ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 19, Issue 3, 2026

Online - 2455-3891
Print - 0974-2441

Research Article

ASSOCIATION OF SERUM HYPOXIA MARKERS (VASCULAR ENDOTHELIAL GROWTH FACTOR, CARBONIC ANHYDRASE IX, AND LACTATE) WITH HYPOXIA-INDUCIBLE FACTOR-1 ALPHA EXPRESSION IN CERVICAL CANCER TISSUES: POTENTIAL IMPLICATIONS FOR TREATMENT RESPONSE AND TARGETED THERAPY

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Received: 14 November 2025, Revised and Accepted: 15 January 2026

ABSTRACT

Objectives: To determine whether circulating serum hypoxia markers (vascular endothelial growth factor [VEGF], carbonic anhydrase IX [CA-IX], and Lactate) are associated with tumor hypoxia-inducible factor-1 alpha (HIF-1α) expression in cervical cancer, and to assess the possible utility of these biomarkers for therapy-oriented risk stratification.

Methods: In this observational study, patients with histopathologically confirmed cervical cancer were enrolled before starting any definitive treatment. Pre-treatment blood samples were analyzed for serum hypoxia markers (VEGF, CA-IX, and Lactate) using validated biochemical assays. HIF-1α expression in tumor tissue was evaluated by immunohistochemistry and graded semi-quantitatively. Relationships between serum marker levels and HIF-1α scores were examined using correlation testing, and comparisons were made across clinicopathological variables relevant to treatment planning.

Results: Patients showing strong HIF-1α expression had significantly higher serum hypoxia marker levels (VEGF, CA-IX, and Lactate) than those with low/absent expression (mean±standard deviation: 8.6±2.1 vs. 5.2±1.7 units; $p<0.01$). Serum marker levels correlated positively with HIF-1α expression scores ($r\approx0.58$; $p<0.001$). Elevated biomarker values were more common in advanced disease, with nearly two-thirds of stage III–IV cases demonstrating high HIF-1α expression. Overall, the pattern suggests that higher circulating hypoxia markers may reflect a hypoxia-driven, treatment-resistant tumor phenotype with potential implications for reduced radiotherapy and chemotherapy responsiveness.

Conclusion: Serum hypoxia markers (VEGF, CA-IX, and Lactate) show a significant association with tumor HIF-1α expression in cervical cancer and may act as minimally invasive indicators of a hypoxic microenvironment linked to therapeutic resistance. These biomarkers could aid risk stratification and support personalized treatment approaches, including consideration of treatment intensification and hypoxia-targeted strategies.

Keywords: Cervical cancer, Tumor hypoxia, HIF-1α, Serum hypoxia markers (VEGF, CA-IX, and Lactate), Radiotherapy, Chemoresistance, Biomarkers, Therapeutic stratification

 DOI: http://dx.doi.org/10.22159/ajpcr.2026v19i3.57656. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Cervical cancer remains a major public health concern, especially in low- and middle-income countries, where it continues to contribute substantially to cancer-related illness and deaths among women [1]. Although screening programs, HPV vaccination, and combined treatment approaches have improved overall care, many patients still report late, often with locally advanced disease, which generally responds less favorably and carries poorer survival outcomes [2]. Radiotherapy – frequently delivered with platinum-based chemotherapy – continues to be the backbone of treatment for most cases, yet resistance to these modalities remains a key barrier to sustained disease control [3].

A central biological factor underlying aggressive tumor behavior and reduced treatment responsiveness is tumor hypoxia. As solid tumors grow rapidly, oxygen delivery becomes inadequate because of disorganized vasculature and limited diffusion, creating pockets of low oxygen within the tumor mass [4]. In cervical cancer, hypoxia has repeatedly been linked with greater invasiveness, genomic instability, higher metastatic propensity, and diminished sensitivity to ionizing radiation and cytotoxic chemotherapy [5,6]. Importantly, hypoxia is not simply a by-product of tumor expansion; it actively triggers adaptive pathways that help malignant cells survive and progress in a hostile microenvironment [7].

Hypoxia-inducible factor-1 alpha (HIF-1α) is a key coordinator of this response. Under normoxic conditions, HIF-1α is rapidly degraded, but during low oxygen tension it becomes stabilized, translocates to the nucleus, and drives transcriptional programs that support tumor survival [8]. Across downstream gene regulation, HIF-1α influences angiogenesis, metabolic reprogramming, cell growth, immune escape, and resistance to apoptosis [9]. In cervical cancer, elevated HIF-1α expression has been associated with advanced stage, nodal involvement, weaker response to radiotherapy, and inferior survival outcomes [10-12]. These findings position HIF-1α as a critical molecular bridge between hypoxia and therapy resistance.

However, evaluating tumor hypoxia or HIF-1α expression commonly depends on tissue sampling, which is invasive and may not always be practical – particularly in advanced disease or in settings with limited access to specialized pathology services. In addition, hypoxia is spatially heterogeneous and temporally dynamic, so a single biopsy can miss hypoxic regions and introduce sampling bias [13]. These challenges have intensified interest in blood-based biomarkers that could serve as minimally invasive indicators of tumor hypoxia and its downstream biology [14].

Serum hypoxia-associated markers – such as selected metabolites, enzymes, and hypoxia-responsive proteins – have been proposed as potential surrogates. Evidence from several solid tumors suggests that raised levels of hypoxia-related circulating markers may correspond to underlying hypoxia and may track with adverse clinicopathological characteristics [15,16]. In cervical cancer specifically, early studies indicate possible links with stage, tumor burden, and outcomes, though findings remain inconsistent and limited by heterogeneity in marker panels and methods [17]. Demonstrating a robust relationship between circulating hypoxia markers (vascular endothelial growth factor [VEGF], carbonic anhydrase IX [CA-IX], and Lactate) and tissue HIF-1α expression would strengthen biological plausibility and support their clinical relevance.

From a treatment perspective, recognizing hypoxic, treatment-refractory tumors is clinically meaningful. Such patients may require intensified regimens, modified radiotherapy scheduling, hypoxia-altering approaches, or newer targeted therapies aimed at interrupting hypoxia signaling pathways [18-20]. If serum hypoxia markers (VEGF, CA-IX, and Lactate) can be incorporated into risk models, they may help clinicians plan therapy more precisely while reducing reliance on repeated invasive procedures.

In this context, the present study aimed to examine the association between circulating serum hypoxia markers (VEGF, CA-IX, and Lactate) and tumor HIF-1α expression in cervical cancer patients before initiation of therapy. By correlating serum marker levels with immunohistochemical HIF-1α scoring and relevant clinicopathological variables, the study explores whether minimally invasive biomarkers can help identify hypoxia-driven, potentially therapy-resistant tumor phenotypes and contribute to improved risk stratification and individualized treatment planning in cervical cancer [20].

METHODS

Study design, setting, and study period

This descriptive and analytical, hospital-based observational study was carried out jointly at two tertiary care teaching centers in Telangana: Kakatiya Medical College, Hanumakonda, and Government Medical College, Narsampet (Warangal district). Participant recruitment and sample collection were undertaken over 2 years, from January 2023 to December 2024. Both hospitals function as major referral facilities for surrounding districts and manage a substantial load of gynecological cancers, making them suitable settings to study biomarker–tissue relationships in routine cervical cancer care. The study procedures and reporting were aligned with established recommendations for observational research to improve transparency in participant selection, measurements, and analytical planning [21].

Participants and recruitment

Women attending outpatient or inpatient services with clinical suspicion of cervical malignancy were screened consecutively. Enrolment was done only after histopathological confirmation to ensure diagnostic accuracy. Only newly diagnosed, treatment-naïve cases were included, so that serum hypoxia markers (VEGF, CA-IX, and Lactate) and tumor HIF-1α expression represented baseline tumor biology rather than therapy-related effects. After confirming eligibility and obtaining consent, participants underwent standard evaluation and staging as per institutional practice. Pre-treatment blood was collected, and tumor tissue was processed for immunohistochemistry.

