression strongly predicted worse prognosis (log-rank *p* = 0.0074, HR = 1.9, *p*(HR) = 0.0084) (**Figure 1I**). Each group included 146 patients.

A pan-cancer prognostic heatmap of the integrin gene family (*ITGB1*, *ITGB2*, *ITGB5*, *ITGB6*, and *ITGB8*) was generated to evaluate the relationship between gene expression and survival across various cancer types. The results indicated that high *ITGB1* expression was associated with poorer prognosis (red) in cervical cancer (CESC), whereas its prognostic impact varied in other cancers, showing either favorable (blue) or unfavorable (red) effects (**Figure 1H, 1J**). Among all integrin subunits examined, *ITGB1* exhibited the most consistent and significant correlation with adverse survival outcomes in cervical cancer. Overall, these findings indicate that *ITGB1* expression is a potential prognostic biomarker in cervical cancer, correlating with reduced DFS and OS.

Figure 1. Bioinformatic analysis of ITGB1 expression and prognostic significance during cervical disease progression.

Analysis based on the GEO database:

(A–C) Heatmaps of differentially expressed genes between groups. Rows represent genes and columns represent samples. The red and blue shades denote high and low expression, respectively. (A) Normal vs. SIL. (B) SIL vs. cervical cancer. (C) Normal vs. cervical cancer.

(D–F) Volcano plots of differentially expressed genes. Red and blue dots indicate significantly up- and down-regulated genes, respectively ($P < 0.05$, $|\text{FC}| > 1.2$); gray dots represent non-significant genes. (D) Normal vs. SIL. (E) SIL vs. cervical cancer. (F) Normal vs. cervical cancer.

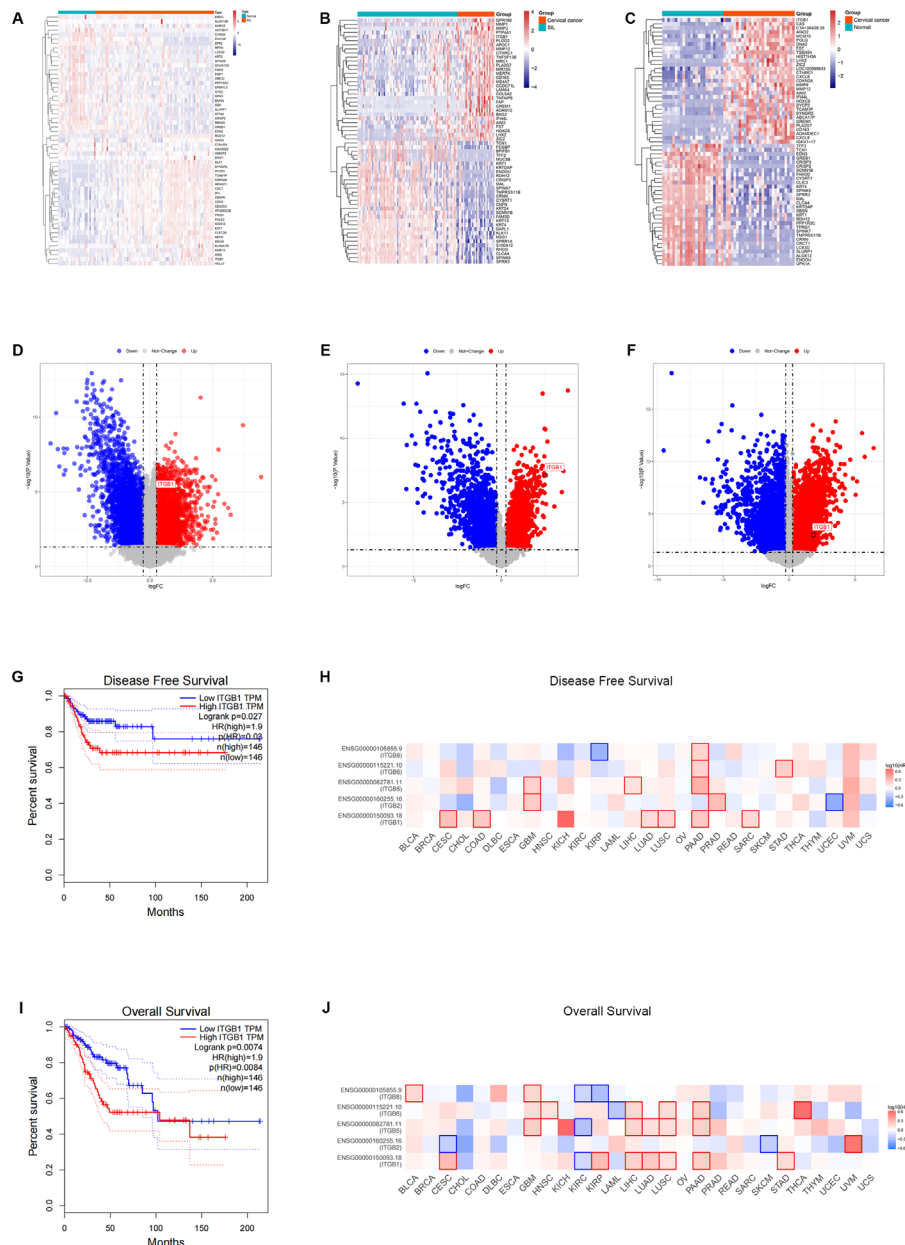
A P value less than 0.05 was considered statistically significant.

