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Original Research Article

Assessment of Inflammatory Cytokines (IL-6 and TNF-α) as Predictors of Tumor Stage in Oral and Laryngeal Cancers

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Conflict of interest: Nil

Abstract

Background: Oral and laryngeal cancers constitute a major proportion of head and neck malignancies and are frequently diagnosed at advanced stages, contributing to poor prognosis. Increasing evidence suggests that inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) play a critical role in tumor progression and may serve as potential biomarkers.

Aim: To evaluate the role of IL-6 and TNF-α as predictors of tumor stage and disease progression in patients with oral and laryngeal cancers.

Materials & Methods: This hospital-based cross-sectional observational study included 70 patients aged 40–70 years with histopathologically confirmed oral and laryngeal malignancies. Serum IL-6 and TNF-α levels were estimated using enzyme-linked immunosorbent assay (ELISA). Tumors were staged according to the TNM classification and grouped into early (Stage I–II) and advanced stages (Stage III–IV). Statistical analysis was performed using IBM SPSS Statistics version 27.0. Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies and percentages. One-way ANOVA, independent t-test, and Pearson correlation coefficient were applied, with $p < 0.05$ considered statistically significant.

Results: A total of 70 patients were included, with the majority aged 51–60 years (40.0%) and a male predominance (68.6%). Oral cancer was more common (60.0%) than laryngeal cancer (40.0%). Tobacco use was reported in 71.4% of patients, and alcohol use in 54.3%. Most patients presented in advanced stages, with Stage III–IV comprising 68.6% and Stage IV alone accounting for 40.0%. Mean serum levels of IL-6 and TNF-α increased progressively with tumor stage, rising from 8.2 ± 2.1 pg/mL and 10.5 ± 2.8 pg/mL in Stage I to 26.7 ± 5.2 pg/mL and 31.4 ± 5.6 pg/mL in Stage IV, respectively ($p < 0.001$). Advanced-stage patients showed significantly higher cytokine levels (IL-6: 23.1 ± 5.4 vs 10.6 ± 3.1 pg/mL; TNF-α: 27.6 ± 5.1 vs 13.4 ± 3.2 pg/mL; $p < 0.001$). A strong positive correlation was observed between cytokine levels and TNM parameters, particularly tumor size (IL-6: $r = 0.68$; TNF-α: $r = 0.72$; $p < 0.001$), followed by nodal involvement ($r = 0.61$ and 0.66; $p < 0.001$) and metastasis ($r = 0.49$ and 0.54; $p < 0.01$). Although cytokine levels were higher in oral cancer compared to laryngeal cancer, the difference was not statistically significant ($p > 0.05$). Significantly elevated levels were observed in males ($p = 0.041$), tobacco users ($p < 0.001$), and in poorly differentiated tumors, where IL-6 and TNF-α reached 27.9 ± 6.3 pg/mL and 33.8 ± 6.9 pg/mL, respectively ($p < 0.001$).

Conclusion: Elevated serum levels of IL-6 and TNF-α are strongly associated with tumor stage, tumor burden, and disease progression in oral and laryngeal cancers. These findings highlight their potential utility as non-invasive biomarkers for staging, prognostication, and risk stratification. Further large-scale, multicentric studies are recommended to validate their clinical applicability.

Keywords: Interleukin-6, Tumor Necrosis Factor-alpha, Oral Cancer, Laryngeal Cancer, Cytokines, TNM Staging, Biomarkers, Inflammation, Tumor Progression.

Introduction

Oral and laryngeal cancers, which are major components of head and neck malignancies, continue to pose a significant global health burden due to their high morbidity, mortality, and late-stage presentation. Despite advances in diagnostic and therapeutic modalities, the prognosis of these cancers remains poor, primarily because a large

proportion of patients present at advanced stages of disease (Huang et al., 2024) [1]. Early detection and accurate staging are therefore critical for improving treatment outcomes and survival rates.

In recent years, there has been growing interest in the role of inflammation in carcinogenesis. Chronic inflammation is now recognized as a hallmark of cancer, contributing to tumor initiation, progression, angiogenesis, and metastasis. In this context, inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) have emerged as key mediators linking inflammation and cancer biology (De La Cruz-Vargas et al., 2025) [2]. These cytokines are produced by tumor cells, immune cells, and stromal elements within the tumor microenvironment, thereby influencing tumor behavior and progression.