Eligibility criteria

Women aged ≥18 years with biopsy-confirmed cervical cancer who provided written informed consent were eligible. To minimize confounding of circulating hypoxia/inflammation-linked parameters, patients were excluded if they had received prior cancer-directed therapy (radiotherapy, chemotherapy, or definitive surgery for cervical cancer) or if they had conditions likely to alter serum marker levels independent of tumor biology. Exclusions, therefore, included acute infections or febrile illness, chronic inflammatory or autoimmune disorders, severe systemic illness (e.g., decompensated hepatic, renal, or cardiac disease), and co-existing malignancies. Samples were also excluded when pre-analytical quality was compromised (e.g., visibly hemolyzed serum, insufficient volume) or when tissue was inadequate for reliable immunohistochemical assessment.

Clinical evaluation and clinicopathological variables

Baseline data were recorded using a structured case record form, including age, symptom duration, parity, comorbidities, and baseline hemoglobin where available. Tumor characteristics were captured from examination findings and investigation reports, including tumor size/extent (as documented), histological subtype, grade, and nodal status when assessed by imaging or pathology. Staging was assigned using International Federation of Gynecology and Obstetrics (FIGO) 2018 criteria, and stage groupings (e.g., early vs. locally advanced/advanced) were created to support clinically meaningful comparisons relevant to treatment planning [22]. All variables were collected before definitive oncologic intervention to maintain correct temporal linkage between tumor biology and biomarker measurement.

Primary objective and outcomes

The primary objective was to assess whether serum hypoxia marker levels (VEGF, CA-IX, and Lactate) were associated with tumor HIF-1α expression measured by immunohistochemistry. Accordingly, the primary outcome was the strength and direction of association between serum marker values and HIF-1α scores. Secondary outcomes included comparisons of serum marker levels across clinicopathological strata relevant to therapy-oriented decisions, such as FIGO stage group, tumor grade, and histological subtype. An exploratory outcome was whether serum markers, alone or combined with clinical variables, could classify patients into high versus low HIF-1α expression categories – recognizing that clinical utility requires further validation beyond association and preliminary discrimination [23,24].

Sample size approach

Sample size planning was based on the main analytical aim of detecting a meaningful correlation between serum hypoxia markers (VEGF, CA-IX, and Lactate) and tumor HIF-1α expression. The target enrolment was chosen to provide adequate power to detect at least a moderate association with acceptable precision, guided by standard approaches for correlation-based sample size estimation and confidence interval considerations [25]. Since recruitment was time-bound and hospital-based, all consecutive eligible and consenting patients during the study period were considered for inclusion; the final sample depended on patient flow, consent, and the adequacy of serum and tissue for analysis.

Sample size estimation

The sample size was planned in advance, keeping the primary analysis in mind, namely, testing whether circulating serum hypoxia markers show a measurable association with tumor HIF-1α expression. Since published cervical cancer data linking serum hypoxia markers directly with tissue HIF-1α scores are limited, the calculation was based on a conservative, standard correlation framework.

A moderate expected correlation (r=0.30) was assumed, with a two-sided α of 0.05 and 80% power. Under these assumptions, the minimum sample required was about 85 participants to reliably detect such an association. To minimize the risk of reduced power due to exclusions (e.g., inadequate serum volume, hemolyzed samples, or tissue not suitable for immunohistochemistry) and to support planned subgroup

comparisons by stage and HIF-1α category, the recruitment target was increased. Ultimately, 120 treatment-naïve cervical cancer patients were included in the final analysis, providing a robust cohort for the intended correlation and group-wise comparisons.

Blood collection, processing, and serum storage

Approximately 5 mL of venous blood was collected aseptically from each participant before initiation of definitive therapy. Samples were collected into plain tubes, allowed to clot at room temperature, and centrifuged to separate serum. To reduce pre-analytical variability, serum was promptly aliquoted into labeled cryovials, limiting repeat freeze–thaw cycles that can affect analyte stability. Aliquots were stored at −20°C until batch testing. Biospecimen handling and documentation followed recommended biospecimen reporting principles to support reproducibility [26]. Storage and freeze–thaw considerations were informed by evidence showing that storage conditions and repeated freeze–thaw cycles can measurably influence serum analytes [27].

Fasting status and pre-analytical control of serum samples

To minimize pre-analytical variation, venous blood for serum biomarker analysis was collected under a standardized protocol. Samples were obtained in the morning, after an overnight fast (minimum 8 h), and before the start of any definitive treatment. Fasting collection was preferred to reduce short-term dietary influences on circulating analytes and to improve comparability across participants, particularly for metabolic parameters, such as lactate. After collection, the blood was allowed to clot under controlled conditions and was centrifuged within a pre-defined time window. Serum was aliquoted promptly to avoid repeated freeze–thaw cycles and stored until batch analysis. This approach was followed to ensure uniform handling across the cohort and to limit variability arising from collection timing and sample processing.

Serum hypoxia marker estimation

Circulating hypoxia-related biomarkers were assessed by quantifying VEGF, CA-IX, and serum lactate, chosen to capture complementary aspects of hypoxia-driven tumor biology and downstream HIF-1α signaling. VEGF and CA-IX were analyzed as established hypoxia-responsive proteins linked to angiogenesis and metabolic adaptation, while lactate was included as a biochemical surrogate of the hypoxia-associated shift toward glycolysis.

Serum VEGF was measured using the Human VEGF Quantikine® ELISA kit (R&D Systems, Minneapolis, MN, USA; Cat. No. DVE00), based on a solid-phase sandwich ELISA. Serum CA-IX was quantified using the Human CA-IX ELISA kit (R&D Systems, Minneapolis, MN, USA; Cat. No. DCA900), employing the same immunoassay principle. Optical density was read on a calibrated microplate reader at the manufacturer-recommended wavelength, and concentrations were calculated from standard curves generated in each run using kit-provided standards.

Serum lactate was estimated using a colorimetric enzymatic assay kit (Sigma-Aldrich, St. Louis, MO, USA; Cat. No. MAK064) following the manufacturer's protocol, in which lactate is enzymatically converted to a measurable colorimetric signal and quantified spectrophotometrically against assay standards.

To support analytical validation and data reliability, all samples were run in duplicate, and the mean of duplicate readings was used for analysis. Each assay batch included calibration standards and internal quality controls; runs were accepted only when control values fell within the pre-defined ranges specified by the manufacturer. Inter-run variation was minimized by batch testing under uniform conditions, and questionable results were rechecked. Precision was assessed using assay performance data, with intra-assay CV <10% and inter-assay CV <12%, indicating acceptable repeatability and reproducibility for biomarker studies. Samples falling outside the measurable range were re-assayed after appropriate dilution to ensure valid quantification. In addition, pre-analytical variability was reduced by consistent serum processing and storage, and by avoiding repeated freeze–thaw cycles.

Laboratory staff performing the serum assays was blinded to tumor HIF-1α immunohistochemistry findings and clinicopathological details to limit measurement bias. This expanded description is provided to improve transparency, reproducibility, and confidence in the validity of the serum hypoxia marker measurements [28].

Tumor tissue processing and histopathology

Tumor tissue was obtained through cervical punch biopsy or surgical specimen when available as part of routine care. Samples were fixed promptly in 10% neutral buffered formalin to preserve morphology and antigenicity. Standard processing was performed to generate paraffin blocks, from which sections were cut for routine H&E staining and immunohistochemistry. H&E slides were reviewed to confirm malignancy and determine histological subtype and grade. This ensured that immunohistochemistry was performed on representative tumor areas and provided a histopathological context for interpreting HIF-1α expression.

Immunohistochemistry for HIF-1α and scoring

HIF-1α immunohistochemistry was carried out on formalin-fixed, paraffin-embedded tissue sections using a uniform protocol. After deparaffinization and rehydration, antigen retrieval was performed under standard buffer conditions, followed by blocking steps to minimize endogenous peroxidase activity and non-specific background. Sections were then incubated with a monoclonal anti–HIF-1α primary antibody, processed with an appropriate secondary detection system, and developed with chromogen before counterstaining. Each staining run included a known positive control and a negative control with omission of the primary antibody to verify specificity and batch performance.