Analysis based on the GEPIA database:

(G, I) Kaplan–Meier survival curves of patients with cervical cancer stratified by ITGB1 expression levels. Log-rank p -values and hazard ratios (HR) are indicated.

(H, J) Prognostic heatmap of ITGB1 and integrin family genes (ITGB2, ITGB5, ITGB6, and ITGB8) across multiple cancer types. Rows: genes; columns: cancer types. Red indicates that high expression correlates with poor prognosis; blue indicates a protective effect. The color intensity reflects the strength of the correlation.

A two-sided significance level of $\alpha = 0.05$ was applied to all statistical tests.



ITGB1 expression is significantly upregulated in cervical cancer tissues

To investigate the expression pattern of ITGB1 in cervical carcinoma, IHC staining was performed on six paired samples of tumor and adjacent non-tumor tissues. Quantitative analysis showed that ITGB1 expression was significantly higher in tumor tissues than in adjacent normal tissues ($P < 0.001$) (**Figure 2A, B**). These findings indicate that ITGB1 is strongly upregulated during the progression of cervical cancer, suggesting its potential role in promoting tumor development and contributing to the pathogenesis of this malignancy.

Elevated ITGB1 expression in serum and PBMCs correlates with cervical disease progression

ITGB1 expression was quantitatively evaluated in serum and PBMCs from patients in the control, SIL, and cervical cancer groups. A consistent increase in ITGB1 levels was observed across disease stages in both sample types. In serum samples, ITGB1 levels gradually increased from the control group to the SIL group ($P < 0.05$) and reached the highest levels in the cervical cancer group ($P < 0.001$). Significant differences were also observed between the SIL and cervical cancer groups ($P < 0.001$) (**Figure 2C**).

Similarly, Western blotting analysis of PBMCs showed that ITGB1 protein levels were significantly higher in the SIL group than in the control group ($P < 0.05$), with a further pronounced increase in the cervical cancer group ($P < 0.001$). The expression levels in the cervical cancer group were also significantly higher than those in the SIL group ($P < 0.01$) (**Figure**

2D). These findings indicate that ITGB1 expression steadily increases in serum and PBMCs as cervical disease progresses, suggesting its potential as a bio-marker associated with cervical carcinogenesis.

Univariate analysis of clinical characteristics across the control, SIL, and cervical cancer groups

A univariate analysis was conducted on clinical data from the control ($n = 8$), SIL ($n = 31$), and cervical cancer ($n = 28$) groups, examining HPV infection status, obstetric history, and complete blood count (CBC) parameters.

The HPV infection status significantly differed among the groups ($\chi^2 = 37.908$, $P < 0.001$). The proportion of HPV-positive cases was 89.3% in the cervical cancer group and 93.5% in the SIL group, both of which were substantially higher than the control group. In contrast, 100.0% of the control group were HPV-negative (**Table 1**). These findings indicate a high prevalence of HPV infection in patients with cervical cancer or SIL, supporting the well-established role of HPV in cervical carcinogenesis.

