

Deciphering The Angiogenic and Inflammatory Profiles of Gingival Enlargement in Adolescents: An Immunohistochemical Study

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Abstract

Background:

Gingival enlargement in adolescents may involve complex inflammatory and vascular tissue responses, but the relationship between angiogenesis and local inflammation remains incompletely characterized. This study evaluated the expression of angiogenic and inflammatory markers in gingival enlargement tissues and examined their associations with histopathological inflammatory changes.

Materials & Methods:

A comparative study was conducted among 60 adolescents, comprising 30 participants with clinically evident gingival enlargement and 30 age- and sex-matched healthy controls. Gingival tissue specimens were evaluated histopathologically and by immunohistochemistry. Vascular endothelial growth factor (VEGF) and CD34-positive microvessel density (MVD) were assessed as indicators of angiogenic activity, while interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) were evaluated as inflammatory markers. Immunohistochemical expression was assessed using H-scores, staining intensity, and marker positivity. Correlation and multivariable analyses were performed to examine relationships between marker expression and gingival enlargement.

Results:

The gingival enlargement group demonstrated significantly higher gingival enlargement, inflammatory infiltrate, vascularity, and fibrosis scores than the control group probability ($p < 0.001$ for all). VEGF expression was significantly greater in the gingival enlargement group (186.4 ± 31.8) than in controls (92.7 ± 24.6 ; $p < 0.001$), while CD34-positive MVD was also higher (28.6 ± 6.7 vs. 17.4 ± 4.9 vessels/ high-power field (HPF); $p < 0.001$). Similarly, IL-6 and TNF- α H-scores were significantly elevated in gingival enlargement specimens (171.8 ± 35.2 vs. 79.6 ± 22.8 and 159.3 ± 33.7 vs. 72.4 ± 20.9 , respectively; $p < 0.001$). VEGF and CD34 positivity, as well as IL-6 and TNF- α positivity, were significantly more frequent in the gingival enlargement group. Significant positive correlations were observed between VEGF and CD34 MVD ($r = 0.68$, $p < 0.001$), VEGF and IL-6 ($r = 0.59$, $p = 0.001$), and VEGF and TNF- α ($r = 0.54$, $p = 0.002$). IL-6 demonstrated the strongest correlation with the histopathological inflammatory infiltrate score ($r = 0.73$, $p < 0.001$). Multivariable analysis showed that VEGF, CD34 MVD, IL-6, and TNF- α remained independently associated with gingival enlargement.

Conclusion:

Gingival enlargement in adolescents is characterized by increased angiogenic and inflammatory activity, accompanied by greater vascularity, inflammatory infiltration, and fibrosis. The significant associations among VEGF, CD34, IL-6, and TNF- α suggest an interconnected angiogenic-inflammatory response that may contribute to the histopathological changes observed in gingival enlargement.

Keywords: Angiogenesis, CD34, Gingival enlargement, Histopathology, Immunohistochemistry, Inflammation, Interleukin-6, Tumor necrosis factor-alpha, Vascular endothelial growth factor.

Introduction

Gingival enlargement represents an abnormal increase in gingival volume and may arise from inflammatory, drug-related, hereditary, systemic, neoplastic, or other localized pathological conditions. Although the clinical appearance may be similar among different forms of gingival enlargement, the underlying tissue response can vary considerably. Plaque-associated inflammation remains an important contributor to gingival enlargement, while medications and genetically determined alterations in gingival connective-tissue metabolism may further modify its severity and clinical expression [1]. In adolescents, gingival enlargement is of particular clinical importance

because changes in gingival contour may interfere with oral hygiene, eruption and alignment of teeth, periodontal health, mastication, speech, and facial aesthetics.

The biological basis of gingival enlargement is complex and involves interactions between inflammatory mediators, gingival fibroblasts, extracellular-matrix (ECM) turnover, epithelial responses, and vascular changes. Drug-associated gingival overgrowth, for example, has been linked to altered fibroblast activity, impaired collagen degradation, accumulation of ECM components, and variable degrees of inflammation [2]. The magnitude and histological characteristics of the enlargement may also be influenced by individual susceptibility, local plaque accumulation, medication-related effects, and differences in fibroblast phenotype [3]. Consequently, gingival enlargement should not be regarded simply as an increase in tissue mass but rather as the outcome of a dynamic biological process involving several interconnected cellular and molecular pathways. Inflammation has a central role in this process. Persistent exposure to microbial biofilm can stimulate vascular, epithelial, and immune-cell responses within the gingival tissues, resulting in the recruitment of inflammatory cells and release of cytokines and growth factors. Experimental and clinical investigations have demonstrated alterations in endothelial adhesion molecules and inflammatory-cell populations during the progression of gingival inflammation [4]. Furthermore, inflammatory stimulation of gingival fibroblasts can modify the expression of vascular and lymphatic growth factors, suggesting that inflammation may actively influence the vascular microenvironment of the enlarged gingival. These observations provide a biological basis for investigating inflammatory activity together with structural changes in gingival tissue [5].

Angiogenesis is another potentially important component of gingival tissue remodeling. Vascular endothelial growth factor (VEGF) is a major mediator of angiogenesis and promotes endothelial-cell proliferation, migration, vascular permeability, and survival [6]. Increased VEGF expression has been demonstrated in inflamed gingival tissues, and its expression has been associated with alterations in gingival vascularity and inflammatory status [7]. Immunohistochemical investigations have additionally demonstrated increased expression of angiogenesis-related markers, including VEGF and endothelial markers such as CD31, together with increased microvascular activity in diseased gingival tissues. These findings suggest that vascular remodeling may accompany or contribute to the inflammatory changes occurring within gingival tissues [8].

Importantly, angiogenesis and inflammation are not independent biological events. Inflammatory cytokines and cellular mediators can stimulate angiogenic pathways, while newly formed or activated vascular endothelium can facilitate the recruitment and trafficking of inflammatory cells into affected tissues. The resulting interaction may contribute to persistence of inflammation and continued tissue remodeling. Studies of periodontal tissues have demonstrated increased expression of angiogenesis-associated proteins and microvascular parameters in inflammatory periodontal disease, supporting the concept that vascular alterations form an integral component of the gingival response to chronic inflammation [9].

Despite increasing understanding of the molecular mechanisms involved in gingival overgrowth, comparatively limited attention has been directed toward the simultaneous characterization of angiogenic and inflammatory profiles in gingival enlargement among adolescents. Most available investigations have focused on drug-induced gingival overgrowth, periodontal inflammation, or adult populations, while the immunohistochemical relationship between vascular activity and inflammatory alterations in adolescent gingival enlargement remains insufficiently characterized. Adolescence also represents a biologically relevant period in which hormonal changes, oral-hygiene practices, medication exposure, and tissue remodeling may interact and influence gingival responses. Therefore, an immunohistochemical assessment of gingival enlargement may provide valuable insight into the tissue-level mechanisms underlying its development and progression. Evaluation of angiogenic markers such as VEGF and CD31, together with selected inflammatory markers, can help characterize the vascular and inflammatory microenvironment of enlarged gingiva. Such an approach may improve understanding of the biological heterogeneity of gingival enlargement and establish potential associations between clinical enlargement, histopathological changes, angiogenic activity, and inflammatory burden. The present study was therefore undertaken to decipher the angiogenic and inflammatory profiles of gingival enlargement in adolescents using immunohistochemical analysis, with the aim of providing a more comprehensive understanding of the molecular and tissue-level events associated with this clinically relevant condition.