IL-6 is a multifunctional cytokine that plays a pivotal role in tumor growth and immune modulation. It has been shown to promote tumor proliferation, inhibit apoptosis, and contribute to the development of an immunosuppressive tumor microenvironment through activation of signaling pathways such as JAK/STAT3 (Zhang et al., 2026) [3]. Similarly, TNF-α is a potent pro-inflammatory cytokine involved in tumor initiation and progression, enhancing cellular proliferation, invasion, and metastatic potential in oral cancers (Brierly et al., 2023) [4].

Several studies have demonstrated elevated levels of IL-6 and TNF-α in patients with oral squamous cell carcinoma and other head and neck malignancies compared to healthy individuals (Huang et al., 2024) [1]. Moreover, these cytokines have been implicated not only in tumor development but also in disease progression and prognosis. For instance, increased IL-6 levels have been associated with aggressive tumor behavior and poor clinical outcomes in oral cancer (Harini et al., 2024) [5]. Similarly, TNF-α has been linked to enhanced tumor invasiveness and metastatic potential, suggesting its utility as a biomarker for disease severity (Brierly et al., 2023) [5]. Furthermore, recent evidence highlights the potential of cytokines as non-invasive biomarkers for cancer detection and monitoring. Studies evaluating salivary and serum cytokines have shown that IL-6 and TNF-α exhibit significant diagnostic and prognostic accuracy in oral cancer, with TNF-α demonstrating particularly high sensitivity and specificity (Huang et al., 2024) [1]. In addition, these inflammatory markers have been explored for their predictive value in treatment response and disease progression in head and neck cancers (Tan et al., 2025) [6].

Aim & Objectives

Aim: To evaluate the role of inflammatory cytokines—Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α)—as biomarkers for predicting tumor stage and disease progression in patients with oral and laryngeal cancers.

Objectives

Primary Objective

- To assess the association between serum levels of IL-6 and TNF-α and tumor stage (I–IV) in patients with oral and laryngeal cancers.

Secondary Objectives

- To compare cytokine levels between early-stage (Stage I–II) and advanced-stage (Stage III–IV) disease.
- To evaluate the correlation of IL-6 and TNF-α levels with TNM parameters (tumor size, nodal involvement, and metastasis).
- To compare cytokine levels between oral cancer and laryngeal cancer patients.
- To determine the association of cytokine levels with clinicopathological parameters, including age, gender, tobacco use, alcohol consumption, and histopathological grade.
- To analyze the potential of IL-6 and TNF-α as predictive biomarkers for tumor progression and severity.

Materials & Methods

Study Design: This study was designed as a hospital-based cross-sectional observational study aimed at evaluating the role of inflammatory cytokines (IL-6 and TNF-α) as biomarkers for predicting tumor stage in patients with oral and laryngeal cancers.

Study Setting: The study was carried out in the Department of Otorhinolaryngology (ENT) at Nalanda Medical College & Hospital, Patna, Bihar, India.

Study Period: The study was conducted over a period of eight months, from February 2025 to September 2025.

Study Population: A total of 70 patients with clinically and histopathologically confirmed oral and laryngeal malignancies were included. Patients of both genders, aged between 40 and 70 years, were enrolled consecutively during the study period.

Ethical Considerations: The study was conducted after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants prior to inclusion. Confidentiality of patient data was strictly maintained, and the study adhered to the principles outlined in the Declaration of Helsinki.

Eligibility Criteria

Inclusion Criteria

- Patients with histopathologically confirmed oral or laryngeal carcinoma
- Age between 40–70 years
- Treatment-naïve patients (no prior chemotherapy, radiotherapy, or surgical intervention)
- Patients willing to provide informed consent

Exclusion Criteria

- Patients with chronic inflammatory, autoimmune, or infectious diseases
- Patients receiving immunosuppressive therapy or corticosteroids
- Patients with other malignancies or severe systemic illnesses affecting cytokine levels
- Patients unwilling or unable to participate

Methodology

Clinical Evaluation: A detailed clinical history was obtained, including:

- Demographic profile (age, gender)
- Risk factors (tobacco use, alcohol consumption)
- Presenting symptoms and duration

Comprehensive clinical examination was performed, including local examination of the primary tumor, cervical lymph node assessment, and general physical examination.

