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Evaluation of NCBP2 Gene Expression in Patients with Oral Squamous Cell Carcinoma Compared to Healthy Individuals

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is a widespread, aggressive disease with low survival rates due to late diagnosis and a lack of effective, noninvasive biomarkers. The nuclear cap-binding protein subunit 2 (NCBP2), involved in mRNA regulation, has been implicated in tumorigenesis. This study aimed to evaluate NCBP2 mRNA expression in plasma samples from patients with OSCC to assess its potential as a circulating diagnostic biomarker.

Materials and Methods: Fifteen patients with histologically proven OSCC and fifteen age-matched healthy controls participated in a case-control study. Plasma was isolated from peripheral blood in an RNase-free environment. Total RNA was extracted and reverse-transcribed into cDNA. Gene-specific primers and SYBR Green chemistry were used in quantitative real-time PCR. Using GAPDH as the reference gene, relative expression was computed using the 2^−ΔΔCt technique. Independent t-tests were used to examine the data, with a significance level of $p<0.05$.

Results: There was a 1.89-fold increase in NCBP2 mRNA expression in the OSCC group when compared to controls ($p<0.001$). Ten out of fifteen OSCC patients had positive NCBP2 expression, compared to five out of fifteen healthy controls who had detectable levels. Age, sex, and smoking status did not show significant correlations with gene expression.

Conclusion: The observed overexpression of circulating NCBP2 mRNA in OSCC patients supports its potential as a non-invasive biomarker for early detection. Integration of NCBP2 testing into liquid biopsy protocols could enhance diagnostic accuracy and improve patient outcomes. Further studies with larger sample sizes and functional validation are recommended.

Keywords: Oral squamous cell carcinoma (OSCC); Nuclear cap-binding protein2 (NCBP2); liquid biopsy; RT-qPCR; Circulating mRNA; Non-invasive biomarker.

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Introduction

Oral squamous cell carcinoma (OSCC) accounts for about 95% of all oral malignancies and is the 6th most common cancer around the world, contributing to roughly 3% of all cancers [3–4]. Despite advances in therapeutic innovation, the worldwide five-year survival rate for OSCC has remained stagnant in recent decades [4–5]. Early-stage detection significantly improves prognosis, with survival rates reaching 60–80%, yet the majority of cases—nearly two-thirds—are diagnosed at advanced stages (stage III or IV), primarily due to the asymptomatic nature of early lesions and their resemblance to benign inflammatory conditions [5,7].

Through varied degrees of epithelial dysplasia, well-known premalignant lesions like leukoplakia, erythroplakia, and erythroleukoplakia can advance to OSCC. [5]. The high recurrence rate of around 33% further highlights the need for more effective diagnostic and prognostic tools [5]. Although histopathological examination and biopsy remain the gold standards for diagnosis, their invasiveness and limitations in early detection necessitate the development of alternative approaches [8]. Current OSCC management is primarily guided by the TNM staging system, which does not accurately reflect the biological behavior of tumors [4–5]. Treatment usually consists of surgical excision, which is frequently followed by radiation therapy or chemotherapy, both of which can severely affect patients' quality of life, particularly in terms of speech, swallowing, and appearance [4–5].

In recent years, efforts have intensified to discover novel biomarkers and therapeutic targets that can facilitate the development of personalized medicine. Identifying molecular signatures associated with OSCC could improve diagnostic accuracy, enable early detection, and aid in stratifying patients for tailored treatment strategies—minimizing overtreatment in low-risk cases while ensuring aggressive therapy for high-risk individuals [5–6]. Cancer results from a combination of genetic and epigenetic changes in tumor suppressor genes and oncogenes, which cause uncontrolled proliferation, impaired differentiation, and resistance to apoptosis, according to cumulative data [1,17]. Environmental and lifestyle factors—particularly tobacco and alcohol consumption—also play crucial roles in OSCC development [4–5]. The nuclear cap-binding protein complex subunit 2 (NCBP2) has been implicated in several biological processes, including mRNA processing and export. While previous studies have evaluated NCBP2 expression in tumor tissues, the present study focuses on investigating NCBP2 gene expression in plasma samples from patients with OSCC and healthy individuals [10,13,26,27]. This non-invasive approach may provide valuable insights into the gene's diagnostic potential and its role in the pathogenesis of OSCC.