Materials & Methods

Study Design and Setting

A comparative observational study was conducted to investigate the histopathological and immunohistochemical characteristics of gingival enlargement in adolescents, with particular emphasis on angiogenic and inflammatory responses. The study was carried out in the Department of Periodontology in collaboration with the Department of Oral and Maxillofacial Pathology at a tertiary dental care centre. The study period extended from January to March 2026. The study protocol was reviewed and approved by the Institutional Ethics Committee before commencement. Written informed consent was obtained from the parents or legal guardians of all participants, and assent was obtained from the adolescents before enrolment. All procedures were performed in accordance with the ethical principles of the Declaration of Helsinki.

Study Population and Sample Size

The study included 60 adolescents who were allocated into two groups. The gingival enlargement group consisted of 30 adolescents presenting with clinically evident gingival enlargement, whereas the control group comprised 30 age- and sex-matched adolescents without clinically detectable gingival enlargement or significant periodontal abnormalities. Participants were recruited consecutively from patients attending the dental outpatient department. Controls were selected from adolescents undergoing routine dental examination and were frequency matched to the study group according to age and sex. Eligibility was determined after a detailed medical and dental history, clinical examination, and assessment of the gingival tissues.

Inclusion Criteria

Gingival Enlargement Group

Participants were included in the gingival enlargement group when they fulfilled all of the following criteria:

- Adolescents within the predefined study age range
- Presence of clinically evident gingival enlargement involving one or more gingival sites
- Gingival enlargement characterized by an increase in gingival volume beyond the normal anatomical contour
- Presence of sufficient gingival tissue for histopathological and immunohistochemical evaluation
- No clinical evidence suggesting an acute necrotizing periodontal condition
- No requirement for emergency periodontal treatment at the time of tissue collection
- Availability of adequate clinical and histopathological information for analysis
- Willingness of the participant and parent/legal guardian to participate in the study
- Provision of written informed consent by the parent or legal guardian and assent by the adolescent

Control Group

Participants were included in the control group when they met the following criteria:

- Adolescents within the same predefined age range as the study group
- Absence of clinically apparent gingival enlargement
- Clinically healthy gingival tissues without significant periodontal destruction
- No history or clinical evidence of a condition requiring gingival tissue biopsy
- Age- and sex-matched with participants in the gingival enlargement group
- Adequate clinical records and willingness to participate.
- Provision of informed consent by the parent or legal guardian and assent by the adolescent.

Exclusion Criteria

Participants were excluded if any of the following conditions were present:

- Known systemic diseases or medical disorders that could independently affect gingival tissues or modify inflammatory and vascular responses
- Current or recent use of medications known to cause or substantially influence gingival enlargement, unless the medication-related etiology was specifically intended for evaluation
- Previous periodontal surgery or extensive gingival surgical treatment at the proposed sampling site

- Previous radiotherapy or chemotherapy involving the head and neck region
- Known hematological, immunological, or connective-tissue disorders that could influence periodontal or inflammatory findings
- Presence of acute oral infections or extensive ulcerative lesions at the tissue sampling site
- Severe generalized periodontal disease unrelated to the clinical diagnosis under investigation
- Presence of clinically suspected malignant or potentially malignant lesions at the sampling site
- Recent use of systemic anti-inflammatory, immunosuppressive, or antimicrobial medications that could substantially alter local inflammatory responses.
- Inability to obtain an adequate gingival tissue specimen for histopathological or immunohistochemical examination
- Inadequate or poorly preserved tissue specimens
- Refusal of the participant or parent/legal guardian to provide consent or assent
- Incomplete clinical, histopathological, or immunohistochemical data

Clinical Assessment

A standardized clinical examination was performed under adequate illumination using a mouth mirror and periodontal probe. Gingival tissues were examined for changes in contour, colour, consistency, size, and surface characteristics. The extent of gingival enlargement was recorded according to a predefined clinical scoring system. Gingival enlargement was assessed at the selected sites by evaluating the degree to which the enlarged gingiva covered the anatomical crown or altered the normal gingival contour. Clinical findings were recorded before tissue sampling to minimize the possibility of bias arising from knowledge of histopathological or immunohistochemical findings. Demographic characteristics, relevant medical history, medication history, oral hygiene status, and periodontal findings were also documented.

Gingival Tissue Collection

Gingival tissue specimens were obtained from clinically representative sites in participants with gingival enlargement when tissue removal was clinically indicated as part of the planned periodontal management. For the control group, tissue specimens were obtained only when clinically justified, such as during a planned dental procedure in which removal of a small amount of otherwise healthy gingival tissue was required. Following administration of local anaesthesia, the tissue was carefully excised using standard periodontal surgical techniques. Hemostasis was achieved using routine measures. Each specimen was immediately placed in an appropriately labelled container containing 10% neutral buffered formalin for fixation. Specimens were coded using study identification numbers so that laboratory assessment could be performed without direct knowledge of the participant's clinical group whenever feasible.

Histopathological Processing

Fixed tissue specimens were routinely processed, dehydrated through graded concentrations of alcohol, cleared in xylene, and embedded in paraffin wax. Serial sections approximately 4–5 µm thick were prepared using a rotary microtome. Sections were mounted on glass slides and stained with hematoxylin and eosin for routine histopathological assessment. The stained sections were examined under light microscopy for epithelial and connective-tissue alterations, inflammatory cell infiltration, vascular changes, fibrosis, and other microscopic features relevant to gingival enlargement.

Histopathological Assessment

Histopathological evaluation was performed using a standardized semiquantitative scoring system. The degree of inflammatory cellular infiltration was assessed according to the density and distribution of inflammatory cells within the gingival connective tissue. Vascularity was assessed based on the number and prominence of identifiable vascular structures within representative microscopic fields. Fibrosis was evaluated according to the relative extent of collagen-rich connective tissue and the degree of fibrotic alteration. For each specimen, representative areas were selected after examination of the entire section. Histopathological scoring was performed by an examiner who was blinded to the immunohistochemical findings. When applicable, multiple high-power fields (HPFs) were evaluated and the mean score was used for statistical analysis.

Immunohistochemical Evaluation

Immunohistochemical analysis was performed to investigate angiogenic and inflammatory activity within the gingival tissues. The angiogenic markers evaluated were VEGF and CD34, while interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were assessed as inflammatory markers. Paraffin-embedded tissue sections were mounted on positively charged slides and subjected to routine deparaffinization and rehydration. Antigen retrieval was performed using an appropriate heat-induced antigen retrieval procedure according to the requirements of each antibody. Endogenous peroxidase activity was blocked before incubation with the respective primary antibodies. Sections were subsequently incubated with appropriate secondary detection reagents, followed by chromogenic development. The sections were counterstained with hematoxylin, dehydrated, cleared, and mounted. Positive and negative controls were included during immunohistochemical processing to verify the specificity and adequacy of staining.

Assessment of VEGF Expression

VEGF immunoreactivity was evaluated in representative gingival connective-tissue areas. Positive staining was identified according to the characteristic brown chromogenic reaction within the relevant cellular and tissue compartments. The intensity and distribution of staining were assessed microscopically. VEGF expression was quantified using an H-score-based approach incorporating both staining intensity and the proportion of immunopositive cells. The H-score provided a semiquantitative measure of overall VEGF expression and was subsequently used for comparison between the gingival enlargement and control groups.

Assessment of CD34-Positive Micro Vessel Density (MVD)

CD34 immunostaining was used to identify vascular endothelial cells and quantify MVD. Areas demonstrating the greatest concentration of immunopositive microvessels were identified during initial scanning of the tissue section. Representative high-power fields were subsequently examined, and CD34-positive microvessels were counted. Each distinct CD34-positive vascular structure with a clearly identifiable endothelial lining was considered a microvessel, irrespective of the presence or absence of a visible vascular lumen. The mean number of CD34-positive microvessels per high-power field was calculated for each specimen and recorded as the CD34-positive MVD.