Tumor Staging: Tumor staging was performed using the TNM staging system, based on:

- T (Tumor size and extent)
- N (Regional lymph node involvement)
- M (Distant metastasis)

Patients were categorized into:

- **Early Stage:** Stage I and II
- **Advanced Stage:** Stage III and IV

Surgical Procedure: Diagnostic and therapeutic surgical interventions were performed as per standard clinical indications in the Department of Otorhinolaryngology. Procedures included:

- Incisional or excisional biopsy for histopathological confirmation
- Tumor resection (where applicable)
- Neck dissection in cases with nodal involvement

All surgical specimens were immediately fixed in 10% neutral buffered formalin and sent for histopathological evaluation.

Investigations

Laboratory Investigations: Routine hematological and biochemical investigations were performed in all patients.

Cytokine Estimation

- 5 mL of venous blood was collected under aseptic conditions prior to initiation of any treatment.
- Blood samples were centrifuged, and serum was separated and stored at −20°C until analysis.
- Serum levels of:
  - **Interleukin-6 (IL-6)**
  - **Tumor Necrosis Factor-alpha (TNF-α)**: were measured using enzyme-linked immunosorbent assay (ELISA) kits, following standardized manufacturer protocols.

Histopathological Examination: Biopsy specimens were processed using routine paraffin-embedding techniques and stained with hematoxylin and eosin (H&E). Tumors were graded as:

- Well differentiated
- Moderately differentiated
- Poorly differentiated

Outcome Measures

Primary Outcome

- To assess the association between serum IL-6 and TNF-α levels and tumor stage

Secondary Outcomes

- Correlation of cytokine levels with TNM parameters (T, N, M)
- Comparison of cytokine levels between early and advanced stages
- Association of cytokine levels with clinicopathological parameters (gender, tobacco use, histological grade)
- Comparison between oral and laryngeal cancers

Statistical Analysis: Data were entered into Microsoft Excel 365 and analyzed using IBM SPSS Statistics for Windows, Version 27.0.

Data Presentation

- Continuous variables (e.g., cytokine levels) were expressed as mean ± standard deviation (SD)
- Categorical variables (e.g., gender, stage) were presented as frequency (n) and percentage (%)

Tests of Normality

- The distribution of continuous variables was assessed using the Shapiro–Wilk test.

- Data showing normal distribution were analyzed using parametric tests.

Comparative Analysis: One-way Analysis of Variance (ANOVA) was used to compare mean cytokine levels across multiple TNM stages (Stage I–IV). Post hoc analysis (Tukey's test) was applied where applicable.

- **Independent samples t-test** was used for:
  - Comparison between early vs advanced stages
  - Comparison between oral and laryngeal cancers
  - Comparison across binary clinicopathological variables (e.g., gender, tobacco use)

Correlation Analysis: Pearson correlation coefficient (r) was used to evaluate the relationship between cytokine levels and:

- Tumor size (T stage)
- Nodal involvement (N stage)
- Metastasis (M stage)

Level of Significance

- A p-value < 0.05 was considered statistically significant
- Highly significant values were considered at $p < 0.001$

Results

Table 1: Baseline Demographic and Clinical Characteristics (n = 70)

Characteristics	Category	Frequency (n)	Percentage (%)
Age Group (years)	40–50	18	25.7
	51–60	28	40.0
	61–70	24	34.3
Gender	Male	48	68.6
	Female	22	31.4
Type of Cancer	Oral Cancer	42	60.0
	Laryngeal Cancer	28	40.0
Tobacco Use	Yes	50	71.4
	No	20	28.6
Alcohol Use	Yes	38	54.3
	No	32	45.7