Analysis of CBC parameters showed that the percentages of neutrophils (%NEUT) and lymphocytes (%LYMPH) varied significantly among the three groups ($P < 0.05$), suggesting a possible relationship between these inflammatory markers and the progression of cervical lesions (**Table 2**).

Subcellular localization of ITGB1 in PBMCs from patients with cervical cancer

Immunofluorescence analysis of PBMCs from patients with cervical cancer showed that ITGB1, de-

Table 1. Comparison of HPV infection status and obstetric characteristics among study cohorts

Classification		Group [n, (%)]			Total (n=67)	χ^2	P
		CON (n=8)	SIL (n=31)	CC (n=28)			
Number of pregnancies	≤ 2	4(50.0%)	14(45.2%)	14(50.0%)	32(47.8%)	0.156	0.925
	> 2	4(50.0%)	17(54.8%)	14(50.0%)	35(52.2%)		
Abortion	Yes	5(62.5%)	25(80.6%)	21(75.0%)	51(76.1%)	1.185	0.553
	No	3(37.5%)	6(19.4%)	7(25.0%)	16(23.9%)		
Number of full-term births	≤ 2	8(100.0%)	29(93.5%)	27(96.4%)	64(95.5%)	0.711	0.701
	> 2	0(0.0%)	2(6.5%)	1(3.6%)	3(4.5%)		
Premature labor	Yes	8(100.0%)	26(83.9%)	25(89.3%)	59(88.1%)	1.642	0.440
	No	0(0.0%)	5(16.1%)	3(10.7%)	8(11.9%)		
HPV infection	Yes	0(0.0%)	29(93.5%)	25(89.3%)	54(80.6%)	37.908	0.000***
	No	8(100.0%)	2(6.5%)	3(10.7%)	13(19.4%)		

Data are presented as frequencies and percentages. Differences between groups were determined using the chi-square test. A P value less than 0.05 was considered statistically significant when compared to the controls. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2. ITGB1 expression in cervical tissues, serum, and PBMCs.

(A) Representative images of ITGB1 immunohistochemical staining in cervical cancer tissues and adjacent non-tumor tissues (n = 6). The right panels show cervical squamous cell carcinoma tissue, and the left panels show adjacent normal cervical tissue from the same patient.

(B) Quantitative analysis of ITGB1 expression levels based on IHC staining intensity.

(C) ITGB1 concentration in serum samples from control (n = 7), SIL (n = 9), and cervical cancer (n = 8) groups, as determined by ELISA.

(D) Western blotting analysis of ITGB1 protein expression in PBMCs from the control (n = 11), SIL (n = 11), and cervical cancer (n = 10) groups, with corresponding quantitative densitometry.

Data are presented as mean $\pm$ SD. Differences between groups were determined using a paired two-tailed Student's t-test or one-way ANOVA. The data shown are representative of three biologically independent experiments unless otherwise stated. A P value less than 0.05 was considered statistically significant when compared to the controls. *P < 0.05, **P < 0.01, ***P < 0.001.