Assessment of IL-6 and TNF- α Expression

IL-6 and TNF- α immunoreactivity was assessed in the gingival tissue sections using the same standardized microscopic approach. Positive staining was identified by the presence of specific brown immunoreactivity within the relevant cellular compartments. For each marker, staining intensity and the proportion of positive cells were recorded. An H-score was subsequently calculated to provide a composite estimate of immunohistochemical expression. In addition, specimens were categorized according to the presence or absence of detectable immunopositivity for comparative analysis between groups.

Immunohistochemical Scoring and Blinding

All immunohistochemical sections were examined independently of the clinical assessment wherever feasible. The evaluator was blinded to participant group allocation during microscopic scoring. Staining intensity was categorized according to a predefined ordinal scale, while the percentage of positive cells was estimated within representative fields. The H-score was calculated by combining staining intensity with the corresponding proportion of immunoreactive cells. This approach allowed both the extent and intensity of tissue expression to be incorporated into a single semiquantitative measure.

Interobserver and Intraobserver Reliability

To assess the reproducibility of microscopic evaluation, a subset of randomly selected sections was reassessed by the same examiner after an interval without access to the original scores. Where more than one examiner participated in microscopic assessment, the same subset was independently evaluated by a second examiner. Agreement was assessed using an appropriate reliability statistic. Any discrepancy was resolved by joint review of the corresponding microscopic field according to the predefined scoring criteria.

Outcome Measures

The primary outcomes were the differences in angiogenic and inflammatory marker expression between adolescents with gingival enlargement and healthy controls.

The principal angiogenic outcomes included:

- VEGF immunohistochemical H-score
- Proportion of VEGF-positive specimens
- CD34-positive MVD

The principal inflammatory outcomes included:

- IL-6 immunohistochemical H-score
- Proportion of IL-6-positive specimens
- TNF- α immunohistochemical H-score
- Proportion of TNF- α -positive specimens

Secondary histopathological outcomes included inflammatory infiltrate, vascularity, and fibrosis scores.

Associations among angiogenic markers, inflammatory markers, and histopathological inflammatory changes were also evaluated.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), IBM SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, NY, USA). Continuous variables such as age were summarized using mean and standard deviation (SD). Between-group comparisons of continuous variables were performed using the independent-samples t-test when assumptions of normality were satisfied; categorical variables were compared using the Fisher's exact test, as appropriate. Correlations between immunohistochemical parameters were assessed using Pearson's correlation coefficient. A multivariable regression model was used to identify independent associations with gingival enlargement. A two-sided probability (p)-value <0.05 was considered statistically significant.

Results

Study Population

Table 1 & Figure 1 present the distribution of the 60 adolescents included in the study, comprising 30 participants with clinically evident gingival enlargement and 30 age- and sex-matched healthy controls. Gingival tissue specimens from both groups underwent histopathological examination and immunohistochemical analysis to evaluate angiogenic and inflammatory responses. VEGF and CD34 were investigated as indicators of angiogenesis, whereas IL-6 and TNF- α were examined to assess inflammatory activity. The mean age of participants was 15.23 ± 1.41 years in the gingival enlargement group and 15.10 ± 1.36 years in the healthy control group. The difference between the groups was not statistically significant ($p = 0.712$). The sex distribution was also similar across both groups, with no significant difference observed ($p = 0.793$), demonstrating comparable baseline demographic characteristics.

Table 1: Demographic characteristics of the study participants

Variable	Gingival enlargement (n=30)	Control (n=30)	p-value
Age (years), Mean $\pm$ SD	15.23 $\pm$ 1.41	15.10 $\pm$ 1.36	0.712
Male, n (%)	16 (53.3)	15 (50.0)	0.793
Female, n (%)	14 (46.7)	15 (50.0)	0.793

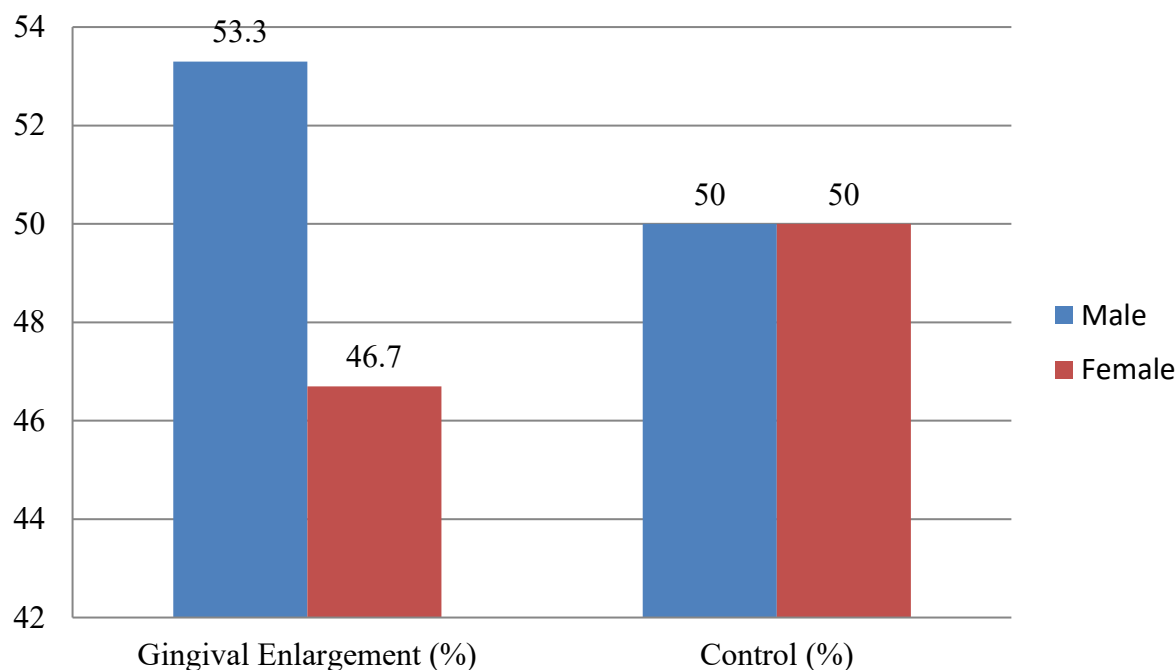


Figure 1: Schematic representation of study design, participant grouping and histopathological and immunohistochemical evaluation of angiogenic and inflammatory markers in adolescent gingival enlargement

Table 2 & Figure 2 summarize the clinical and histopathological findings in the gingival enlargement and control groups. The gingival enlargement group demonstrated significantly greater alterations across all assessed parameters compared with the control group. The mean gingival enlargement score was 2.43 ± 0.57 in the gingival enlargement group and 0.47 ± 0.51 in the control group, corresponding to a mean difference of 1.96 ($p < 0.001$). The inflammatory infiltrate score was also significantly higher in the gingival enlargement group (2.10 ± 0.66) than in the control group (0.73 ± 0.45), with a mean difference of 1.37 ($p < 0.001$). Similarly, the mean vascularity score was 2.27 ± 0.58 in the gingival enlargement group compared with 1.03 ± 0.41 among controls, representing a mean difference of 1.24 ($p < 0.001$). The fibrosis score was likewise elevated in participants with gingival enlargement (1.70 ± 0.60) compared with controls (0.83 ± 0.38), with a mean difference of 0.87 ($p < 0.001$). Overall, participants with gingival enlargement exhibited significantly higher scores for all evaluated clinical and histopathological parameters, reflecting greater inflammatory cell infiltration, vascular proliferation, and fibrotic alterations within the gingival tissues.

