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Potential of IP-10, EGFR, CK17 and ANXA1 as Non-Invasive Biomarkers for the Diagnosis of Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity. Early diagnosis and the identification of non-invasive biomarkers for disease monitoring are essential. This study aimed to evaluate the expression of genes involved in inflammation (IP-10), cellular proliferation (EGFR), epithelial differentiation (CK17), and cellular regulation (ANXA1) in the peripheral blood of OSCC patients compared with healthy individuals.

Materials and Methods: In this case–control study, peripheral blood samples were collected from 30 OSCC patients and 30 healthy controls. Total RNA was extracted and reverse-transcribed into cDNA. Relative expression levels of IP-10 (CXCL10), EGFR, CK17, and ANXA1 were quantified using real-time polymerase chain reaction (Real-Time PCR). Statistical analyses were performed using SPSS software.

Results: The expression levels of EGFR and CK17 were significantly increased in OSCC patients compared with controls ($p < 0.001$). Similarly, IP-10 expression was significantly upregulated in the patient group ($p < 0.001$). In contrast, ANXA1expression was significantly downregulated in OSCC patients ($p < 0.001$).

Conclusion: The altered expression patterns of IP-10, EGFR, CK17, and ANXA1 in the peripheral blood of OSCC patients indicate the potential of these markers as non-invasive molecular diagnostic biomarkers. The concurrent upregulation of EGFR and CK17 may play a critical role in tumor progression, while the downregulation of ANXA1 may reflect impaired anti-inflammatory and apoptotic processes. These findings support the future application of PCR-based blood tests for OSCC screening or monitoring; however, further confirmatory studies are recommended.

Keywords: Oral squamous cell carcinoma; Peripheral blood; Gene expression; EGFR; CK17; IP-10; ANXA1; Real-time PCR.

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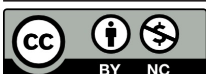

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Introduction

Oral squamous cell carcinoma (OSCC) accounts for more than 90% of oral malignancies and is recognized as the most common cancer of the head and neck region [1]. Despite advances in diagnostic approaches and therapeutic modalities, the prognosis of OSCC—particularly in advanced stages—remains unfavorable, with reported 5-year survival rates ranging from approximately 50% to 60% [2]. This persistent clinical burden highlights the critical need for the development of early, non-invasive, and reliable diagnostic strategies. Carcinogenic compounds present in tobacco smoke contribute to both the initiation and progression of oral cancer by inducing persistent genomic damage and promoting a state of chronic inflammation in the oral mucosa [3,4]. These pathological processes result in dysregulation of complex molecular networks, including genes involved in cellular proliferation, inflammation, metastasis, and apoptosis [5].

Epidermal growth factor receptor (EGFR) is a cell-surface tyrosine kinase receptor that plays a pivotal role in epithelial cell growth and survival. Overexpression or mutation of EGFR, reported in more than 80% of OSCC cases, has been associated with uncontrolled cellular proliferation, increased metastatic potential, and poor clinical prognosis [6,7]. Cytokeratin 17 (CK17) is a cytoskeletal protein commonly expressed in basal epithelial cells. Elevated CK17 expression has been observed in several epithelial cancers, including OSCC, and has been proposed as a marker of tumor invasiveness, resistance to apoptosis, and adverse prognosis [8,9]. Interferon gamma-induced protein 10 (IP-10 or CXCL10) is a vital chemokine primarily induced by interferon-γ. IP-10 plays a dual role in cancer biology: on the one hand, it can exert antitumor effects by recruiting immune effector cells, such as T lymphocytes and natural killer cells; on the other hand, it can facilitate tumor progression by promoting angiogenesis and metastasis [10]. Annexin A1 (ANXA1) is a calcium- and phospholipid-binding protein involved in the regulation of inflammation, induction of apoptosis, and inhibition of cell migration. Reduced expression of ANXA1 has been reported in multiple malignancies and has been correlated with tumor progression, metastasis, and poor prognosis [11,12]. The present study was designed to evaluate the relative expression patterns of IP-10, EGFR, CK17, and ANXA1 genes in peripheral blood samples from OSCC patients compared healthy individuals, without making a priori assumptions about etiological factors such as smoking, as these data were not collected.