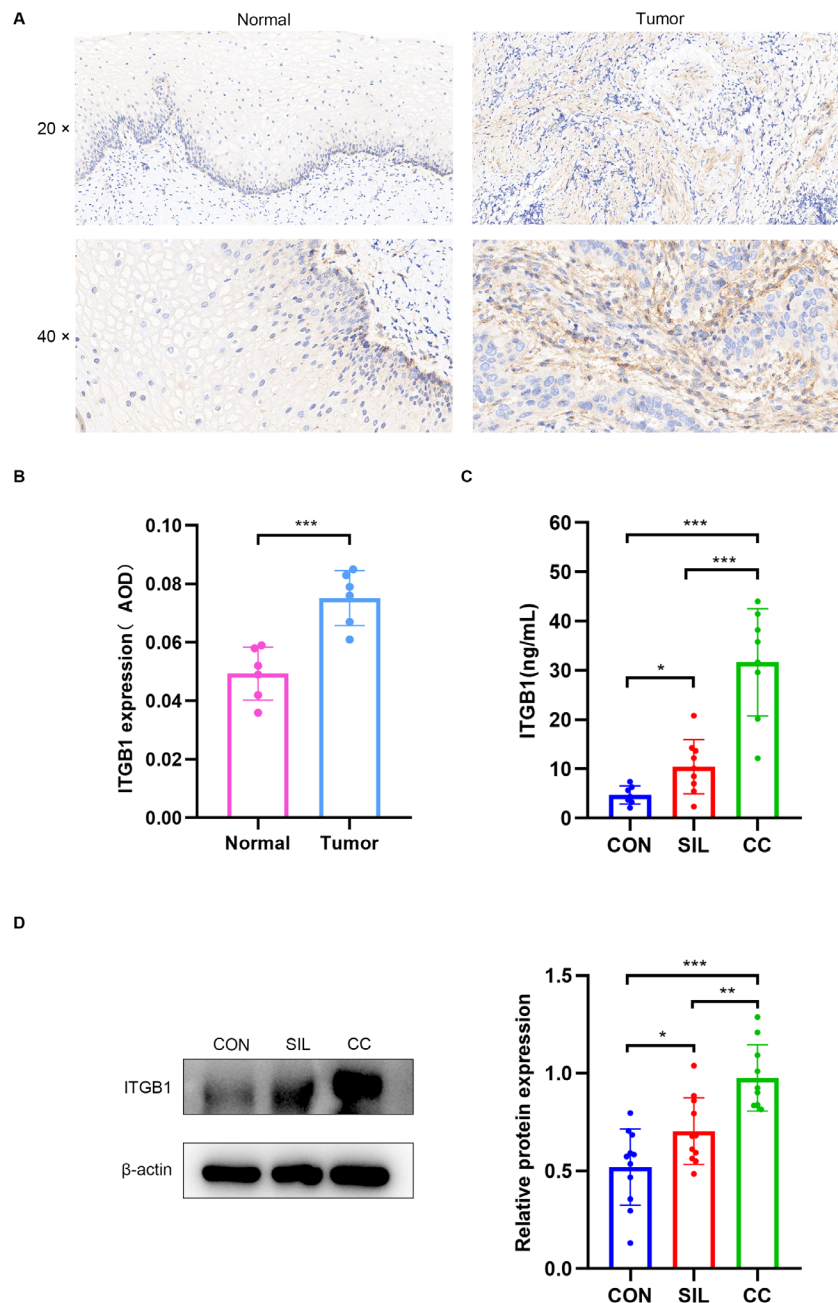


Table 2. Comparison of CBC among the study cohorts

Classification		Group (mean $\pm$ SD)			F	P
		CON (n=8)	SIL (n=31)	CC (n=28)		
WBC	$10^9/L$	5.69 $\pm$ 1.63	5.35 $\pm$ 1.72	6.18 $\pm$ 1.80	1.643	0.201
RBC	$10^{12}/L$	4.53 $\pm$ 0.47	4.35 $\pm$ 0.34	4.25 $\pm$ 0.37	1.960	0.149
HGB	g/L	125.50 $\pm$ 18.05	127.19 $\pm$ 15.59	117.68 $\pm$ 18.51	2.360	0.103
HCT	%	0.39 $\pm$ 0.05	0.39 $\pm$ 0.04	0.36 $\pm$ 0.05	3.017	0.056
PLT	$10^9/L$	297.00 $\pm$ 80.89	264.71 $\pm$ 59.52	275.32 $\pm$ 88.87	0.603	0.550
%NEUT	%	65.48 $\pm$ 11.00	59.21 $\pm$ 9.25	66.47 $\pm$ 10.89	4.026	0.023*
%LYMPH	%	26.54 $\pm$ 8.77	32.91 $\pm$ 8.28	26.48 $\pm$ 9.63	4.304	0.018*
MCV	fL	85.26 $\pm$ 7.96	88.30 $\pm$ 7.20	84.40 $\pm$ 7.19	2.202	0.119
MCH	pg	27.83 $\pm$ 3.49	29.24 $\pm$ 2.98	27.65 $\pm$ 3.20	2.061	0.136
MCHC	g/L	324.75 $\pm$ 12.58	330.32 $\pm$ 11.67	326.71 $\pm$ 13.86	0.914	0.406
RDW	%	13.95 $\pm$ 3.03	12.91 $\pm$ 1.69	13.81 $\pm$ 3.59	0.942	0.395
PDW	%	13.95 $\pm$ 2.63	13.76 $\pm$ 2.75	14.98 $\pm$ 2.25	1.776	0.178

Data are presented as mean $\pm$ SD. Differences between groups were determined using one-way ANOVA. A *P* value less than 0.05 was considered statistically significant when compared to the controls. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

tected with green fluorescence, was mainly distributed in the plasma membrane and cytoplasm. A distinct ring-like fluorescence signal around the cell periphery indicated that ITGB1 was strongly localized on the plasma membrane (**Figure S1**). These findings confirm that ITGB1 is primarily expressed on the cytomembrane of PBMCs.