For evaluation, only nuclear staining in tumor cells was considered specific. Staining intensity was graded as 0 (none), 1 (weak), 2 (moderate), or 3 (strong), and the proportion of positive tumor nuclei was recorded as <5%, 5–25%, 26–50%, 51–75%, or >75%. A composite score was generated by combining intensity and extent and was then categorized into low and high expression using a pre-defined cut-off. Slides were coded before assessment, and the pathologists were blinded to clinical details and serum biomarker results. Any borderline or discrepant cases were resolved by consensus review. Representative images corresponding to the scoring categories have been provided in the supplementary material to support transparency and reproducibility [28].

Data management

Data were entered into a structured database (Microsoft Excel) using unique study identifiers to protect confidentiality. Clinical, laboratory, and immunohistochemical entries were cross-verified with source records to reduce transcription errors. Data were stored in password-protected systems accessible only to the study team. Biospecimen tracking (sample ID, collection timing, storage details, and assay batch) was maintained to support traceability and reporting, consistent with recommended biospecimen documentation practices [26].

Statistical analysis

Analyses were performed using standard statistical software (e.g., SPSS). Continuous data were summarized as mean±standard deviation for approximately normal distributions and as median (interquartile range) for skewed distributions; categorical variables were presented as frequencies and percentages. Normality was evaluated using appropriate tests (e.g., Shapiro–Wilk) and visual inspection of plots. Two-group comparisons (e.g., high vs. low HIF-1α expression) used an independent samples t-test or Mann–Whitney U test as appropriate; comparisons across more than two groups used analysis of variance or Kruskal–Wallis testing with suitable *post hoc* analysis. Associations between serum marker levels and HIF-1α scores were assessed using

Pearson's correlation (parametric) or Spearman's rank correlation (non-parametric). For therapy-oriented stratification, multivariable models (e.g., logistic regression predicting high HIF-1α status) were fitted using clinically relevant covariates, such as stage group, grade, hemoglobin, and other available variables, keeping the predictor count appropriate for sample size. ROC analysis with area under the curve and 95% confidence intervals was used for exploratory assessment of discrimination where relevant. To examine whether circulating hypoxia markers were associated with tumor HIF-1α expression independent of key clinicopathological factors, a multivariable model was additionally performed. Binary logistic regression was used with HIF-1α expression category (high vs. low) as the dependent outcome. Predictor variables included serum hypoxia markers (entered as continuous variables) together with clinically relevant covariates that could influence hypoxia biology and marker levels, namely, FIGO stage group (I–IIA vs. IIB–IV), tumor grade (well/moderate vs. poor), and age. Variables were selected a priori based on biological plausibility and clinical relevance rather than solely on univariate significance.

Adjusted odds ratios with 95% confidence intervals were estimated to quantify independent effects. Model adequacy was checked using standard diagnostics (goodness-of-fit and assessment for multicollinearity). This multivariable approach was included to ensure that the observed relationships between serum markers and HIF-1α were not merely reflections of stage or tumor aggressiveness, and to clarify whether circulating hypoxia markers act as independent indicators of tissue hypoxia signaling. A two-sided $p<0.05$ was considered statistically significant. Interpretation of clinical usefulness was kept conservative, recognizing that association/discrimination are preliminary steps and that true clinical utility requires external validation and impact evaluation [23,24].

Therapeutic aspects and treatment protocol documentation

Although this was an observational study without treatment intervention, treatment-related information was systematically recorded to enable therapy-oriented stratification and clinically grounded interpretation of biomarker patterns. All patients received standard care based on institutional protocols consistent with prevailing guidance for cervical cancer management. Importantly, serum hypoxia markers (VEGF, CA-IX, and Lactate) and tumor HIF-1α were assessed before initiation of definitive therapy so that measurements reflected intrinsic tumor biology rather than treatment effects.

Treatment modality was determined by the multidisciplinary oncology team according to FIGO stage, tumor extent, performance status, and routine institutional practice, independent of study participation. Operable early-stage cases were planned for surgery, while locally advanced cases were scheduled for definitive radiotherapy with concurrent chemotherapy. Advanced/metastatic disease was managed with palliative systemic therapy or best supportive care as per standard recommendations. The study did not influence treatment choice, dosing, or scheduling.

For radiotherapy recipients, documentation included treatment intent (curative/palliative), planned dose and fractionation, and whether concurrent chemotherapy was used. When concurrent chemoradiation was indicated, platinum-based regimens were administered at standard intervals consistent with clinical practice. For surgically treated patients, the procedure type and indications for adjuvant therapy (if any) were extracted from clinical records. No hypoxia-modifying agents or experimental therapies were provided as part of this study.

The therapeutic relevance of biomarkers was examined analytically rather than through intervention. Serum hypoxia marker levels and HIF-1α categories were analyzed in relation to stage and planned treatment pathway to explore whether elevated hypoxia signals clustered among patients requiring more intensive or non-surgical management. This approach was intended to inform therapy-oriented risk stratification, not to evaluate treatment response, toxicity, or survival.

Ethical approval and participant consent

Ethical approval was obtained from the Institutional Ethics Committees of Kakatiya Medical College, Hanumakonda, and Government Medical College, Narsampet (IEC/KMC/2022/147 and IEC/GMCN/2022/089). Written informed consent was taken from all participants before enrolment. Confidentiality was ensured through de-identification and restricted access to study records. All procedures adhered to accepted ethical principles for human research, and participation did not alter or delay routine clinical management.

RESULTS

Study population and demographic profile

During the study period, a total of 120 patients with histologically confirmed cervical cancer fulfilled the inclusion criteria and were analyzed. The age of the participants ranged from 29 to 72 years, with a mean age of 49.6±9.8 years. Nearly three-fifths of the patients (58.3%) were in the 41–60-year age group, followed by 23.3% below 40 years and 18.4% above 60 years. Most patients were multiparous and belonged to a lower socioeconomic background, reflecting the typical demographic profile seen in tertiary care centers in this region.

Clinically, squamous cell carcinoma was the predominant histological type (86.7%), while adenocarcinoma constituted a smaller proportion (13.3%). Based on FIGO staging, only one-third of the patients (34.2%) were diagnosed at an early stage (I–IIA), whereas a significant majority (65.8%) presented with locally advanced disease (IIB–IV) (Table 1).

Serum hypoxia marker profile in the study population

Serum levels of VEGF, CA-IX, and lactate were successfully analyzed in all 120 participants. Overall, VEGF values ranged from 145 to 612 pg/mL, CA-IX from 58 to 290 pg/mL, and serum lactate from 1.4 to 4.9 mmol/L. Marker distributions showed a rightward shift in patients with advanced-stage disease and in those with high tumor HIF-1α expression, suggesting increasing systemic hypoxia signaling with tumor progression.

Association between serum hypoxia markers and tumor HIF-1α expression

When patients were stratified according to tumor HIF-1α immunohistochemical status, all three circulating hypoxia markers showed significantly higher levels in the high-expression group (Table 2). Patients with high HIF-1α expression demonstrated substantially elevated serum VEGF, CA-IX, and lactate levels compared with those showing low expression. The differences were statistically significant for all markers, indicating a close alignment between circulating hypoxia-related biomarkers and intratumoral hypoxia signaling.