Table 2: Clinical and histopathological findings

Parameter	Gingival enlargement group (n=30)	Control group (n=30)	Mean Difference	p-value
Gingival enlargement score, Mean $\pm$ SD	2.43 ± 0.57	0.47 ± 0.51	1.96	<0.001
Inflammatory infiltrate score, Mean $\pm$ SD	2.10 ± 0.66	0.73 ± 0.45	1.37	<0.001
Vascularity score, Mean $\pm$ SD	2.27 ± 0.58	1.03 ± 0.41	1.24	<0.001
Fibrosis score, Mean $\pm$ SD	1.70 ± 0.60	0.83 ± 0.38	0.87	<0.001

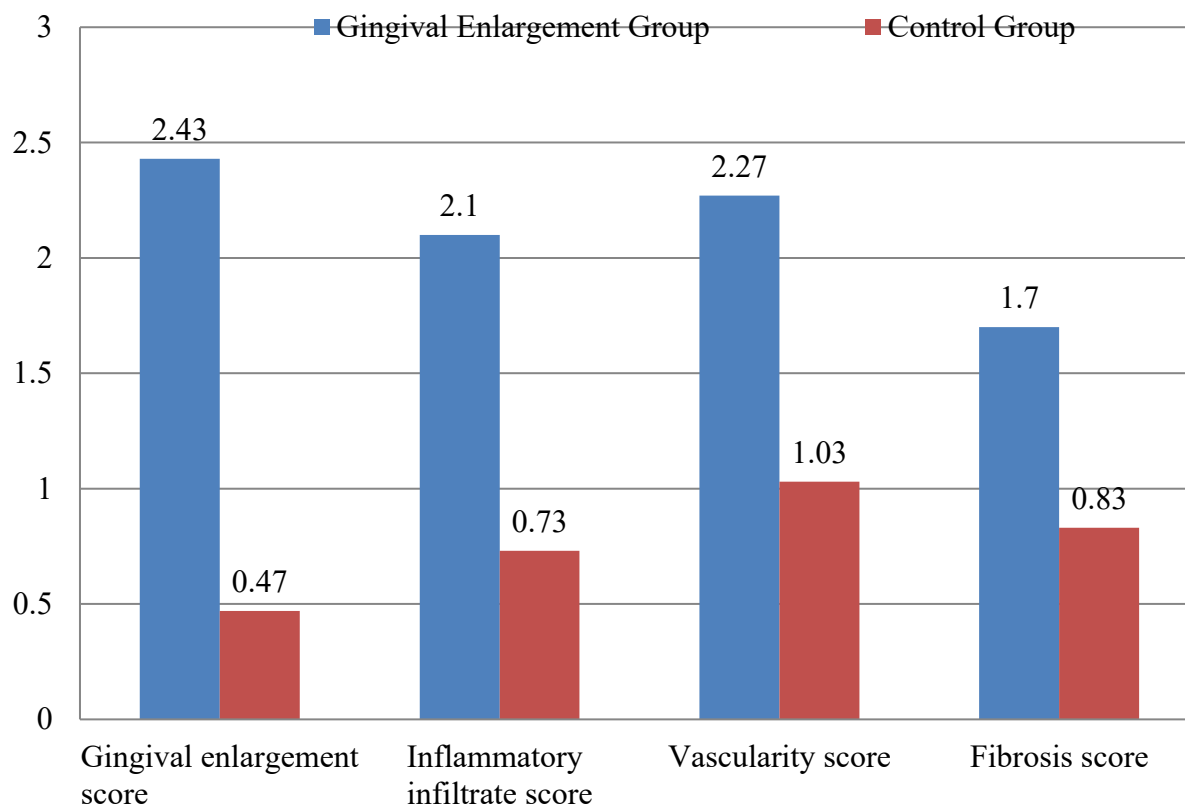


Figure 2: Clinical and histopathological findings in gingival enlargement and control groups

Table 3 & Figure 3 present the immunohistochemical findings, which revealed substantially higher expression of angiogenesis-related markers in the gingival enlargement group than in the control group. The mean VEGF H-score was 186.4 ± 31.8 in specimens from the gingival enlargement group, compared with 92.7 ± 24.6 in the control group. The corresponding mean difference was 93.7, which was statistically significant ($p < 0.001$). A similar pattern was observed for CD34-positive MVD. The mean MVD was 28.6 ± 6.7 vessels /HPF in the gingival enlargement group, compared with 17.4 ± 4.9 vessels /HPF in the control group. The between-group mean difference was 11.2 vessels/ HPF, and the difference was statistically significant ($p < 0.001$). Overall, the increased VEGF immunoreactivity and higher CD34-positive MVD observed in the gingival enlargement specimens indicate enhanced angiogenic activity within the affected gingival tissues.

Table 3: Immunohistochemical expression of angiogenic markers

Marker / parameter	Gingival enlargement	Control	Mean Difference	p-value
VEGF H-score, Mean $\pm$ SD	186.4 ± 31.8	92.7 ± 24.6	93.7	<0.001
CD34 MVD Mean $\pm$ SD	28.6 ± 6.7	17.4 ± 4.9	11.2	<0.001

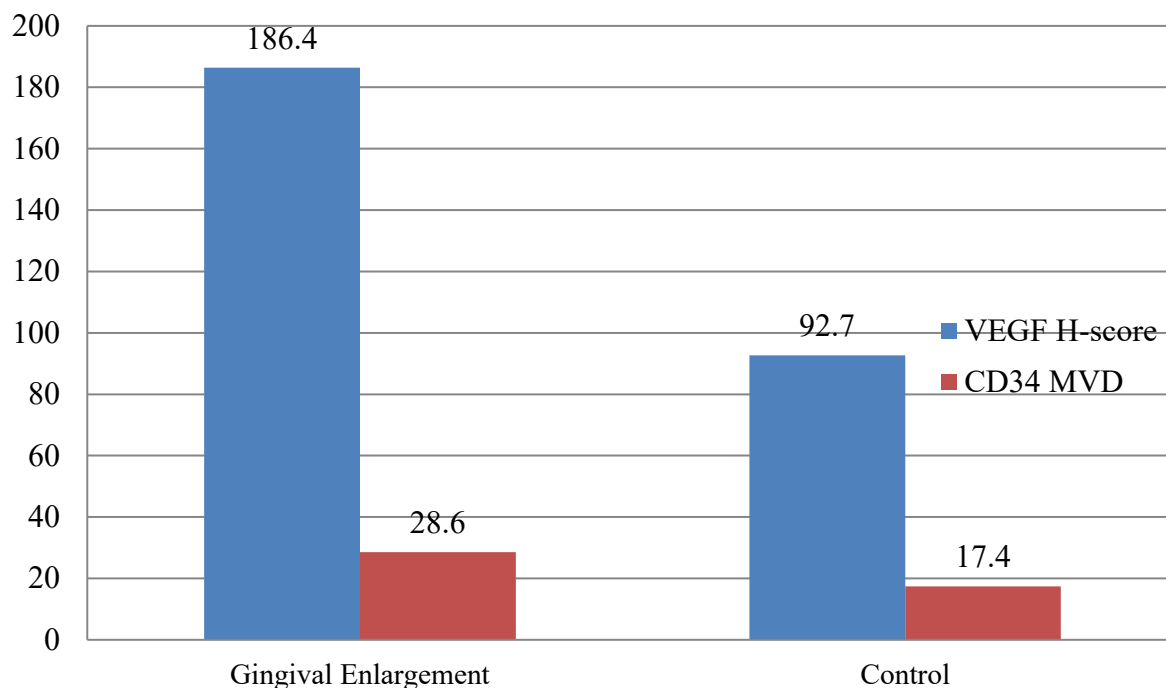


Figure 3: Comparative immunohistochemical expression of VEGF and CD34-positive MVD in gingival enlargement and control groups

Table 4 & Figure 4 summarize the frequency distribution of angiogenic marker expression in the gingival enlargement and control groups. VEGF positivity was detected in 27 (90.0%) specimens from the gingival enlargement group, compared with 14 (46.7%) specimens from the control group. This difference was statistically significant ($p < 0.001$).