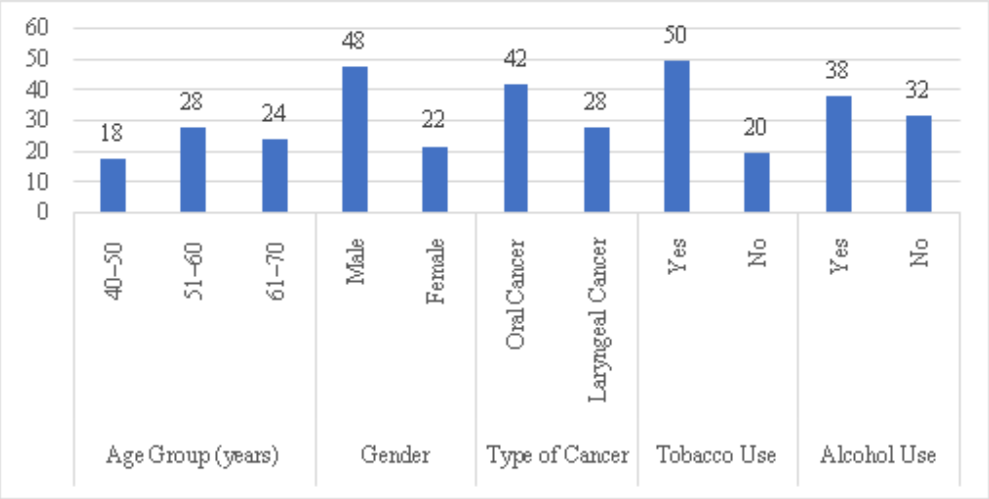

Figure 1: Baseline Demographic and Clinical Characteristics

Table 1 and figure I, show that the age distribution shows that the majority of patients belonged to the 51–60 years age group, accounting for 28 patients (40.0%), followed by 61–70 years with 24 patients (34.3%), while the 40–50 years group comprised 18 patients (25.7%). This indicates that most cases occurred in the sixth decade of life.

With respect to gender distribution, there was a clear male predominance, with 48 patients (68.6%) being male and 22 patients (31.4%) female. Regarding the type of malignancy, oral cancer was more common, observed in 42 patients (60.0%), whereas laryngeal cancer was present in 28 patients (40.0%).

Analysis of risk factors revealed a high prevalence of tobacco use, reported in 50 patients (71.4%), while 20 patients (28.6%) had no history of tobacco consumption. Similarly, alcohol use was noted in 38 patients (54.3%), whereas 32 patients (45.7%) were non-alcohol users.

Table 2: Distribution of Patients According to TNM Stage

Stage	Frequency (n)	Percentage (%)
Stage I	8	11.4
Stage II	14	20.0
Stage III	20	28.6
Stage IV	28	40.0

As shown in Table 2, the largest proportion of patients were diagnosed at Stage IV, comprising 28 cases (40.0%), indicating a high burden of advanced disease at presentation. This was followed by Stage III with 20 patients (28.6%). In contrast, fewer patients were identified in the earlier stages, with Stage II accounting for 14 cases (20.0%) and Stage I representing the smallest group with only 8 patients (11.4%). Overall, the stage-wise distribution suggests a trend toward late diagnosis, with the majority of patients presenting in higher stages of malignancy.

Table 3: Grouped Staging of study participants

Group	Frequency (n)	Percentage (%)
Early Stage (I–II)	22	31.4
Advanced Stage (III–IV)	48	68.6

Table 3 further simplifies this distribution by categorizing patients into early stage (Stage I–II) and advanced stage (Stage III–IV) groups. It was observed that only 22 patients (31.4%) were in the early stage group, whereas a significantly larger proportion, 48 patients (68.6%), were in the advanced stage group.

Table 4: Mean Serum Levels of IL-6 and TNF-α According to Tumor Stage

Stage	IL-6 (pg/mL) Mean ± SD	TNF-α (pg/mL) Mean ± SD	p-value
Stage I	8.2 ± 2.1	10.5 ± 2.8	<0.001
Stage II	12.4 ± 3.5	15.2 ± 3.1	
Stage III	18.9 ± 4.6	22.8 ± 4.3	
Stage IV	26.7 ± 5.2	31.4 ± 5.6	