Discussion

Cervical cancer continues to pose a significant health threat to women worldwide. Although China has implemented the “Two Cancers” screening program [10, 11] and the health initiative “Accelerating the Elimination of Cervical Cancer by 2030” [12], the morbidity and mortality of cervical cancer continue to increase. Early detection, standardized treatment, and effective follow-up management remain crucial for improving the survival and prognosis of patients with cervical intraepithelial lesions and cervical cancer.

Infection with high-risk HPV is the main cause of cervical cancer. The development of HPV-related cervical cancer is closely associated with the functions of the viral early proteins, collectively referred to as the E protein family. These proteins are essential for the viral life cycle and contribute to host cell malignant transformation. Specifically, E1, E2, and E4 are primarily responsible for viral integration, replication, and transcriptional regulation, whereas E5, E6, and E7 function as oncoproteins that directly promote malignant transformation and contribute to cervical carcinogenesis [13]. In addition, the E proteins inter-

act with several critical cellular signaling pathways, playing a crucial role in malignant transformation and tumor progression. Pathways such as phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), wingless-related integration site (Wnt), and Notch are key regulators of cell proliferation, survival, differentiation, and metastatic potential. By influencing these signaling networks, E proteins promote tumor cell survival and growth, thereby supporting tumor initiation and progression [14].

Despite advances in HPV vaccination and ongoing improvements in treatment strategies, which have improved the prevention and clinical management of cervical cancer [15], substantial challenges remain. Tumor heterogeneity and differences in biological behavior continue to pose metastasis and recurrence risks. Moreover, patients with advanced-stage disease often have a poor prognosis due to limited treatment options and the generally limited efficacy of radiotherapy and chemotherapy.

ITGB1 appears to play a central role in the development of cervical cancer. Growing evidence shows that ITGB1 is significantly upregulated in cervical cancer tissues, and its high expression is closely associated with increased tumor invasion, metastatic potential, and poor prognosis. These findings indicate that ITGB1 may act as a key mediator of HPV-driven oncogenesis, possibly through its role in extracellular matrix remodeling, cell adhesion, and activation of pro-oncogenic signaling pathways. Targeting ITGB1 represents a promising therapeutic strategy, particularly for aggressive or recurrent forms of cervical can-

cer [16]. Recent studies indicate that ITGB1 influences cervical cancer cell behavior, likely by modulating the PI3K/AKT signaling pathway. This pathway is a critical intracellular cascade that controls cell proliferation, survival, and apoptosis. Its dysregulation, often observed as constitutive activation in cervical cancer, may be a key mechanism through which ITGB1 drives tumor progression and maintains the malignant phenotype [17]. ITGB1 overexpression in cervical cancer promotes tumor growth and aggressiveness by continuously activating the PI3K/AKT signaling pathway, resulting in reduced apoptosis and increased cell proliferation [16]. When ITGB1 binds to extracellular matrix (ECM) ligands such as fibronectin, laminin, and collagen, it triggers downstream signal transduction by activating focal adhesion kinase (FAK). Autophosphorylation of FAK at key sites, including Tyr397, enables the recruitment and activation of phosphoinositide 3-kinase (PI3K). Activated PI3K catalyzes the production of phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which subsequently activates protein kinase B (AKT). Activation of AKT then drives multiple downstream processes that support cell survival, proliferation, and metabolic reprogramming [16, 18, 19]. ITGB1 also plays a key role in promoting the migration and invasion of cervical cancer cells. It regulates cytoskeletal dynamics and enhances cell motility, allowing cancer cells to penetrate the basement membrane, invade surrounding tissues and blood vessels, and ultimately establish distant metastasis. In addition, the expression and activity of ITGB1 are regulated post-transcriptionally by specific microRNAs in cervical carcinogenesis. For instance, miR-183-5p directly targets ITGB1, significantly reducing the migratory and invasive abilities of cervical cancer cells. Consistent with this regulatory correlation, ITGB1 expression is inversely associated with miR-183-5p levels in cervical cancer [20]. These observations highlight ITGB1 as a promising therapeutic target for the treatment of cervical cancer.