Likewise, high CD34 expression was identified in 25 (83.3%) cases in the gingival enlargement group, whereas it was observed in 12 (40.0%) cases among controls. The difference between the groups was statistically significant ($p < 0.001$). Overall, both VEGF positivity and high CD34 expression occurred substantially more frequently in the gingival enlargement group, further supporting the presence of enhanced angiogenic activity in the affected gingival tissues.

Table 4: Comparison of Angiogenic Marker Expression between Study Groups — Frequency and Percentage

Marker / Parameter	Gingival Enlargement Group (n=30)	Control Group (n=30)	p-value
VEGF positive cases, n (%)	27 (90.0)	14 (46.7)	<0.001
CD34 high-expression cases, n (%)	25 (83.3)	12 (40.0)	<0.001

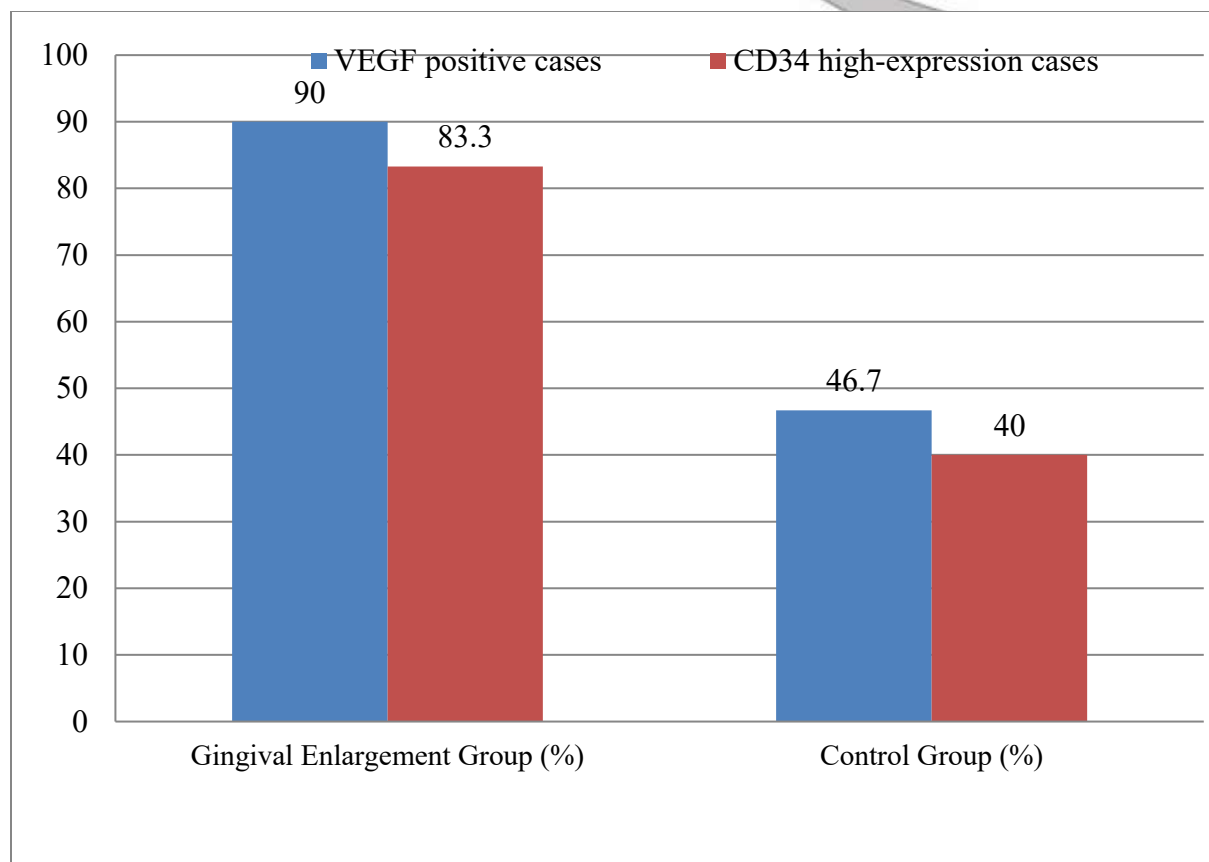


Figure 4: Frequency of VEGF positivity and high CD34 expression in gingival enlargement and control groups.

Table 5 & Figure 5 present the immunohistochemical evaluation of inflammatory marker expression in the gingival enlargement and control groups. The gingival enlargement specimens demonstrated significantly greater expression of both inflammatory markers than the control specimens. The mean IL-6 H-score was 171.8 ± 35.2 in the gingival enlargement group, compared with 79.6 ± 22.8 in the control group, yielding a mean difference of 92.2, which was statistically significant ($p < 0.001$). Similarly, the mean TNF- α H-score was higher in the gingival enlargement group (159.3 ± 33.7) than in the control group (72.4 ± 20.9). The mean difference between the groups was 86.9, and this difference was statistically significant ($p < 0.001$). Overall, the elevated IL-6 and TNF- α expression observed in the gingival enlargement specimens indicates increased local inflammatory activity within the affected gingival tissues.

Table 5: Immunohistochemical expression of inflammatory markers

Marker	Gingival enlargement	Control	Mean Difference	p-value
IL-6 H-score, Mean $\pm$ SD	171.8 ± 35.2	79.6 ± 22.8	92.2	<0.001
TNF- α H-score, Mean $\pm$ SD	159.3 ± 33.7	72.4 ± 20.9	86.9	<0.001

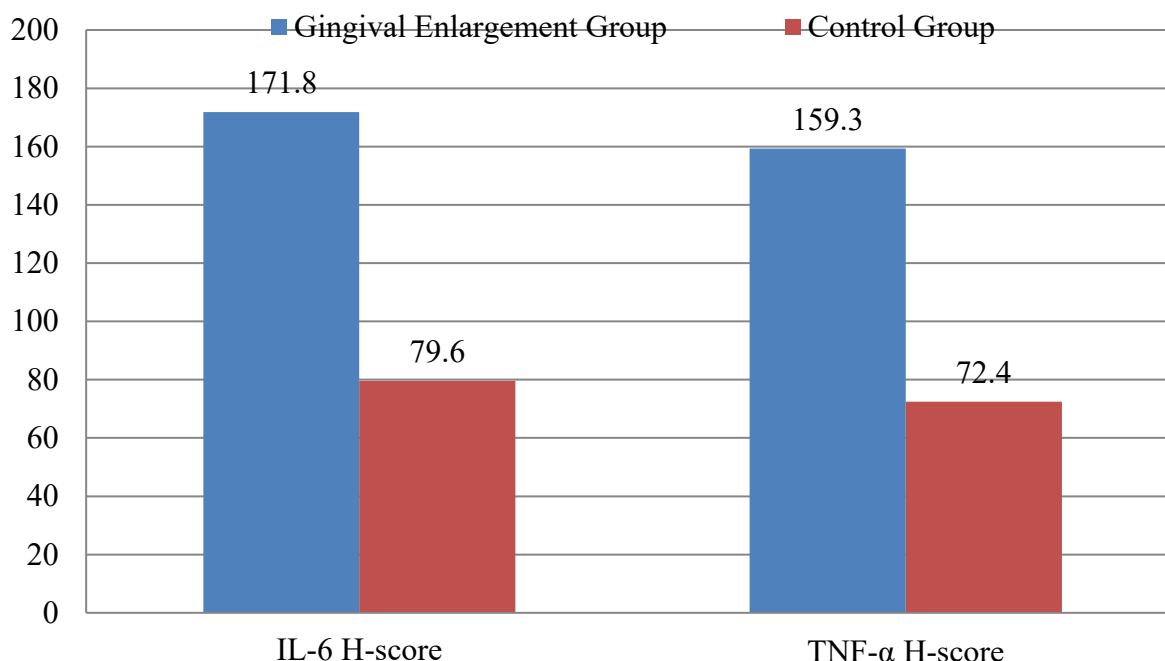


Figure 5: Comparative immunohistochemical expression of IL-6 and TNF-α in gingival enlargement and control groups.