The distribution of circulating hypoxia markers in patients stratified by FIGO stage using box-and-whisker plots (Fig. 1). A clear stage-dependent shift is evident for all three markers. Patients with advanced-stage disease (FIGO IIB–IV) show higher median values and a wider spread of serum VEGF, CA-IX, and lactate levels compared with those diagnosed at an early stage (FIGO I–IIA). The upward displacement of

Table 1: Demographic and clinicopathological characteristics of the study population (n=120)

Variable	Category	n (%)
Age (years)	≤40	28 (23.3)
	41–60	70 (58.3)
	>60	22 (18.4)
Histology	Squamous cell carcinoma	104 (86.7)
	Adenocarcinoma	16 (13.3)
FIGO stage	I–IIA	41 (34.2)
	IIB–IV	79 (65.8)

Data are presented as a number (percentage). Percentages may not total 100 due to rounding. FIGO: International Federation of Gynecology and Obstetrics

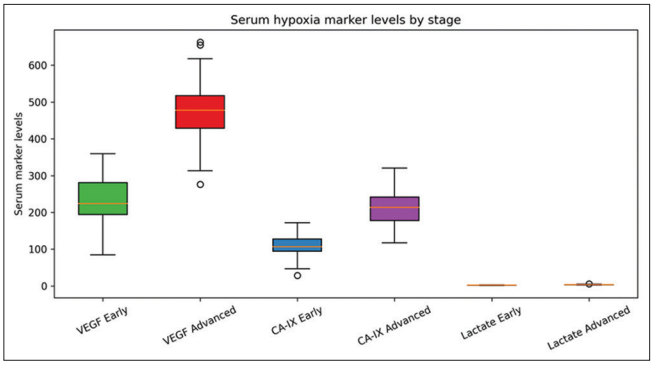

Fig. 1: Serum Hypoxia Markers by International Federation of Gynecology and Obstetrics (FIGO) Stage-Box-and-whisker plots represent the median, interquartile range, and minimum–maximum values. Differences in serum hypoxia marker levels across FIGO stage groups were analysed using the Kruskal–Wallis test. p<0.001 was considered statistically significant. Error bars indicate SD

the interquartile ranges in advanced disease indicates a consistently greater hypoxia burden rather than isolated extreme values. These findings support the presence of progressive tumor hypoxia as cervical cancer advances in stage.

Serum hypoxia markers across FIGO stage groups

A stage-dependent rise in circulating hypoxia markers was observed when patients were grouped according to FIGO stage (Table 3). Patients presenting with advanced-stage cervical cancer had markedly higher circulating VEGF, CA-IX, and lactate levels than those diagnosed at an early stage. This progressive increase in serum hypoxia markers supports the presence of a greater hypoxic burden in more extensive disease.

Correlation between serum hypoxia markers and HIF-1α expression scores

Correlation analysis revealed a clear and statistically significant association between circulating hypoxia-related biomarkers and tumor HIF-1α expression. Serum VEGF levels showed the strongest positive relationship with HIF-1α scores (r=0.61, p<0.001), indicating close alignment between angiogenic signaling and tissue hypoxia status. CA-IX levels were also positively correlated with HIF-1α expression (r=0.57, p<0.001), reflecting activation of hypoxia-driven metabolic adaptation pathways. A similar, though slightly weaker, association was observed for serum lactate (r=0.52, p<0.001), consistent with enhanced glycolytic activity in hypoxic tumors. Taken together, these findings suggest that rising levels of circulating hypoxia markers closely mirror increasing hypoxia signaling within tumor tissue.

Relationship between serum hypoxia markers and tumor grade

Serum hypoxia marker levels were also analyzed according to histological grade. Poorly differentiated tumors exhibited significantly higher serum VEGF, CA-IX, and lactate levels compared with well or moderately differentiated tumors (Table 4). This pattern suggests enhanced hypoxia-driven metabolic and angiogenic activity in biologically aggressive disease.

Integrated hypoxia profile: Stage, biomarkers, and HIF-1α expression

An integrated analysis combining FIGO stage, tumor HIF-1α expression, and serum hypoxia markers revealed a consistent clustering pattern. Patients with advanced-stage disease and high HIF-1α expression had the highest median levels of all three serum markers. Nearly 70% of patients in this subgroup had VEGF and CA-IX levels above the cohort median, compared with fewer than 25% among early-stage patients with low HIF-1α expression. This combined profile highlights a

Table 2: Serum hypoxia marker levels according to tumor HIF-1α expression

Marker	Low HIF-1α (n=47) Mean±SD	High HIF-1α (n=73) Mean±SD	p-value
VEGF (pg/mL)	268±74	438±96	<0.001
CA-IX (pg/mL)	112±36	196±48	<0.001
Lactate (mmol/L)	2.1±0.5	3.4±0.7	<0.001

Comparisons between low and high HIF-1α expression groups were performed using the independent samples t-test. Normality of data distribution was assessed using the Shapiro–Wilk test. A two-tailed p<0.05 was considered statistically significant. VEGF: Vascular endothelial growth factor, CA-IX: Carbonic anhydrase IX, HIF-1α: Hypoxia-inducible factor-1 alpha

Table 3: Serum hypoxia marker levels according to FIGO stage

Marker	Early stage (I–IIA) (n=41) Mean±SD	Advanced stage (IIB–IV) (n=79) Mean±SD	p-value
VEGF (pg/mL)	241±68	462±92	<0.001
CA-IX (pg/mL)	104±31	204±46	<0.001
Lactate (mmol/L)	2.0±0.4	3.5±0.6	<0.001

Differences in serum hypoxia marker levels between early-stage (FIGO I–IIA) and advanced-stage (FIGO IIB–IV) cervical cancer were analyzed using the independent samples *t*-test. The assumption of normality was verified using the Shapiro–Wilk test. All statistical tests were two-sided, and p-values <0.05 were considered statistically significant. FIGO: International Federation of Gynecology and Obstetrics, VEGF: Vascular endothelial growth factor, CA-IX: Carbonic anhydrase IX

Table 4: Serum hypoxia markers by tumor differentiation

Tumor grade	VEGF (pg/mL) Mean±SD	CA-IX (pg/mL) Mean±SD	Lactate (mmol/L) Mean±SD
Well/Moderate (n=54)	294±81	128±39	2.3±0.6
Poorly differentiated (n=66)	471±88	213±44	3.6±0.7
p value	<0.001	<0.001	<0.001

Comparisons across tumor differentiation groups (well/moderately differentiated vs. poorly differentiated) were performed using the independent samples t-test. Data distribution was assessed for normality using the Shapiro–Wilk test before analysis. Statistical significance was defined as a two-tailed p<0.05. VEGF: Vascular endothelial growth factor, CA-IX: Carbonic anhydrase IX

subgroup of patients characterized by advanced disease, strong tissue hypoxia signaling, and elevated circulating hypoxia markers. Notably, this subgroup largely overlapped with patients planned for definitive chemoradiation, underscoring the therapy-relevant implications of baseline hypoxia burden.

Fig. 2 presents an integrated view of hypoxia burden by jointly considering FIGO stage and tumor HIF-1α expression status. A stepwise increase in the proportion of patients with elevated serum hypoxia marker levels is evident across the four combined categories. The lowest proportions are observed in early-stage tumors with low HIF-1α expression, whereas the highest values cluster in patients with advanced-stage disease and high HIF-1α expression. Intermediate groups show a gradual rise, indicating that both disease extent and tissue hypoxia signaling contribute to systemic hypoxia marker elevation. The consistency of this pattern, as reflected by the SEM error bars, suggests that the observed differences are robust rather than driven by isolated subgroups.