This study shows that evaluating ITGB1 expression in PBMCs and serum provides a non-invasive and repeatable approach for supporting the diagnosis and monitoring of cervical cancer and SIL. Blood-based biomarkers, including ITGB1, HPV antibodies, and inflammatory indicators, enable early detection, treatment response evaluation, and recurrence monitoring. When combined with traditional diagnostic methods, such as cytology and colposcopy, blood tests can improve diagnostic accuracy and inform

personalized treatment strategies. Incorporating ITGB1 detection into clinical practice could significantly enhance cervical cancer screening in high-risk populations, enable timely interventions, and ultimately improve patient outcomes.

This study uniquely emphasizes the precancerous stage of cervical cancer to enable early detection and intervention using blood-based biomarkers before the disease progresses to invasive carcinoma. Unlike previous studies that primarily focused on established cervical cancer, we examined dynamic changes in ITGB1 expression in PBMCs and serum across disease stages, finding a marked increase with disease severity. These results indicate that ITGB1 may play a crucial role in cervical carcinogenesis and could serve as a valuable biomarker for monitoring disease progression and guiding clinical decision-making. Moreover, to the best of our knowledge, this is the first study to systematically examine the relationship between ITGB1 expression and both SIL and cervical cancer, highlighting its potential as a novel biomarker for early diagnosis and risk stratification. Early detection and intervention at the precancerous stage are essential for timely treatments and reducing cervical cancer incidence. Additional blood parameters, such as %NEUT and %LYMPH, also differed significantly, supporting their potential role as auxiliary indicators in the preliminary screening of SIL and cervical cancer. As key components of the immune system, neutrophils and lymphocytes actively participate in disease pathogenesis, further supporting the clinical relevance of these measures.

In addition to monitoring ITGB1 expression in the peripheral blood, we explored its mechanistic role in cervical cancer at the cellular level. Immunofluorescence analysis showed that ITGB1 is primarily localized to the plasma membrane of PBMCs, with additional presence in the cytoplasm. Membrane localization suggests that ITGB1 may facilitate its role in cell-extracellular matrix adhesion and intercellular communication, whereas its cytoplasmic distribution may indicate involvement in intracellular signaling pathways and cytoskeletal remodeling. This dual localization also reflects ITGB1 recycling or stimulus-dependent redistribution. These findings provide valuable insights into the molecular functions of ITGB1 in cervical cancer pathogenesis and lay the foundation for further functional investigation.

This study has several limitations that should be considered. First, the sample collection was limited to Shandong Province, which may restrict the general-

izability of the findings. Regional differences in genetic background, environmental factors, or health-care practices could affect disease presentation and biomarker performance. Including multi-regional cohorts in future research would enhance the broader applicability of these results. Second, the overall sample size was relatively small, particularly within the SIL subgroup. Although consistent trends in ITGB1 upregulation with disease severity were observed, larger cohorts would enable more robust statistical analyses and subgroup comparisons, especially between LSIL and HSIL. Third, all data were obtained from human samples without *in vitro* functional validation. The challenge of obtaining normal cervical epithelial cells limited the direct comparison with cervical cancer cell lines (e.g., HeLa, SiHa). Future cell-based studies are necessary to clarify the precise mechanistic roles of ITGB1 in cervical carcinogenesis. Finally, because of the limited number of cases, LSIL and HSIL cases were combined for analysis. Although preliminary results indicated higher ITGB1 expression in HSIL than in LSIL, which is consistent with the overall trend, future studies with sufficient samples should separately analyze these groups to identify potential differences in ITGB1 expression and function across SIL subtypes.