Table 6 & Figure 6 present the frequency distribution of inflammatory marker positivity in the gingival enlargement and control groups. IL-6 positivity was identified in 26 (86.7%) cases in the gingival enlargement group, compared with 11 (36.7%) cases in the control group. This difference was statistically significant ($p < 0.001$). Similarly, TNF-positivity was observed in 25 (83.3%) cases in the gingival enlargement group, whereas only 10 (33.3%) cases in the control group showed positive expression. The difference between the groups was statistically significant ($p < 0.001$). Overall, the markedly higher frequency of IL-6 and TNF-positivity in gingival enlargement specimens indicates enhanced local inflammatory activity and suggests a potential role for these pro-inflammatory cytokines in the underlying tissue changes associated with gingival enlargement.

Table 6: Comparison of immunohistochemical expression of inflammatory markers between study groups — frequency and percentage

Marker	Gingival enlargement	Control	p-value
IL-6 positive cases, n (%)	26 (86.7)	11 (36.7)	<0.001
TNF-α positive cases, n (%)	25 (83.3)	10 (33.3)	<0.001

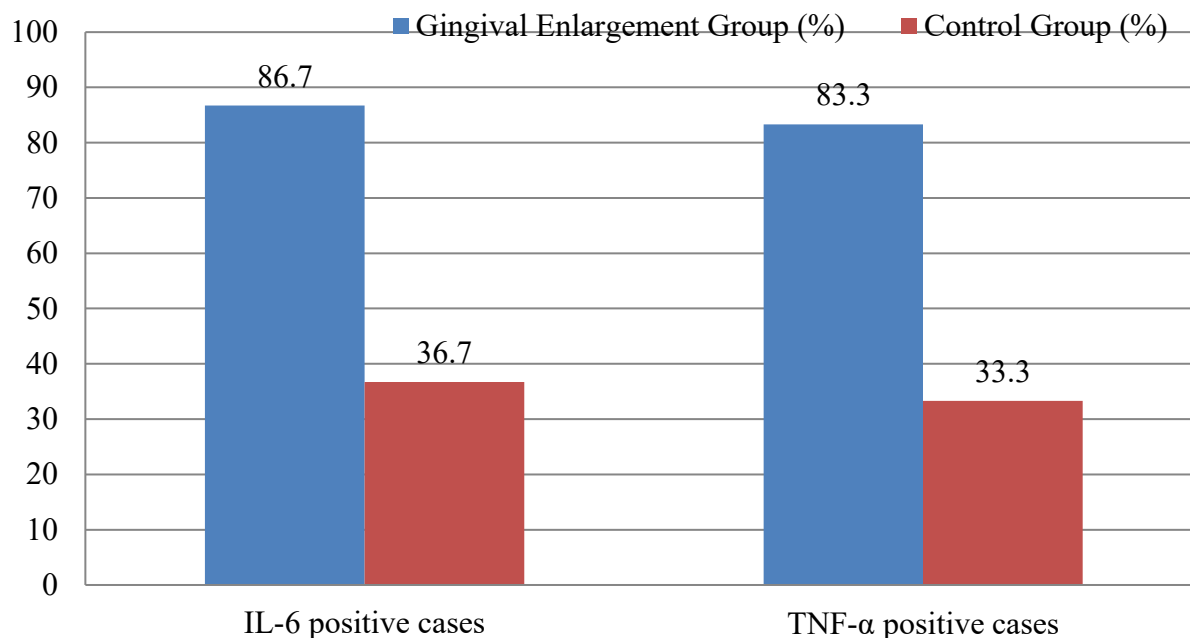


Figure 6: Frequency of IL-6 and TNF-α positivity in gingival enlargement and control groups

Table 7 & Figure 7 illustrate the distribution of immunohistochemical staining intensity for the three evaluated markers and demonstrate a clear predominance of moderate and strong immunoreactivity in gingival enlargement specimens. For VEGF, negative staining was observed in only 3 (10.0%) cases in the gingival enlargement group, compared with 16 (53.3%) controls. Moderate staining was identified in 11 (36.7%) gingival enlargement cases and 5 (16.7%) controls, whereas strong staining was observed in 11 (36.7%) and 1 (3.3%) cases, respectively.

For IL-6, moderate staining was detected in 10 (33.3%) and strong staining in 11 (36.7%) gingival enlargement specimens, compared with 4 (13.3%) and 1 (3.3%) controls, respectively. Negative staining was considerably less frequent among gingival enlargement specimens, occurring in 4 (13.3%) cases, compared with 19 (63.3%) controls. A similar pattern was observed for TNF-α, with moderate and strong staining present in 9 (30.0%) and 11 (36.7%) gingival enlargement specimens, respectively, compared with 4 (13.3%) and 1 (3.3%) controls. Negative staining was recorded in 5 (16.7%) gingival enlargement cases compared with 20 (66.7%) control specimens.

Table 7: Comparison of immunohistochemical staining intensity between the group

	Marker	Negative n (%)	Mild n (%)	Moderate n (%)	Strong n (%)
VEGF	Gingival enlargement	3 (10.0)	5 (16.7)	11 (36.7)	11 (36.7)
	Control	16 (53.3)	8 (26.7)	5 (16.7)	1 (3.3)
IL-6	Gingival enlargement	4 (13.3)	5 (16.7)	10 (33.3)	11 (36.7)
	Control	19 (63.3)	6 (20.0)	4 (13.3)	1 (3.3)
TNF-α	Gingival enlargement	5 (16.7)	5 (16.7)	9 (30.0)	11 (36.7)
	Control	20 (66.7)	5 (16.7)	4 (13.3)	1 (3.3)