Materials and Methods

Study Design and Participants

This case–control study investigated the expression of interferon gamma–inducible protein 10 (IP-10/CXCL10), epidermal growth factor receptor (EGFR), cytokeratin 17 (CK17), and annexin A1 (ANXA1) genes in peripheral blood samples obtained from patients diagnosed with oral squamous cell carcinoma (OSCC) compared with healthy controls. Ethical approval was obtained from the institutional Ethics Committee (Approval Code: IR.SBMU.NRITLD.REC.1404.105). Written informed consent was obtained from all participants before enrollment. A total of 60 participants were included in the study: 30 patients with OSCC and a history of (case group) and 30 age- and sex-matched healthy individuals without a history of oral or systemic malignant diseases (control group). Clinicopathological characteristics including TNM stage (AJCC 8th edition), tumor grade, and anatomical site were recorded for all OSCC patients. Patients were recruited from the Department of Oral and Maxillofacial Pathology, School of Dentistry, Tehran University of Medical Sciences.

Sample Collection

Following ethical approval and informed consent, 2 mL of peripheral venous blood was collected from each participant into EDTA-containing tubes. Blood samples were processed immediately for RNA extraction. [13-15].

RNA Extraction and Quantification

Total RNA was extracted from peripheral blood samples using the RNA Blood Mini Kit (Qiagen, Cat. No. 52304) in accordance with the manufacturer's instructions [15-17]. All procedures were conducted under RNase-free conditions using certified RNase-free microtubes and filter tips. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (NanoDrop Technologies) [16,17].

cDNA Synthesis

Complementary DNA (cDNA) was synthesized from 1 µg RNA, using the Viva 2-Step RT-PCR Kit (Cat. No. RTPL12). The 18S rRNA gene was used as the internal reference gene for normalization, according to the manufacturer's instructions. Synthesized cDNA was stored at −20 °C until real-time RT-PCR analysis.

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Quantitative Real-Time RT-PCR

Quantitative real-time RT-PCR was performed using CinnaGreen qPCR Mix 2X (Cat. No. MM2041). The target genes (IP-10, EGFR, CK17, and ANXA1) and their specific primers are listed in Table 1. Each PCR reaction was prepared in a final volume of 20 μL containing 4 μL master mix, 1 μL of forward primer, 1 μL of reverse primer, 2 μL cDNA, and nuclease-free distilled water.

Thermal cycling conditions were as follows:

- Initial denaturation at 95 °C for 5 minutes.
- 40 cycles of:
    - Denaturation at 95 °C for 15 seconds.
    - Primer annealing at 56 °C for 60 seconds.
    - Extension at 72 °C for 25 seconds.
- Final extension at 72 °C for 5 minutes.

Following amplification, results were interpreted based on amplification curves and melting peak analyses to confirm specificity.

Relative Gene Expression Analysis

Relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method. The threshold cycle (Ct) was defined as the cycle number at which fluorescence exceeded the threshold level. ΔCt values were obtained by subtracting the Ct of the reference gene from that of the target gene, while ΔΔCt represented the difference between ΔCt values of OSCC patients and healthy controls. Fold changes in gene expression were calculated using the $2^{-}\Delta\Delta Ct$ formula.

Statistical Analysis

Statistical analyses were performed using SPSS software version 20. Mean values and standard deviations were calculated. Differences in gene expression levels between groups and associations with clinicopathological characteristics were evaluated using paired t-tests. A p-value ≤ 0.05 was considered statistically significant.