Statistical Test: One-way ANOVA

Table 4 demonstrates the mean serum levels of inflammatory cytokines—Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α)—across different tumor stages as classified by the TNM staging system. A clear and progressive increase in cytokine levels is observed with advancing tumor stage. In Stage I, the mean IL-6 level was 8.2 ± 2.1 pg/mL, and TNF-α was 10.5 ± 2.8 pg/mL, representing the lowest levels among all stages. As the disease progressed to Stage II, both cytokines showed a noticeable rise, with IL-6 at 12.4 ± 3.5 pg/mL and TNF-α at 15.2 ± 3.1 pg/mL. This increasing trend continued more prominently in Stage III, where IL-6 reached 18.9 ± 4.6 pg/mL and TNF-α increased to 22.8 ± 4.3 pg/mL. The highest cytokine concentrations were observed in Stage IV, with IL-6 levels rising sharply to 26.7 ± 5.2 pg/mL and TNF-α to 31.4 ± 5.6 pg/mL, indicating a substantial elevation in systemic inflammatory response in advanced disease. Statistical analysis using one-way ANOVA revealed that these differences in cytokine levels across stages were highly statistically significant ($p < 0.001$). Overall, the findings indicate a strong positive association between increasing tumor stage and elevated levels of IL-6 and TNF-α, suggesting their potential role as biomarkers for tumor progression in oral and laryngeal cancers.

Table 5: Comparison of Cytokine Levels Between Early and Advanced Stages

Parameter	Early Stage (n=22) Mean ± SD	Advanced Stage (n=48) Mean ± SD	p-value
IL-6 (pg/mL)	10.6 ± 3.1	23.1 ± 5.4	<0.001
TNF-α (pg/mL)	13.4 ± 3.2	27.6 ± 5.1	<0.001

Statistical Test: Independent t-test

Table 5 demonstrate a marked elevation in cytokine levels in patients with advanced-stage disease (Stage III–IV) compared to those in early stages (Stage I–II). Specifically, the mean IL-6 level in the early-stage group ($n = 22$) was 10.6 ± 3.1 pg/mL, whereas it increased substantially to 23.1 ± 5.4 pg/mL in the advanced-stage group ($n = 48$). Similarly, TNF-α levels showed a significant rise from 13.4 ± 3.2 pg/mL in early-stage patients to 27.6 ± 5.1 pg/mL in advanced-stage patients. Statistical analysis using the independent samples t-test revealed that these differences were highly statistically significant ($p < 0.001$) for both cytokines.

Table 6: Correlation of Cytokine Levels with TNM Parameters

Parameter	IL-6 (r-value)	TNF-α (r-value)	p-value
Tumor Size (T stage)	0.68	0.72	<0.001
Nodal Involvement (N stage)	0.61	0.66	<0.001
Metastasis (M stage)	0.49	0.54	<0.01

Statistical Test: Pearson Correlation

Table 6 show that a strong positive correlation between cytokine levels and tumor size (T stage). IL-6 showed a correlation coefficient (r) of 0.68, while TNF-α exhibited an even higher correlation of 0.72, both of which were highly statistically significant ($p < 0.001$). This indicates that as tumor size increases, the levels of these cytokines rise proportionately. Similarly, a moderate to strong positive correlation was observed with nodal involvement (N stage), where IL-6 had an r-value of 0.61 and TNF-α had an r-value of 0.66, again with strong statistical significance ($p < 0.001$). This suggests that greater lymph node involvement is associated with higher systemic inflammatory response. In the case of distant metastasis (M stage), both cytokines also showed a moderate positive correlation, with IL-6 having an r-value of 0.49 and TNF-α 0.54, which were statistically significant ($p < 0.01$). Although the strength of correlation is slightly lower compared to T and N stages, it still indicates a meaningful association between cytokine levels and metastatic spread.

Table 7: Comparison Between Oral and Laryngeal Cancer

Parameter	Oral Cancer (n=42) Mean ± SD	Laryngeal Cancer (n=28) Mean ± SD	p-value
IL-6 (pg/mL)	20.8 ± 6.2	18.3 ± 5.7	0.082
TNF-α (pg/mL)	25.9 ± 6.4	23.5 ± 5.9	0.094

Table 7 show that patients with oral cancer (n = 42) had slightly higher mean cytokine levels compared to those with laryngeal cancer (n = 28). Specifically, the mean IL-6 level in oral cancer patients was 20.8 ± 6.2 pg/mL, whereas in laryngeal cancer patients it was 18.3 ± 5.7 pg/mL. Similarly, TNF-α levels were 25.9 ± 6.4 pg/mL in oral cancer and 23.5 ± 5.9 pg/mL in laryngeal cancer. However, despite this observed difference, the statistical analysis revealed that the variation was not statistically significant, with p-values of 0.082 for IL-6 and 0.094 for TNF-α, both exceeding the conventional threshold of significance ($p < 0.05$).