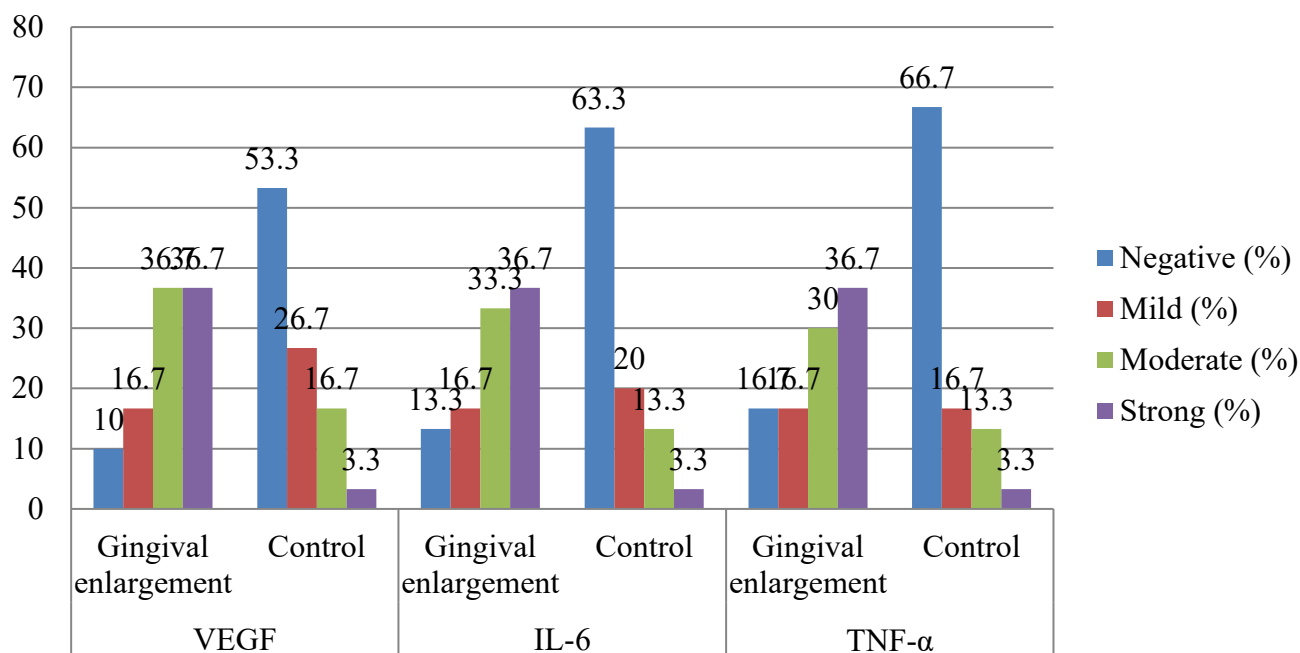


Figure 7: Comparative distribution of immunohistochemical staining intensity for VEGF, IL-6, and TNF-α in gingival enlargement and control groups

Table 8 summarizes the relationships between the assessed angiogenic and inflammatory biomarkers. VEGF expression was strongly and positively associated with CD34-positive MVD ($r=0.68$, $p<0.001$). Positive moderate correlations were also observed between VEGF and IL-6 ($r=0.59$, $p=0.001$) and between VEGF and TNF-α ($r=0.54$, $p=0.002$), indicating an association between angiogenic activity and pro-inflammatory cytokine expression. CD34-positive MVD demonstrated significant moderate positive correlations with IL-6 ($r=0.49$, $p=0.006$) and TNF-α ($r=0.45$, $p=0.012$). Among all evaluated relationships, the strongest association was observed between IL-6 and TNF-α ($r=0.71$, $p<0.001$), reflecting a strong positive relationship between these inflammatory mediators. Taken together, the findings demonstrate significant positive associations across the angiogenic and inflammatory parameters examined. The observed relationships indicate that increased vascular activity and inflammatory mediator expression may occur concurrently within gingival enlargement tissues, supporting an integrated angiogenic-inflammatory response.

Table 8: Correlation between angiogenic and inflammatory markers

Correlation	r-value	p-value	Interpretation
VEGF vs CD34 MVD	0.68	<0.001	Strong positive correlation
VEGF vs IL-6	0.59	0.001	Moderate positive correlation
VEGF vs TNF-α	0.54	0.002	Moderate positive correlation
CD34 MVD vs IL-6	0.49	0.006	Moderate positive correlation
CD34 MVD vs TNF-α	0.45	0.012	Moderate positive correlation
IL-6 vs TNF-α	0.71	<0.001	Strong positive correlation

Table 9 presents the results of the multivariable analysis assessing the independent associations between the immunohistochemical parameters and gingival enlargement. After adjustment for potential confounding factors, all four evaluated markers remained significantly associated with gingival enlargement. The VEGF H-score demonstrated a significant adjusted association (adjusted odds ratio OR=1.42, 95% confidence interval CI: 1.18–1.71; $p<0.001$). Likewise, CD34-positive MVD remained independently associated with gingival enlargement (adjusted OR=1.31, 95% CI: 1.12–1.54; $p=0.001$). Among the evaluated parameters, IL-6 H-score showed the strongest adjusted association with gingival enlargement (adjusted OR=1.56, 95% CI: 1.25–1.94; $p<0.001$). TNF- α H-score also demonstrated a statistically significant independent association (adjusted OR=1.28, 95% CI: 1.08–1.51; $p=0.004$). Overall, the multivariable findings indicate that higher levels of both angiogenic markers (VEGF and CD34) and inflammatory markers (IL-6 and TNF- α) were independently associated with gingival enlargement, even after accounting for potential confounding factors.

Table 9: Association of marker expression with histopathological inflammation

Marker	Correlation with inflammatory infiltrate score (r)	p-value
VEGF	0.56	0.001
CD34 MVD	0.61	<0.001
IL-6	0.73	<0.001
TNF- α	0.69	<0.001

Table 10 presents the correlation analysis between the immunohistochemical markers and the histopathological inflammatory infiltrate score. VEGF expression demonstrated a moderate positive correlation with the inflammatory infiltrate score ($r=0.56$, $p=0.001$), indicating that higher VEGF expression was associated with greater inflammatory cell infiltration. A strong positive correlation was observed between CD34-positive MVD and the inflammatory infiltrate score ($r=0.61$, $p<0.001$). Among the inflammatory markers, IL-6 showed the strongest association with the inflammatory infiltrate score ($r=0.73$, $p<0.001$), followed by TNF- α ($r=0.69$, $p<0.001$). These findings indicate that greater expression of the evaluated pro-inflammatory cytokines was associated with more pronounced histopathological inflammatory infiltration. Overall, all four markers exhibited statistically significant positive correlations with the inflammatory infiltrate score. The results suggest that increased angiogenic activity and heightened inflammatory cytokine expression are closely related to the severity of histopathological inflammation in gingival enlargement tissues.

Table 10: Multivariable analysis

Predictor	Adjusted β / OR	95% CI	p-value
VEGF H-score	1.42	1.18–1.71	<0.001
CD34 MVD	1.31	1.12–1.54	0.001
IL-6 H-score	1.56	1.25–1.94	<0.001
TNF- α H-score	1.28	1.08–1.51	0.004

Discussion

Gingival enlargement during adolescence may involve a combination of inflammatory activity, vascular alterations, and connective-tissue remodeling. In the present study, these changes were evaluated through histopathological examination and immunohistochemical assessment of VEGF, CD34, IL-6, and TNF- α . The findings demonstrated that gingival enlargement was associated with coordinated alterations in both angiogenic and inflammatory

parameters, providing evidence that the increase in gingival volume was accompanied by underlying biological changes within the tissue. The two study groups were comparable with respect to age and sex. This comparability is important because gingival tissues may undergo physiological and developmental changes during adolescence. Therefore, the significant differences observed in histopathological and immunohistochemical parameters are unlikely to be explained by differences in these basic demographic characteristics. The use of age- and sex-matched controls consequently strengthened the validity of the group comparisons.

Histopathological examination revealed significantly greater gingival enlargement, inflammatory infiltrate, vascularity, and fibrosis in the affected group. These findings indicate that the clinically evident increase in gingival volume was accompanied by substantial microscopic alterations. The increased inflammatory infiltrate suggests active local inflammation, whereas greater vascularity indicates involvement of the gingival microcirculation. The associated increase in fibrosis further suggests that connective-tissue remodeling may contribute to the persistence and structural characteristics of gingival enlargement. Similar histopathological inflammatory and hyperplastic changes have been documented in gingival overgrowth occurring in younger populations by Aspriello SD et al. (2009) [10], supporting the relevance of microscopic tissue assessment in pediatric and adolescent gingival enlargement.