Results

Participant Characteristics

A total of 60 participants were enrolled, including 30 patients diagnosed with oral squamous cell carcinoma (OSCC) (case group) and 30 healthy individuals (control group). The cohorts were comparable in baseline demographics, with mean ages of 58.5 ± 2.7 years for cases and 54.1 ± 8.3 years for controls ($p > 0.05$). Gender distribution did not differ significantly between groups, confirming balanced comparability.

Gene Expression Profiles

Real-time quantitative PCR analysis and ΔΔCt calculations revealed that the relative expression patterns (RQ values) of all four target genes differed significantly between OSCC patients and healthy controls (Figures 1-2). EGFR was positive in 28 of 30 OSCC patients, compared with 12 of 30 healthy individuals. CK17 was positive in 26 of 30 patients and 11 of 30 controls. IP-10 was positive in 25 of 30 patients and 8 of 30 controls. Conversely, ANXA1 was positive in 10 of 30 patients and 22 of 30 controls. Between-group comparisons using independent t-tests demonstrated highly significant differences for all four biomarkers (all $p < 0.001$; Figures 1-4).

Fold Change Analysis

Relative expression calculated using the $2^{-}\Delta\Delta Ct$ method revealed that EGFR expression increased 3.0-fold, CK17 by 2.6-fold, and IP-10 by 2.3-fold in OSCC patients compared to healthy controls, whereas ANXA1 expression decreased by 2.1-fold (all $p < 0.001$). Boxplot distributions demonstrated clear separation between the patient and control cohorts with minimal overlap. Levene's test indicated consistent variance across groups (all $p > 0.10$), confirming the assumptions for parametric testing (Figures 5-8).

Clinical Correlations

Strong positive correlations were observed between EGFR and CK17 expression levels. Moderate positive correlations were noted between IP-10 and EGFR. No significant correlations were found between ANXA1 and any of the other three genes. Overall, the results indicate a distinct gene expression signature in the peripheral blood of OSCC patients, characterized by coordinated upregulation of pro-tumorigenic genes EGFR and CK17, increased expression of the inflammatory chemokine IP-10, and downregulation of the tumor suppressor gene ANXA1. The combination of these four markers in a single panel may provide high diagnostic potential for differentiating OSCC patients from healthy individuals, although further validation in larger cohorts is warranted.

Table 1. Primer sequences and amplicon sizes.

Parameters	IP-10	CK17	EGFR	ANXA1	18s rRNA
Forward Primer (3′–5′)	AGA ACG GTG CGC TGC AC	ATCCTGCTGGAT-GTGAAGACGC	AACACCCTG-GTCTGGAAG-TACG	GCGAAACAATG-CACAGCGTCAAC	GTAACCCGTT-GAACCCCATT
Length of primer	17	22	22	23	20
Reverse Primer (3′–5′)	CCT ATG GCC CTG GGT CTC A	TCCACAATGG-TACGCACCTGAC	TCGTTGGACAG-CCTTCAAGACC	CAACCTCCT-CAAGGTGACCT-GT	CCATCCAATCGG-TAGTAGCG
Length of primer	19	22	22	22	20
Annealing Temperature (°C)	58.4°C	56.5°C	55.5°C	54.5°C	53.5°C

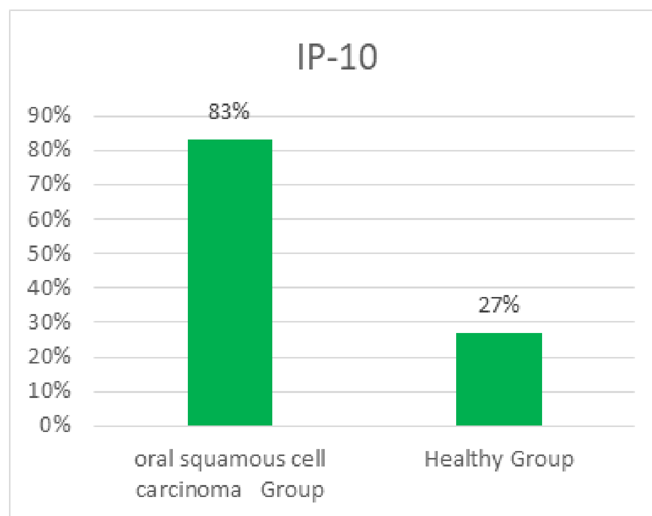

Figure 1. IP-10 levels in OSCC versus healthy individuals.