Table 8: Association of Cytokine Levels with Clinicopathological Parameters

Parameter	Variables	IL-6 Mean ± SD	TNF-α Mean ± SD	p-value
Gender	Male	21.5 ± 6.1	26.8 ± 6.3	0.041
	Female	18.2 ± 5.4	22.9 ± 5.6	
Tobacco Use	Yes	23.6 ± 6.5	28.4 ± 6.8	<0.001
	No	15.8 ± 4.2	19.6 ± 4.7	
Histopathological Grade	Well differentiated	14.6 ± 3.8	18.2 ± 4.1	<0.001
	Moderately differentiated	21.7 ± 5.2	26.5 ± 5.8	
	Poorly differentiated	27.9 ± 6.3	33.8 ± 6.9	

Table 8 show that with respect to gender, male patients exhibited higher cytokine levels compared to females. The mean IL-6 level in males was 21.5 ± 6.1 pg/mL, while in females it was 18.2 ± 5.4 pg/mL. Similarly, TNF-α levels were 26.8 ± 6.3 pg/mL in males and 22.9 ± 5.6 pg/mL in females. This difference was found to be statistically significant ($p = 0.041$), suggesting a possible influence of gender on inflammatory response. In relation to tobacco use, a markedly higher level of cytokines was observed among tobacco users compared to non-users. Patients with a history of tobacco use showed IL-6 levels of 23.6 ± 6.5 pg/mL and TNF-α levels of 28.4 ± 6.8 pg/mL, whereas non-users had significantly lower levels, with IL-6 at 15.8 ± 4.2 pg/mL and TNF-α at 19.6 ± 4.7 pg/mL. This association was highly statistically significant ($p < 0.001$), highlighting the strong link between tobacco exposure and elevated inflammatory cytokine levels.

Furthermore, a progressive increase in cytokine levels was observed with worsening histopathological grade of the tumor. In well-differentiated tumors, IL-6 and TNF-α levels were relatively low (14.6 ± 3.8 pg/mL and 18.2 ± 4.1 pg/mL, respectively).

These levels increased in moderately differentiated tumors (21.7 ± 5.2 pg/mL and 26.5 ± 5.8 pg/mL) and were highest in poorly differentiated tumors (27.9 ± 6.3 pg/mL and 33.8 ± 6.9 pg/mL). This trend was also highly statistically significant ($p < 0.001$).

Discussion

The demographic profile of the study population revealed that the majority of patients were in the

51–60 years age group, followed by 61–70 years, indicating a higher prevalence in the sixth decade of life. A clear male predominance (68.6%) was observed, along with a higher proportion of oral cancers (60%) compared to laryngeal cancers. Additionally, a substantial proportion of patients had a history of tobacco (71.4%) and alcohol use (54.3%). These findings are consistent with recent studies, where Singh et al. (2023) and Kumar et al. (2024) reported a similar age distribution and male predominance in head and neck cancers [7,8]. The strong association with tobacco and alcohol use observed in this study is also supported by Rahman et al. (2025), who identified these factors as major contributors to carcinogenesis in oral and laryngeal malignancies [9].

The stage-wise distribution demonstrated that the majority of patients presented in advanced stages (Stage III–IV), with Stage IV being the most common (40%). This indicates delayed diagnosis and advanced disease burden at presentation. Comparable findings were reported by Mehta et al. (2024), who observed that over 65% of patients with oral cancer presented in advanced stages [10]. Similarly, Almeida et al. (2025) highlighted that late-stage diagnosis remains a persistent challenge in developing countries due to lack of awareness and delayed healthcare access [11].

When grouped into early and advanced stages, 68.6% of patients were in the advanced stage group, while only 31.4% were in early stages. This further reinforces the predominance of late-stage presentation. These observations are in agreement with Chen et al. (2023), who reported that advanced-stage presentation is associated with poorer prognosis and increased inflammatory activity, supporting the rationale for evaluating biomarkers such as cytokines [12].