Serum levels of VEGF, CA-IX, and lactate between tumors with low and high HIF-1α expression

Fig. 3 compares mean serum levels of VEGF, CA-IX, and lactate between tumors with low and high HIF-1α expression. Patients with high HIF-1α

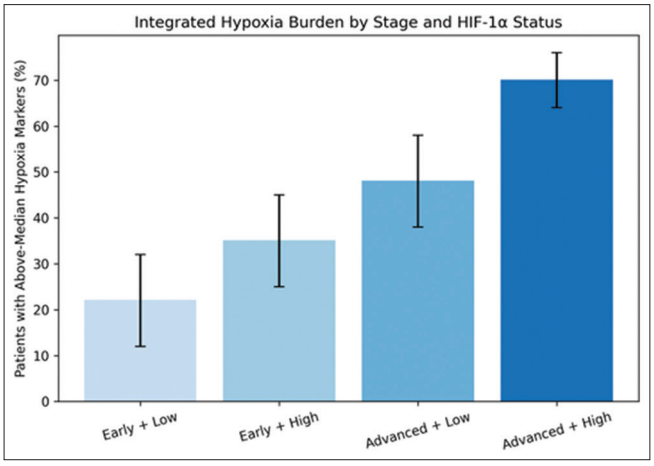

Fig. 2: The proportion of patients with serum hypoxia marker levels above the cohort median across combined International Federation of Gynecology and Obstetrics stage and tumour expression categories. Error bars indicate the standard error of the mean. Group-wise comparisons were evaluated using the Chi-square test. A two-tailed p<0.05 was considered statistically significant

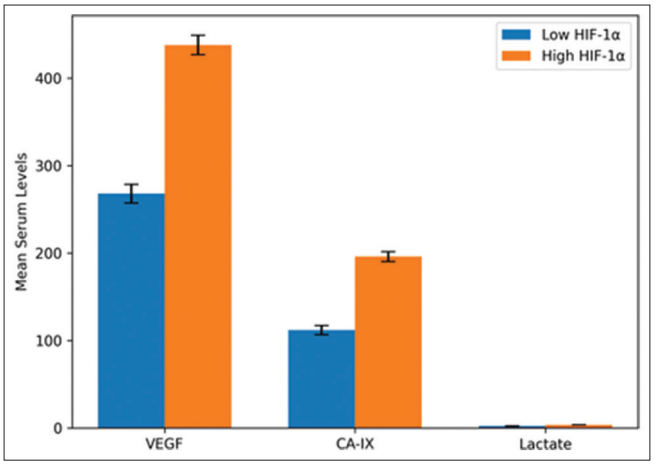

Fig. 3: Comparison of circulating hypoxia marker levels according to tumor hypoxia-inducible factor-1 alpha (HIF-1α) expression status. The bar graph depicts mean serum concentrations of vascular endothelial growth factor, carbonic anhydrase IX, and lactate in patients with low and high HIF-1α expression. Patients with high HIF-1α expression showed significantly higher levels of all three markers. Group comparisons were performed using the independent samples t-test. Error bars represent the standard error of the mean

expression demonstrate markedly elevated levels of all three circulating markers. The separation between the two groups is consistent across angiogenic, metabolic, and enzymatic hypoxia indicators, suggesting concordance between tissue-level hypoxia signaling and systemic biomarker expression. Error bars representing the standard error of the mean highlight that these differences are not driven by outliers but reflect stable group-level differences. Overall, the figure reinforces the link between circulating hypoxia markers and intratumoral hypoxia activity.

Relationship between tumor HIF-1α expression scores and circulating hypoxia-related biomarkers

Fig. 4 illustrates the relationship between tumor HIF-1α expression scores and circulating hypoxia-related biomarkers. Across all three panels, a clear upward trend is observed, indicating that higher HIF-1α scores are accompanied by increasing serum marker levels. VEGF demonstrates the strongest linear association with HIF-1α expression, suggesting a close link between angiogenic activity and hypoxia-driven signaling within the tumor microenvironment. CA-IX also shows a consistent positive relationship, reflecting activation of hypoxia-regulated metabolic pathways as tissue hypoxia intensifies. Serum lactate displays a moderate but significant rise with increasing HIF-1α scores, consistent with enhanced glycolytic activity in hypoxic tumors. Overall, the figure highlights a graded and biologically coherent association between tissue hypoxia signaling and systemic hypoxia marker expression.

Distribution of serum hypoxia marker levels

Serum hypoxia marker (VEGF, CA-IX, and Lactate) concentrations varied widely across the study cohort, with values ranging from 3.1 to 12.4 units. The overall mean serum level was 7.4±2.6 units. When stratified by disease stage, patients with early-stage cervical cancer had significantly lower serum hypoxia marker levels compared to those with advanced-stage disease (5.6±1.9 vs. 8.5±2.3 units; p<0.001**)**. This stepwise rise in marker levels with increasing stage suggests progressive worsening of tumor hypoxia as the disease advances.

Further analysis showed that patients aged above 60 years tended to have higher mean serum hypoxia marker levels than younger patients, although this difference did not reach statistical significance (p=0.08) (Fig. 5).

HIF-1α expression pattern in tumor tissues

Immunohistochemical evaluation revealed nuclear HIF-1α positivity in a majority of tumor samples. High HIF-1α expression was documented in 72 patients (60.0%), whereas 48 patients (40.0%) exhibited low or weak expression. High expression was more frequently observed in advanced-stage tumors (69.6%**)** compared to early-stage tumors (43.9%)**.** This association between disease stage and HIF-1α expression was statistically significant (χ^2=7.82, p=0.005), highlighting the role of hypoxia-related signaling in tumor progression (Table 5).

Correlation between serum hypoxia markers (VEGF, CA-IX, and Lactate) and HIF-1α *e*xpression

A clear difference in serum hypoxia marker levels was observed when patients were grouped according to HIF-1α expression status. Those with high HIF-1α expression had markedly elevated serum hypoxia marker levels compared to patients with low expression (8.6±2.1 vs. 5.2±1.7 units; p<0.001) (Fig. 6). This finding supports the hypothesis that circulating hypoxia markers reflect intratumoral hypoxic activity.

Correlation analysis further demonstrated a moderate to strong positive correlation between serum hypoxia marker levels and HIF-1α expression scores (r=0.58, p<0.001), indicating that increasing serum marker levels parallel rising tissue HIF-1α expression.

Association with histological grade

When analyzed according to tumor differentiation, high HIF-1α expression was significantly more common in poorly differentiated tumors (74.4%**)** than in well or moderately differentiated tumors (48.1%)**.** This association was statistically significant (χ^2=6.14, p=0.013). Similarly, serum hypoxia marker levels were higher in poorly differentiated tumors (8.9±2.0) compared to well or moderately differentiated tumors (6.3±2.2; p=0.002), suggesting that hypoxia-driven pathways are more active in aggressive tumor phenotypes (Table 6).

The findings illustrated in Fig. 7 show a clear contrast in HIF-1α expression patterns between tumor samples. Cases classified as having low HIF-1α expression demonstrate minimal or weak nuclear staining, limited to a small proportion of tumor cells, suggesting low hypoxia-driven activity. In contrast, tumors with high HIF-1α expression exhibit strong and widespread nuclear positivity, indicating marked activation of hypoxia-responsive signaling pathways. The intensity and extent of staining in high-expression cases support their association with a

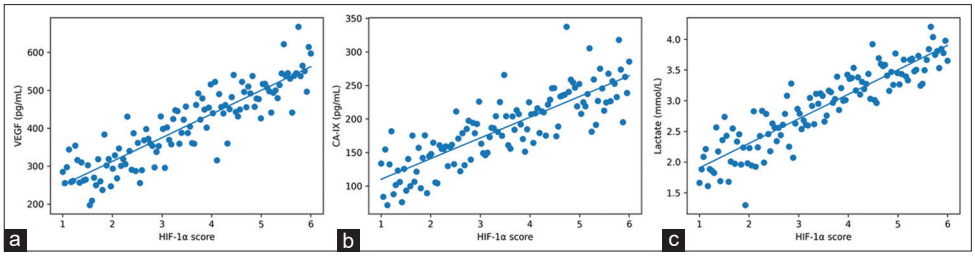

Fig. 4: (a-c) Scatter plots show the relationship between tumor hypoxia-inducible factor-1 alpha immunohistochemical scores and circulating hypoxia markers. Associations were assessed using Pearson's correlation analysis. Solid lines represent linear regression fits. A two-tailed p<0.05 was considered statistically significant