Future research should focus on validating and expanding these findings through larger cohorts, multi-institutional collaborations, and detailed exploration of the cellular and molecular mechanisms underlying ITGB1-related pathways. These efforts will provide a more robust foundation for the early diagnosis, risk assessment, and therapeutic strategies for cervical cancer. Ultimately, this study could support ongoing improvements in the prevention and treatment of cervical cancer, contributing to better health outcomes for women globally.

Conclusions

The content of ITGB1 increases in different sample types as the severity of cervical lesions and the progression of cancer intensify. High ITGB1 predicts shorter disease-free and overall survival. ITGB1 correlates with HPV status and inflammatory markers.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of the Second Affiliated Hospital of Shandong First Medical University (approval No. 2022-016). All participants provided written informed consent before enrollment.

Data availability statement

The patient data used in this study were obtained from the Second Affiliated Hospital of Shandong First Medical University, but these data are restricted due to privacy and ethical considerations and are not publicly available. Relevant data can be accessed upon request by contacting the corresponding authors.

This study also utilized publicly available data from the GEO database and the GEPIA2 database, with dataset numbers GSE63514 and CESC. These datasets can be accessed through the following links:

GSE63514: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgiacc=GSE63514>

TCGA-CESC: <https://portal.gdc.cancer.gov/projects/TCGA-CESC>

Conflict of interest disclosure

The authors declare that there is no conflict of interest regarding this article.

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Authors' contributions

S.Z.Q performed data analysis and was a major contributor to writing the manuscript. G.M.H contributed to the analysis of the data, edited and revised the manuscript. Z.Z.K and L.G.H reviewed and made a critical edition of the manuscript. L.W.N and Z.Y.Y collected and diagnosed the clinical samples. Z.G.G and Z.Y statistically analyzed the clinical information of the subjects. L.C.H and Z.H contributed to the conception, design of the work, edited and revised the manuscript. All authors reviewed the manuscript.

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Conference presentation

Not applicable. All authors have read and approved the final version of the manuscript and agree to its submission to the *Serican Journal of Medicine*. All authors agree to be accountable for all aspects of the work.

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Table S1. The comparison of LSIL and HSIL

		LSIL	HSIL
Cytological characteristics			
Nuclear changes		Nuclei are mildly enlarged with uniformly distributed chromatin and generally regular nuclear membranes.	Marked nuclear enlargement, hyperchromasia with coarsely granular and irregularly distributed chromatin, irregular nuclear contours, and occasionally prominent nucleoli.
Cellular morphology		Cellular polarity is largely maintained with relatively organized cell arrangement. Mild cellular pleomorphism is generally confined to the lower third of the epithelium.	Loss of cellular polarity with significant disorganization. Moderate to severe pleomorphism involving from the lower two-thirds to the full thickness of the epithelium.
Cell size		Minimal change in cell size; slightly increased nuclear-to-cytoplasmic (N/C) ratio.	Marked variation in cell size with a significantly increased N/C ratio.
Predominant cell types		Mainly superficial or intermediate squamous cells.	Primarily parabasal or immature metaplastic cells, occasionally with abnormal keratinization.
Etiology		Commonly associated with transient low-risk HPV infection (e.g., types 6, 11).	Strongly linked to persistent high-risk HPV infection (e.g., types 16, 18, 31, 33), which carries a high potential for malignant transformation.
Risk of malignancy		Low; most cases regress spontaneously.	High; may progress to invasive carcinoma if left untreated.
Clinical management		Regular follow-up with cervical cytology and high-risk HPV testing every 6–12 months. Colposcopy may be considered if persistent or symptomatic.	Colposcopy with directed biopsy is recommended. Excisional or ablative treatment (e.g., LEEP, CKC) is often indicated.
Treatment approaches		Active surveillance is first-line; physical ablation (e.g., cryotherapy, laser) or medical therapy may be used in selected cases.	Surgical excision (e.g., LEEP, CKC) is standard; laser ablation or cryotherapy may be alternatives in specific scenarios.
Screening recommendations		Regular screening with cervical cytology and high-risk HPV co-testing.	Immediate colposcopic evaluation and biopsy are indicated following cytological diagnosis of HSIL.

Figure S1. Subcellular localization of ITGB1 in PBMCs from cervical cancer patients.

Nuclei were stained with DAPI (blue), and ITGB1 was detected using a FITC-conjugated antibody (green). The merged image demonstrates predominant membrane localization of ITGB1.