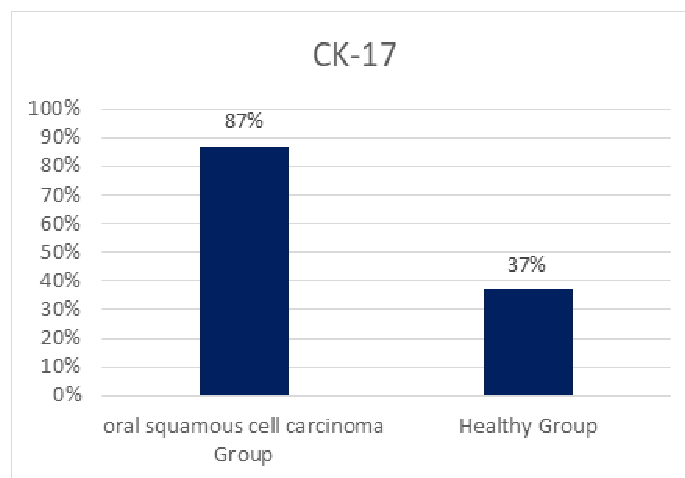

Figure 2. CK17 expression in OSCC compared with controls.

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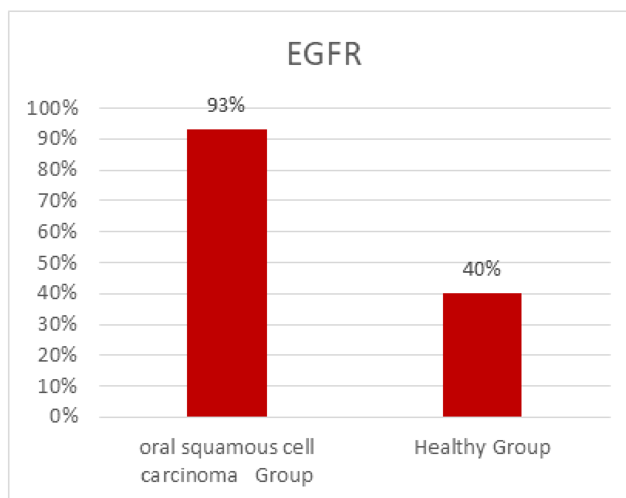

Figure 3. EGFR expression in OSCC versus healthy controls.

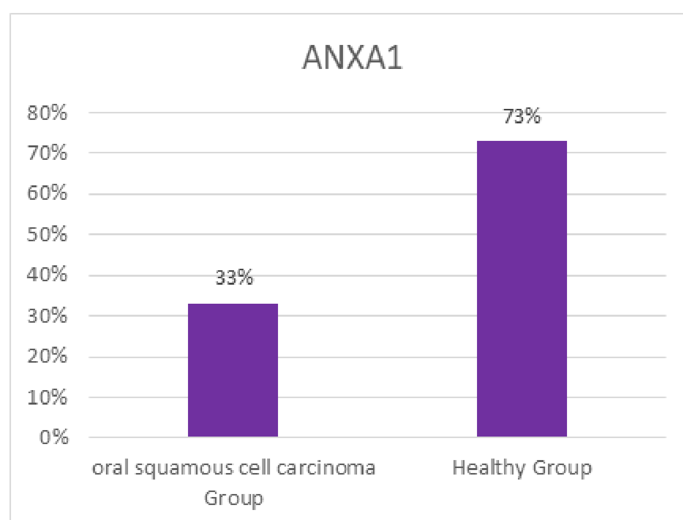

Figure 4. ANXA1 expression in OSCC versus controls.