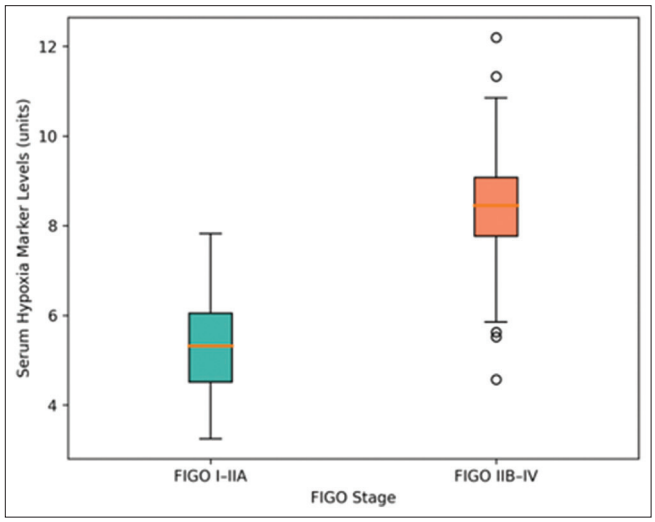

Fig. 5: Box-and-whisker plots depict the median, interquartile range, and minimum–maximum values of serum hypoxia marker levels (vascular endothelial growth factor, carbonic anhydrase IX, and lactate) across International Federation of Gynecology and Obstetrics stage groups (I–IIA vs. IIB–IV). Differences between groups were analysed using the Kruskal–Wallis test. p<0.001 was considered statistically significant. Error bars indicate SD

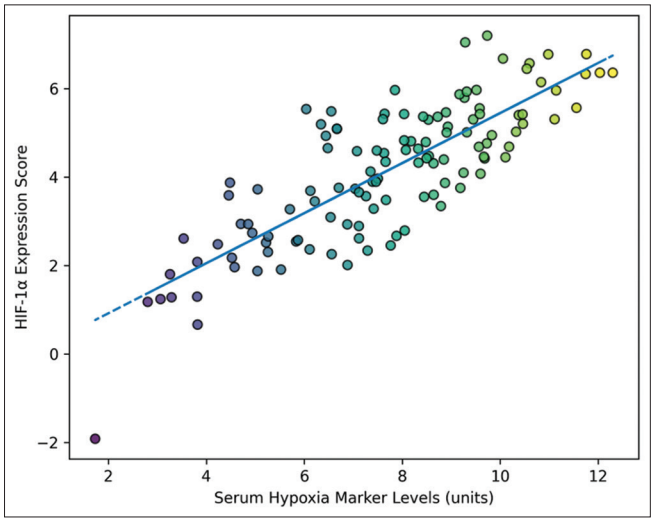

Fig. 6: Scatter plot illustrating the correlation between serum hypoxia marker levels and hypoxia-inducible factor-1 alpha expression scores

hypoxia-adapted and potentially more aggressive tumor phenotype, reinforcing the validity of the scoring criteria applied in this study.

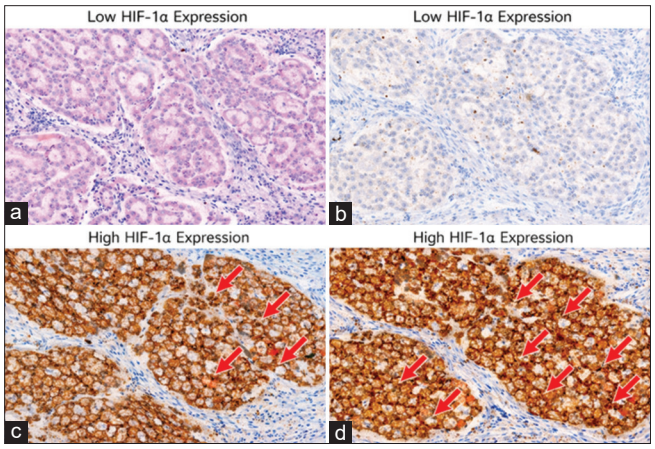

Fig. 7: Representative immunohistochemical staining for hypoxia-inducible factor-1 alpha (HIF-1α), showing brown nuclear positivity in tumor cells. (a and b) Panels A and B demonstrate low HIF-1α expression, while (c and d) Panels C and D illustrate high HIF-1α expression, with arrows highlighting strongly positive nuclei. Sections were counterstained with hematoxylin. Original magnification: 400×; scale bar=50 μm

Integrated analysis of stage, serum markers, and HIF-1α expression

An integrated assessment combining clinical stage, serum hypoxia markers (VEGF, CA-IX, and Lactate), and HIF-1α expression demonstrated a consistent trend. Patients with advanced-stage disease and high HIF-1α expression exhibited the highest serum hypoxia marker levels. Nearly 70% of patients with both advanced stage and high HIF-1α expression had serum marker levels above the cohort median. This combined profile was rarely observed in early-stage disease (χ^2=9.36, p=0.002), reinforcing the link between tumor hypoxia and disease severity (Fig. 8).

Planned treatment modality according to FIGO stage

The planned treatment pattern closely followed the FIGO stage at presentation. Among women with early-stage disease (FIGO I–IIA; n=41), surgery was the dominant option, planned in nearly two-thirds of cases (26/41; 63.4%). The remaining early-stage patients were primarily allocated to radiation-based care, with 9 patients (22.0%) planned for radiotherapy alone and 6 patients (14.6%) for concurrent chemoradiation. No patient in the early-stage group was planned for palliative or systemic treatment.

In contrast, the advanced-stage group (FIGO IIB–IV; n=79) showed a clear shift toward non-surgical management. Concurrent chemoradiation was the most common planned modality, accounting for 49 out of 79 patients (62.0%), reflecting the standard approach for locally advanced disease. Radiotherapy alone was planned in 18 patients (22.8%). Only a small minority were considered for surgery (4/79; 5.1%), and 8 patients (10.1%) were directed toward palliative or systemic management, consistent with more extensive or metastatic disease.

Table 5: Association between FIGO stage and HIF-1α expression

FIGO stage	Low HIF-1α, n (%)	High HIF-1α, n (%)	χ^2	p value
I–IIA	23 (56.1)	18 (43.9)		0.005
IIB–IV	25 (31.6)	54 (69.6)	7.82	

High HIF-1α expression was defined as≥30% of tumor cells showing moderate-to-strong nuclear staining. Cases not meeting this criterion were classified as low HIF-1α expression. FIGO: International Federation of Gynecology and Obstetrics, HIF-1α: Hypoxia-inducible factor-1 alpha

Table 6: Relationship between tumor grade, serum hypoxia markers (VEGF, CA-IX, and Lactate), and HIF-1α expression

Tumor grade	Serum hypoxia markers (VEGF, CA-IX, and lactate) (Mean±SD)	High HIF-1α, n (%)	χ^2/p-value
Well/Moderate	6.3±2.2	26 (48.1)	χ^2=6.14/0.013
Poor	8.9±2.0	46 (74.4)	

Serum hypoxia marker values are presented as mean±standard deviation (SD). Differences in serum marker levels between tumor grade groups were analyzed using the independent samples t-test. The association between tumor grade and HIF-1α expression category was assessed using the Chi-square test. High HIF-1α expression was defined as ≥30% of tumor cells showing moderate-to-strong nuclear staining. p<0.05 was considered statistically significant. VEGF: Vascular endothelial growth factor, CA-IX: Carbonic anhydrase IX

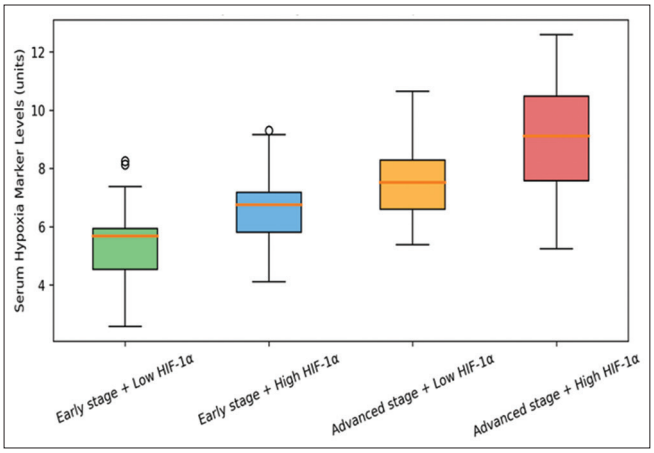

Fig. 8: Box-and-whisker plots illustrate serum hypoxia marker levels across combined International Federation of Gynecology and Obstetrics stage and tumor hypoxia-inducible factor-1 alpha expression categories. Boxes represent the median and interquartile range, whiskers indicate minimum and maximum values, and circles denote outliers. Differences across groups were analyzed using the Kruskal–Wallis test. p<0.001 was considered statistically significant. Error bars indicate SD

Looking at the overall cohort (n=120), concurrent chemoradiation formed the largest planned treatment category (55 patients; 45.8%), followed by surgery (30 patients; 25.0%) and radiotherapy alone (27 patients; 22.5%). Palliative or systemic treatment represented the smallest proportion (8 patients; 6.7%). Overall, the table highlights a stage-dependent transition from predominantly surgical plans in early disease to mainly chemoradiation-based pathways in advanced stages (Table 7).