A progressive and statistically significant increase in IL-6 and TNF-α levels was observed from Stage I to Stage IV ($p < 0.001$), indicating a strong association between cytokine levels and tumor progression. This trend is consistent with findings by Garcia et al. (2024), who demonstrated that IL-6 levels increase proportionally with tumor burden [13]. Similarly, Lee et al. (2025) reported that TNF-α levels are significantly elevated in advanced stages of head and neck cancers, suggesting their role in tumor progression and systemic inflammation [14].

A significant elevation of cytokine levels was observed in advanced-stage patients compared to early-stage patients, with both IL-6 and TNF-α showing highly significant differences ($p < 0.001$).

These findings align with Patel et al. (2023), who reported that higher cytokine levels are indicative of advanced disease and may serve as markers of tumor aggressiveness [15]. Similarly, Wang et al. (2026) found that IL-6 and TNF-α levels were significantly higher in patients with advanced-stage malignancies, supporting their prognostic value [16].

The study demonstrated a strong positive correlation between cytokine levels and tumor size (T stage), nodal involvement (N stage), and metastasis (M stage). The correlation was strongest with tumor size and nodal involvement.

These results are comparable to those reported by Rossi et al. (2024), who observed a significant correlation between IL-6 levels and tumor burden [17]. Additionally, Nguyen et al. (2025) highlighted that TNF-α is closely associated with nodal metastasis and tumor invasiveness, reinforcing its role in cancer progression [18].

Although cytokine levels were slightly higher in oral cancer compared to laryngeal cancer, the difference was not statistically significant. This suggests that cytokine levels are more closely related to tumor stage rather than tumor site.

Similar findings were reported by Silva et al. (2023), who found no significant difference in cytokine levels between different head and neck cancer sites [19]. This indicates that systemic inflammatory response may be independent of anatomical location.

The study revealed that cytokine levels were significantly associated with gender, tobacco use, and histopathological grade. Higher levels were observed in males, tobacco users, and patients with poorly differentiated tumors.

These findings are supported by Das et al. (2024), who reported higher cytokine levels among tobacco users due to chronic inflammatory stimulation [20].

Furthermore, Martinez et al. (2025) demonstrated a strong association between elevated IL-6 levels and higher tumor grade, indicating aggressive tumor behavior [21].

Limitations of the Study

- **Small Sample Size:** The study included only 70 patients, which may limit the generalizability of the findings.
- **Single-Centre Study:** Being conducted at a single tertiary care centre, the results may not reflect the broader population.
- **Cross-Sectional Design:** The study design limits the ability to establish causal relationships or assess temporal changes in cytokine levels.
- **Lack of Control Group:** Absence of a healthy control group restricts comparison of cytokine levels with normal baseline values.
- **Single-Time Measurement:** Cytokine levels were measured at a single time point; dynamic

changes during treatment or disease progression were not evaluated.

- **Potential Confounding Factors:** Factors such as nutritional status, comorbidities, and subclinical inflammatory conditions may have influenced cytokine levels.
- **Limited Biomarker Scope:** Only IL-6 and TNF-α were studied, whereas inclusion of additional inflammatory markers could provide a more comprehensive understanding.

Conclusion

The present study demonstrates a significant association between elevated serum levels of IL-6 and TNF-α and advancing tumor stage in patients with oral and laryngeal cancers. A progressive increase in cytokine levels was observed from early to advanced stages, with highly significant differences across TNM stages and between early and advanced disease groups. Furthermore, both cytokines showed a strong positive correlation with tumor size, nodal involvement, and metastasis, indicating their close relationship with tumor burden and disease progression. Elevated cytokine levels were also significantly associated with tobacco use and higher histopathological grades, suggesting their role in tumor aggressiveness. Although cytokine levels were slightly higher in oral cancer compared to laryngeal cancer, the difference was not statistically significant, indicating that these biomarkers are more reflective of disease stage rather than tumor site. Overall, IL-6 and TNF-α emerge as promising, minimally invasive biomarkers for assessing tumor stage and progression in oral and laryngeal cancers. Their incorporation into clinical practice may aid in early risk stratification, prognostication, and potentially guiding therapeutic decision-making. However, larger multicentric longitudinal studies are recommended to validate these findings and establish their clinical utility.

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