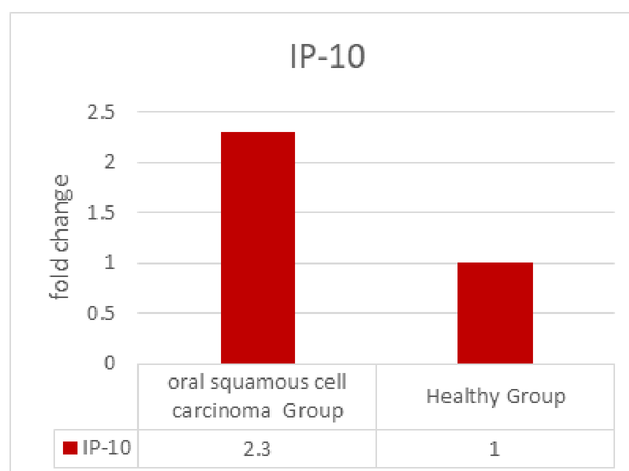

Figure 5. Differences in IP-10 gene expression between OSCC and healthy controls.

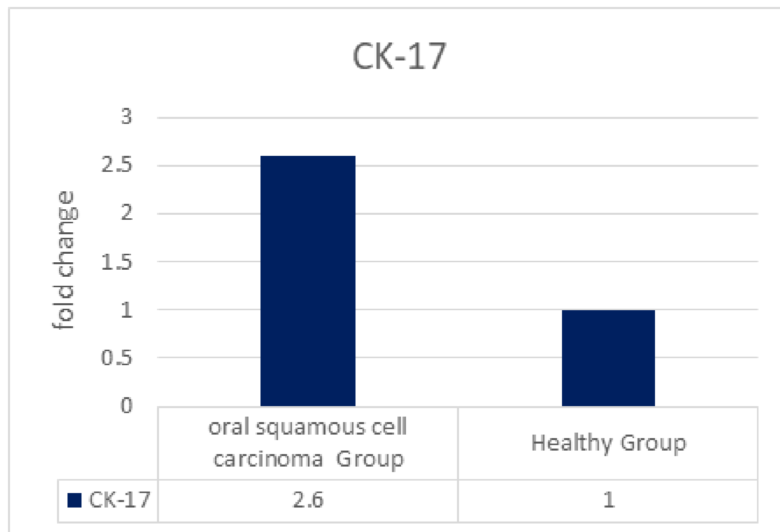

Figure 6. Differences in CK17 gene expression between OSCC and healthy controls.

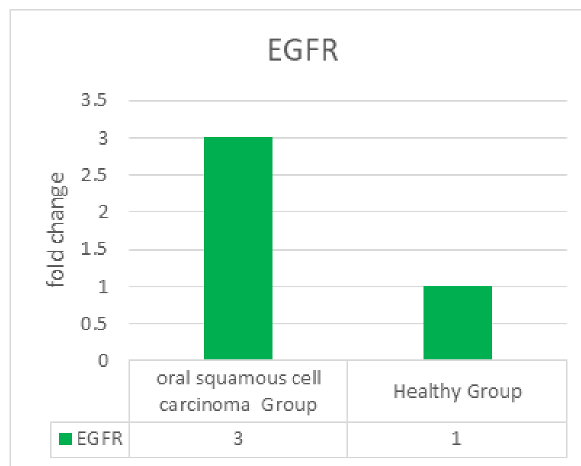

Figure 7. Differences in EGFR gene expression between OSCC and healthy controls.