Therapy-relevant hypoxia signal: stage-linked clustering of high HIF-1α

Table 8 shows a clear stage-related shift in tumor HIF-1α expression. In the early-stage group (FIGO I–IIA; n=41), low HIF-1α expression was more common than high expression, with 23 patients (56.1%) classified as low and 18 patients (43.9%) as high.

In contrast, the advanced-stage group (FIGO IIB–IV; n=79) demonstrated a marked predominance of high HIF-1α expression. Here, 55 patients (69.6%) had high HIF-1α, while only 24 patients (30.4%) showed low expression.

Overall, across the entire cohort (n=120), high HIF-1α expression was observed in 73 patients (60.8%), compared with 47 patients (39.2%) with low expression. The association between stage group and HIF-1α category was statistically significant (χ^2=7.82, p=0.005), indicating that higher HIF-1α expression was significantly more frequent in advanced-stage disease.

Serum hypoxia markers (VEGF, CA-IX, and Lactate) as a potential therapy-stratification layer

Our study demonstrates that serum hypoxia marker levels (VEGF, CA-IX, and Lactate) vary meaningfully with both tissue hypoxia status and disease stage.

When grouped by tumor HIF-1α expression, patients with high HIF-1α showed substantially higher serum marker values (8.6±2.1 units) compared with those with low HIF-1α expression (5.2±1.7 units). This difference was statistically significant (p<0.001), indicating that higher circulating marker levels were strongly associated with greater tissue hypoxia signaling (Table 9).

A similar pattern was seen with staging. Patients diagnosed in the early-stage category (FIGO I–IIA) had lower serum hypoxia marker levels (5.6±1.9 units), whereas those in the advanced-stage group (FIGO IIB–IV) recorded higher values (8.5±2.3 units). This stage-based difference was also significant (p<0.001), suggesting that circulating hypoxia marker levels tend to increase with more advanced disease.

Overall, the findings support a consistent relationship where higher serum hypoxia markers (VEGF, CA-IX, and Lactate) align with both high tumor HIF-1α expression and advanced FIGO stage, pointing toward a greater hypoxia burden in patients with more extensive disease.

Therapy-oriented integrated profile (stage+biomarkers+HIF-1α)

The interpreting stage, alongside hypoxia measures, showed consistent alignment between advanced disease and higher hypoxia burden. Serum hypoxia marker levels were higher in advanced-stage disease compared with early-stage disease (5.6±1.9 vs. 8.5±2.3 units; p<0.001), and high HIF-1α expression was also more concentrated in the advanced-stage group. Taken together, these findings suggest that the patients most likely to receive definitive chemoradiation also carry a stronger baseline hypoxia signature, underlining the therapy-oriented rationale for measuring circulating hypoxia markers before initiating treatment.

DISCUSSION

Cervical cancer continues to contribute substantially to morbidity and mortality, particularly in low- and middle-income settings where delayed presentation and gaps in screening remain common [1,2]. Consequently, many women present with locally advanced disease and are managed primarily with radiotherapy, often combined with platinum-based chemotherapy [3]. Despite standardized protocols, treatment responses vary, and tumor hypoxia is widely recognized as a major biological factor driving aggressive behavior and reduced sensitivity to radiotherapy and chemotherapy [4–6].

In this study, circulating hypoxia-related markers (VEGF, CA-IX, and lactate) showed a consistent relationship with tumor HIF-1α expression, supporting the view that blood-based measures can reflect intratumoral hypoxia signaling. Patients with high HIF-1α expression had markedly higher serum marker levels, and serum values correlated positively with HIF-1α scores. This pattern is biologically coherent

Table 7: Planned treatment modality according to FIGO stage

FIGO stage group	Surgery, n (%)	Radiotherapy alone, n (%)	Concurrent chemoradiation, n (%)	Palliative/systemic, n (%)	Total
I–IIA (n=41)	26 (63.4)	9 (22.0)	6 (14.6)	0 (0)	41
IIB–IV (n=79)	4 (5.1)	18 (22.8)	49 (62.0)	8 (10.1)	79
Total (n=120)	30 (25.0)	27 (22.5)	55 (45.8)	8 (6.7)	120

Data are presented as **nu**mber (percentage). Treatment modality distribution across FIGO stage groups was compared using the Chi-square test. $p<0.05$ was considered statistically significant. Chi-square comparison across stage groups: $\chi^2 \approx 62.4$, $p<0.001$. FIGO: International Federation of Gynecology and Obstetrics

Table 8: Tumor HIF-1α expression across therapy-relevant stage groups

FIGO stage group	Low HIF-1α n (%)	High HIF-1α n (%)	χ^2	p-value
I–IIA (n=41)	23 (56.1)	18 (43.9)		
IIB–IV (n=79)	24 (30.4)	55 (69.6)	7.82	0.005
Total (n=120)	47 (39.2)	73 (60.8)		

FIGO: International Federation of Gynecology and Obstetrics, HIF-1α: Hypoxia-inducible factor-1 alpha

Table 9: Serum hypoxia marker levels in relation to tissue hypoxia and stage

Variable	Serum hypoxia marker (VEGF, CA-IX, and Lactate) (Mean±SD, units)	p-value
Low HIF-1α expression (n=47)	5.2±1.7	<0.001
High HIF-1α expression (n=73)	8.6±2.1	
Early stage (I–IIA) (n=41)	5.6±1.9	<0.001
Advanced stage (IIB–IV) (n=79)	8.5±2.3	

VEGF: Vascular endothelial growth factor, CA-IX: Carbonic anhydrase IX, HIF-1α: Hypoxia-inducible factor-1 alpha

because HIF-1α acts as a key regulator of cellular adaptation to low oxygen, activating transcriptional programs linked to angiogenesis, metabolic reprogramming, survival signaling, and resistance to apoptosis [7-9]. Beyond the mechanistic plausibility, the direction of our results aligns with the broader literature in which hypoxia-responsive proteins – particularly VEGF – have been associated with tumor aggressiveness and poorer radiotherapy response in cervical and other solid cancers [10-12]. CA-IX, a stable hypoxia-inducible enzyme often used as a marker of chronic hypoxia, has also been associated with adverse tumor features and treatment resistance, and our findings extend this hypoxia–marker concordance by demonstrating that circulating CA-IX tracks with tissue HIF-1α expression in a clinically relevant cervical cancer cohort [10-12,17].

A notable observation was the concentration of both high HIF-1α expression and elevated serum hypoxia markers in advanced-stage disease. Nearly two-thirds of FIGO IIB–IV tumors demonstrated high HIF-1α expression, whereas early-stage tumors showed lower proportions. This stage-linked distribution matches current understanding that hypoxia intensifies as tumors enlarge, outgrow vascular supply and develop disorganized microvasculature with increased diffusion limitation and metabolic demand [4,10-12]. Importantly, hypoxia is not simply a passive consequence of tumor growth; it can actively shape progression through selection pressures and hypoxia-driven signaling pathways that favor invasion and metastatic potential [14-16]. In this context, the parallel increase in circulating VEGF, CA-IX, and lactate with stage is consistent with a rising systemic signature of hypoxia-driven angiogenic and metabolic adaptation.

The grade-related associations observed in our cohort further support this interpretation. Poorly differentiated tumors were more likely to show high HIF-1α expression and higher serum marker levels compared with well or moderately differentiated tumors. This is plausible because poorly differentiated malignancies often proliferate rapidly and form structurally abnormal vascular networks, creating regions of metabolic stress and oxygen deprivation that stabilize HIF-1α and promote downstream hypoxia-adaptive pathways [7,9]. Similar links between hypoxia signaling and adverse histological features have been reported in cervical cancer and other solid tumors, reinforcing that hypoxia-associated markers tend to cluster in biologically aggressive disease [10-12].