The angiogenic findings provided additional evidence of enhanced vascular activity. VEGF expression and CD34-positive MVD were significantly higher in the gingival enlargement group than in healthy controls. VEGF is an important mediator of angiogenesis and vascular permeability, while CD34 immunostaining can be used to identify endothelial-associated microvascular structures. The simultaneous increase in both parameters therefore provides complementary evidence of increased angiogenic activity. Artese L et al. (2010) [11] demonstrated increased VEGF expression in diseased periodontal tissues and proposed a relationship between VEGF activity and vascular changes associated with periodontal inflammation. More recently, Vladau M et al. (2016) [12] reported elevated VEGF expression in periodontal disease, further supporting the relevance of VEGF to inflammatory alterations of periodontal tissues.

The increased histopathological vascularity observed in the present study is particularly consistent with the immunohistochemical findings. The parallel elevation of VEGF and CD34-positive MVD suggests that the increased vascularity was accompanied by enhanced biological angiogenic activity rather than representing only passive expansion of the gingival tissue. The observed relationship between vascularity and angiogenic markers is also consistent with evidence that periodontal inflammation can be accompanied by alterations in the gingival microvasculature. Roi C et al. (2002) [13] described significant vascular alterations in gingivitis and chronic periodontitis and emphasized the relationship between vascular responses and persistent inflammatory activity. Nevertheless, because the present investigation was observational and cross-sectional, the findings establish association rather than demonstrating whether angiogenesis precedes gingival enlargement or develops secondary to it.

The inflammatory component of gingival enlargement was also clearly demonstrated by the immunohistochemical findings. IL-6 and TNF- α expression were significantly greater in enlarged gingival tissues than in controls, both according to H-scores and the proportion of positive specimens. The predominance of moderate and strong staining in affected tissues further indicates that increased inflammatory activity was relatively consistent across the gingival enlargement group. These findings are biologically plausible because IL-6 and TNF- α are important mediators of inflammatory responses within periodontal tissues. Noh et al. (2013) [14] demonstrated increased IL-6 and TNF- α levels in inflamed gingival tissues, supporting the present observation of enhanced local inflammatory mediator expression.

The relationship between inflammatory mediator expression and tissue inflammation was further supported by the correlation analysis. IL-6 demonstrated the strongest association with the histopathological inflammatory infiltrate, followed by TNF- α . This pattern indicates that increasing expression of these inflammatory mediators was accompanied by greater cellular inflammatory infiltration. Such findings provide concordance between the immunohistochemical assessment and conventional histopathological examination. They also suggest that cytokine activity may be closely related to the inflammatory tissue phenotype observed in gingival enlargement.

An important finding of the present study was the significant association between angiogenic and inflammatory parameters. VEGF showed a strong positive relationship with CD34-positive MVD, confirming consistency between the two measures of angiogenic activity. Furthermore, both angiogenic parameters were positively correlated with IL-6 and TNF- α . These findings indicate that vascular and inflammatory alterations may occur simultaneously within enlarged gingival tissues. The interaction between inflammatory signaling and angiogenesis is biologically

plausible because inflammatory mediators can influence endothelial activation and vascular permeability, while changes in the vascular environment may modify the local inflammatory response. Neurath N et al. (2021) [15] highlighted the central role of VEGF signaling in angiogenesis and its interaction with inflammatory processes in oral diseases.

The strongest correlation among the evaluated biomarkers was observed between IL-6 and TNF- α . This close association suggests coordinated activity of these two inflammatory mediators within enlarged gingival tissues. Their positive relationships with VEGF and CD34-positive MVD further support the possibility of an interconnected angiogenic-inflammatory response. However, these correlations should not be interpreted as evidence of direct causation. Longitudinal and mechanistic studies will be required to determine the temporal sequence and biological pathways linking inflammatory cytokines with angiogenic changes in gingival enlargement.

All four evaluated markers were significantly correlated with the histopathological inflammatory infiltrate. VEGF and CD34-positive MVD demonstrated positive associations with the extent of inflammatory infiltration, while IL-6 and TNF- α showed stronger relationships. This pattern provides further evidence that the inflammatory tissue phenotype is accompanied by both increased cytokine activity and enhanced vascular responses. The findings therefore establish a consistent relationship between conventional microscopic features and the immunohistochemical profile of the enlarged gingival tissues.

The multivariable analysis further strengthened these observations. After adjustment for potential confounding variables, VEGF, CD34-positive MVD, IL-6, and TNF- α remained independently associated with gingival enlargement. IL-6 demonstrated the strongest adjusted association among the evaluated markers, followed by VEGF, CD34-positive MVD, and TNF- α . These findings suggest that the observed relationship between gingival enlargement and the evaluated biomarkers was not merely a consequence of the interdependence among the markers. Instead, each parameter retained an independent association within the statistical model.

Taken together, the histopathological and immunohistochemical findings provide an integrated interpretation of gingival enlargement in adolescents. Increased inflammatory infiltration and fibrosis indicate structural tissue remodeling, while increased vascularity was supported by elevated VEGF expression and CD34-positive MVD. Simultaneously, increased IL-6 and TNF- α expression demonstrated enhanced inflammatory signaling. The significant relationships among these parameters suggest that inflammation and angiogenesis may interact within the enlarged gingival tissues and collectively contribute to their structural characteristics.

From a clinical perspective, these findings emphasize that adolescent gingival enlargement should not be regarded solely as an increase in gingival volume. Conventional clinical assessment identifies the extent of enlargement, whereas histopathological and immunohistochemical evaluation can provide additional insight into the biological processes accompanying the lesion. The demonstrated association of angiogenic and inflammatory markers with tissue changes may also provide a foundation for future investigations aimed at identifying biomarkers of disease activity and persistence.

Several limitations should be considered when interpreting the findings. The relatively small sample size and single-centre design may restrict generalizability to wider adolescent populations. The cross-sectional nature of the investigation prevents conclusions regarding causality or temporal relationships between inflammation and angiogenesis. Immunohistochemical H-scores and MVD are semiquantitative measures and may be influenced by tissue selection and microscopic assessment. In addition, only a limited number of angiogenic and inflammatory markers were investigated, and other molecular pathways potentially involved in gingival enlargement were not evaluated. Future multicentre studies with larger samples, longitudinal follow-up, broader biomarker panels, and detailed clinical characterization would help validate and expand the present findings. Overall, the present study demonstrates that gingival enlargement in adolescents is accompanied by significant histopathological, angiogenic, and inflammatory alterations. The concurrent increase in VEGF, CD34-positive MVD, IL-6, and TNF- α , together with their significant relationships with inflammatory infiltration, supports the presence of an interconnected angiogenic-inflammatory tissue response. These findings provide a broader biological understanding of adolescent gingival enlargement and indicate that both vascular and inflammatory mechanisms may contribute to its tissue characteristics.

Limitations

This study has several limitations. The relatively limited sample size and single-centre design may restrict the generalizability of the findings to broader adolescent populations. The cross-sectional nature of the study prevents

determination of a temporal or causal relationship between angiogenic and inflammatory responses. Immunohistochemical assessment provides semiquantitative information and may be influenced by tissue processing and observer-related variation. In addition, potential confounding factors such as oral hygiene, duration of gingival enlargement, and individual inflammatory susceptibility may have influenced marker expression. Larger multicentre longitudinal studies incorporating molecular and clinical parameters are warranted to validate these findings and clarify the underlying biological mechanisms.

Conclusion

Gingival enlargement in adolescents is associated with enhanced angiogenic and inflammatory activity accompanied by increased vascularity, inflammatory infiltration, and fibrotic changes. The coordinated expression of VEGF, CD34, IL-6, and TNF- α indicates a close relationship between vascular and inflammatory responses within enlarged gingival tissues. These findings suggest that angiogenic-inflammatory interactions may contribute to the tissue remodeling and pathological changes associated with gingival enlargement. Further longitudinal and multicentre investigations are needed to establish the clinical significance of these mechanisms and their potential relevance to targeted therapeutic strategies.

Conflicts of interest: Nil

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