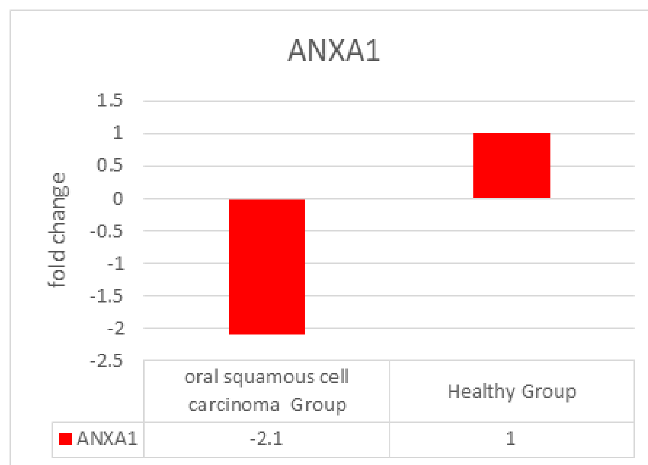

Figure 8. Differences in ANXA1 gene expression between OSCC and healthy controls.

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Discussion

This study demonstrated a significant upregulation of EGFR, CK17, and IP-10 gene expression and a marked downregulation of ANXA1 in peripheral blood samples from patients with oral squamous cell carcinoma (OSCC), compared with healthy controls. The combination of these four markers shows potential for differentiating OSCC patients from healthy individuals, although validation in larger cohorts is needed. The coordinated upregulation of EGFR and CK17 represents a key finding of this study and is consistent with previous reports. EGFR overexpression is a well-established event in OSCC, associated with tumor progression and therapeutic resistance [6]. CK17, widely reported as a tumor-associated marker in histopathological studies of OSCC, may be transcriptionally upregulated downstream of EGFR activation through pathways involving transcription factors such as STAT3, although mechanistic studies are needed to confirm this link [8,12].

The significant increase in IP-10 expression in the patient group is also noteworthy. IP-10 is primarily secreted in response to interferon-γ and has been reported to be elevated in serum in various malignancies [10]. While IP-10 traditionally exerts antitumor effects by recruiting cytotoxic T lymphocytes, accumulating evidence suggests that elevated IP-10 levels may also promote angiogenesis and cell migration, thereby facilitating tumor progression [18]. Conversely, the observed downregulation of ANXA1 in OSCC patients is significant. ANXA1 is a key modulator of inflammation resolution and a potential tumor suppressor. Loss or reduction of ANXA1 expression has been reported across multiple malignancies [11], and its anti-inflammatory effects are primarily mediated via inhibition of NF-ΚB translocation [19]. In OSCC patients, suppression of ANXA1 may have two significant consequences: first, impaired local and systemic control of inflammation, favoring a pro-metastatic microenvironment; and second, reduced activation of apoptotic pathways in neoplastic cells. The mechanisms underlying altered ANXA1 expression in OSCC require further investigation.

Limitations

This study was limited by a modest sample size of 60 participants, which reduced statistical power for multivariate analyses and for adjustment for potential confounders. Peripheral blood analysis may not fully reflect local tissue events, and the lack of protein-level validation restricts functional interpretation. Future studies should include larger, longitudinal cohorts to track dynamic changes in gene expression and validate findings across OSCC subtypes. Integration of complementary molecular techniques, including qRT-PCR, single-cell RNA sequencing, and proteomics, could strengthen the correlation between mRNA and protein levels. Finally, interventional or diagnostic trials evaluating multi-gene panels (EGFR, CK17, IP-10, ANXA1) are needed to confirm their clinical utility as non-invasive biomarkers OSCC patients.

Conclusion

In summary, the present study demonstrates that OSCC patients exhibit a distinct peripheral blood gene expression profile, characterized by coordinated upregulation of pro-tumorigenic genes EGFR and CK17, increased expression of the inflammatory chemokine IP-10, and downregulation of the tumor suppressor ANXA1. This four-gene panel shows promise as a complementary, non-invasive biomarker for the early detection and clinical management of OSCC. Further investigations are needed to clarify the mechanistic links between these molecular changes and smoke-induced OSCC pathogenesis, and to validate the clinical applicability of this panel in larger, independent cohorts with comprehensive exposure assessment.

Conflict of Interest

There is no conflict of interest to declare.

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