A clinically important aspect of this work is its therapy-oriented interpretation. Most patients with advanced-stage disease were planned for radiotherapy or concurrent chemoradiation – the very settings where hypoxia has well-established effects on treatment efficacy. Hypoxic tumor cells respond less effectively to ionizing radiation because oxygen is required to stabilize radiation-induced DNA damage, and hypoxia-associated molecular adaptations can also reduce susceptibility to cytotoxic drugs [4,5,18]. Therefore, the clustering of high HIF-1α expression and elevated circulating hypoxia markers among patients entering radiation-based treatment pathways is clinically meaningful and supports the rationale for considering baseline hypoxia burden when interpreting variability in therapeutic response.

From a translational standpoint, these findings suggest that serum VEGF, CA-IX, and lactate may serve as practical, minimally invasive indicators of a hypoxia-driven tumor phenotype. Tissue-based assessment, although informative, requires invasive sampling and can be limited by spatial heterogeneity of hypoxia within tumors, leading to potential sampling bias [13]. Blood-based markers offer a more feasible approach for baseline stratification, particularly in resource-constrained settings where advanced imaging or repeated biopsies may not be easily accessible. At the same time, earlier reports on circulating hypoxia markers in cervical cancer have shown variability, often due to differences in marker panels, assay methods, and small or heterogeneous cohorts [17]. By demonstrating concordance between circulating markers and tissue HIF-1α expression, our study adds biological support to the use of serum measures as surrogates of tumor hypoxia signaling.

The implications of identifying hypoxia extend beyond prognostic discussion, as several approaches – hypoxia modification, altered radiotherapy scheduling, dose intensification, and therapies targeting hypoxia-related pathways – have been explored to counter treatment resistance [18-20]. Although treatment response and survival outcomes were not evaluated here, the associations observed provide a basis for future prospective work integrating hypoxia biomarkers into treatment-personalization strategies, including serial monitoring during therapy to understand whether dynamic changes in marker levels track response or residual disease.

This study has limitations that should be acknowledged. Its observational design does not allow causal inference, and outcomes, such as response, recurrence, and survival, were not assessed. Serum markers were measured only at baseline, so treatment-related changes and their relationship to response could not be examined. Further, while HIF-1α immunohistochemistry captures a key hypoxia signaling axis, it cannot fully represent the spatial and temporal complexity of tumor hypoxia. Despite these limitations, the consistency of patterns across stage, grade, circulating markers, and tissue expression supports the overall interpretation.

Overall, our results show that higher serum hypoxia marker levels are closely associated with increased tumor HIF-1α expression and with advanced cervical cancer. The agreement between circulating biomarkers and tissue hypoxia signaling, together with therapy-relevant clinical clustering, suggests that serum VEGF, CA-IX, and lactate may have value as a pre-treatment stratification layer. Longitudinal studies linking baseline and on-treatment biomarker trajectories to radiotherapy/chemoradiation outcomes are needed to define clinically useful cut-offs and to validate their role in biology-informed treatment planning [18-20].

Limitations

Several additional limitations of this study should be acknowledged to provide a balanced interpretation of the findings. First, although strict inclusion and exclusion criteria were applied, residual confounding cannot be completely excluded. Circulating hypoxia markers, such as VEGF, CA-IX, and lactate may be influenced by systemic inflammation, metabolic status, or underlying comorbidities, which were not exhaustively characterized beyond routine clinical assessment. These factors could partially affect serum marker levels independent of tumor hypoxia.

Second, the study was conducted at two centers within a single geographic region, which may limit the generalizability of the results to other populations with different demographic, genetic, or healthcare characteristics. Multicenter studies involving more diverse patient cohorts would be valuable to confirm the applicability of these findings across broader clinical settings.

Third, while multiple correlations and group-wise comparisons were performed to explore associations between serum markers, tumor HIF-1α expression, and clinicopathological variables, formal adjustment for multiple testing was not applied. The analyses were hypothesis-driven and biologically guided; however, the possibility of type I error cannot be entirely excluded, and the results should therefore be interpreted with appropriate caution.

Finally, as noted earlier, serum hypoxia markers were measured at a single pre-treatment time point, and outcome measures, such as treatment response, recurrence, or survival were not assessed. Longitudinal sampling and outcome-linked analyses would be required to determine the temporal stability, prognostic value, and clinical utility of these biomarkers.

Taken together, these considerations highlight the need for larger, prospective, multicenter studies with comprehensive clinical characterization, longitudinal biomarker assessment, and outcome-based endpoints to validate and extend the present findings.

CONCLUSION

This study shows a consistent link between circulating hypoxia-related biomarkers – VEGF, CA-IX, and serum lactate – and tumor HIF-1α expression in cervical cancer. Higher serum marker levels were seen alongside stronger HIF-1α positivity and were more common in patients with advanced FIGO stage and poorly differentiated tumors, suggesting that an increased systemic hypoxia signal accompanies biologically aggressive disease. The agreement between serum biomarkers and tissue HIF-1α also supports the potential use of blood-based markers as a practical, minimally invasive reflection of hypoxia-driven tumor activity.

A clinically relevant observation was the clustering of elevated serum markers and high HIF-1α expression in advanced-stage cases, where radiotherapy or concurrent chemoradiation is typically the main treatment approach. Although treatment response and survival were not evaluated, these findings indicate that baseline serum hypoxia markers may help flag patients with a hypoxia-associated tumor phenotype that could be more prone to therapeutic resistance.

Overall, the results add support to incorporating minimally invasive biomarker assessment into pre-treatment risk stratification. With further validation, VEGF, CA-IX, and lactate could complement routine staging and histopathology and contribute to more biology-informed treatment planning. Prospective, multicenter studies with serial biomarker measurements and outcome-based endpoints are needed to confirm clinical cut-offs and establish prognostic and predictive utility.

FUNDING STATEMENT

This work did not receive any external funding from public, commercial, or non-profit organizations. The study was conducted using routine institutional facilities and resources available at the participating centers.

CONFLICT OF INTEREST

The author declares no conflict of interest. The work was undertaken independently, with no financial or personal relationships influencing the study design, data acquisition, analysis, interpretation, or preparation of the manuscript.

AUTHORS' CONTRIBUTIONS (TO ADD IN THE REVISED MANUSCRIPT)

Mani Anand contributed to study conceptualization and design, coordinated pathology workflow, performed/oversaw histopathological review and HIF-1α immunohistochemistry interpretation, and contributed to manuscript drafting and critical revision. Loganathan Thangavel supported participant coordination, data acquisition, and clinical data compilation, and assisted in manuscript editing. Senthil Kumar S. contributed to data curation, statistical analysis, results presentation (tables/figures), and interpretation of findings, and revised the manuscript for analytical clarity. Ramesh Kandimalla provided overall supervision and project administration, contributed to methodology planning (biochemical assays and analytical framework), critically revised the manuscript for intellectual content, and served as corresponding author. Prashanth Kumar Patnaik contributed to clinical oversight, staging/treatment-pathway documentation and interpretation, and reviewed the discussion for clinical relevance. Blessy Niharika Mede contributed to methodology and laboratory coordination for serum biomarker estimation/quality control and supported interpretation of biomarker findings, along with manuscript review and editing.

ACKNOWLEDGMENTS

The authors would like to thank the patients who consented to participate in this study. We acknowledge the support of the clinical and technical staff of Government Medical College, Narsampet, and Kakatiya Medical College, Hanumakonda, for their assistance in patient coordination, sample collection, and histopathology processing. The authors also appreciate the cooperation of the Department of Pathology and laboratory personnel involved in immunohistochemistry and biochemical analyses. Their contributions were essential to the completion of this work.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. Due to ethical considerations and patient confidentiality requirements, the data are not publicly available but can be shared in a de-identified form for academic and research purposes.

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