Materials and Methods

Study Design and Participants

The purpose of this case–control investigation was to compare the expression of the NCBP2 gene in plasma samples from individuals diagnosed with oral squamous cell carcinoma (OSCC) to that of healthy controls. Before sample collection, all participants provided written informed consent. The study was approved by the Ethics Committee of Tehran University of Medical Sciences (Ethics code: IR.TUMS.AMIRALAM.REC.1403.018). A total of thirty individuals were enrolled, including 15 patients with histopathologically confirmed OSCC and 15 age-matched healthy controls with no prior history of cancer or chronic disease. None of the OSCC patients had undergone chemotherapy, radiotherapy, or surgery before blood collection, and all had recently been diagnosed. Participants ranged in age from 15 to 45 years.

Sample Collection and Processing

Peripheral venous blood (2mL) was collected from each participant using sterile syringes and transferred into RNase-free glass test tubes. Blood samples were immediately centrifuged at 2,000 rpm for 10 minutes at 4°C to isolate plasma. The supernatant was then transferred to RNase-free microtubes, followed by a second centrifugation at 2,000×g for 15 minutes at 4°C to remove any residual cells and platelets. The plasma was aliquoted and stored at –80°C until RNA extraction.

RNA Extraction and Quantification

The ALL GENE RNA extraction kit (Zist Baran, Iran) was used to extract total RNA, including tiny RNAs, from 200µL of plasma according to the manufacturer's instructions. All procedures were conducted using approved RNase-free materials in an RNase-free environment. The NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA) was used to determine the RNA concentration and purity, and only samples with an A260/A280 ratio between 1.8 and 2.1 were taken into consideration for subsequent analysis.

cDNA Synthesis and Real-Time PCR

Complementary DNA (cDNA) synthesis was performed from total RNA using a miRNA-specific cDNA synthesis kit (Zist Baran, Iran). Reverse transcription was conducted on the entire RNA content of each sample. Quantitative real-time PCR (qPCR) was carried out using SYBR Green Master Mix (Pars Genome, Iran) and gene-specific primers for NCBP2 and the GAPDH housekeeping gene. Amplification was performed on a Rotor-Gene Q real-time PCR system (Qiagen, Germany) with the following thermal cycling conditions:

- Initial activation: 95 °C for 5 minutes
- 40 cycles of:
  - Denaturation: 95 °C for 5 seconds.
  - Annealing: 62 °C for 20 seconds.
  - Extension: 72 °C for 30 seconds.
- Melt curve analysis: 60–95 °C.
- SPSS software (version XX, IBM Corp., USA) was used for all statistical analyses. The Shapiro–Wilk test was used to determine the normality of the data distribution, while Levene's test was used to assess the homogeneity of the variances.
- The independent samples t-test (for normally distributed data) was used to compare NCBP2 gene expression between OSCC patients and healthy controls. TheSpearman's rank correlation coefficient was used to determine the correlations between NCBP2 expression levels and clinical factors such as age, gender, and smoking habits.
- Statistical significance was defined as $p<0.05$.

Results

The study included 15 patients with OSCC and 15 healthy controls. The mean age in the OSCC group was 32±15.02 years, while the control group had a mean age of 31±9.75 years. Statistical analysis using a test showed no significant age difference between the groups, confirming they were age-matched. Expression of NCBP2 was assessed using real-time PCR and a melting curve analysis. The biomarker was positive in 10 of 15 patients with OSCC and 5 of 15 healthy individuals. A statistical comparison using an independent t-test revealed a significant difference between the groups (p-value<0.001). (Figure 1). The CT (Cycle Threshold) value of each sample was initially acquired to compute the folding change. With the help of the $2^{\Delta\Delta C}$t equation, the relative difference between the two groups was determined. In comparison to the control group, the patient group exhibited a 1.89-fold increase in the relative expression of NCBP2 mRNA (Figure 2).

Table 1. Primer sequences and amplicon sizes.

Gene	*Forward Primer (5›-3›)*	*Reverse Primer (5›-3›)*	*Amplicon Size (bp)*
NCBP2	*AGTTCTGCCAGTGGTGATGC*	*TTGGGAGGAAATGTCGTTGA*	*154*
GAPDH	*GAAGGTGAAGGTCGGAGT*	*GAAGATGGTGATGGGATTTC*	*226*

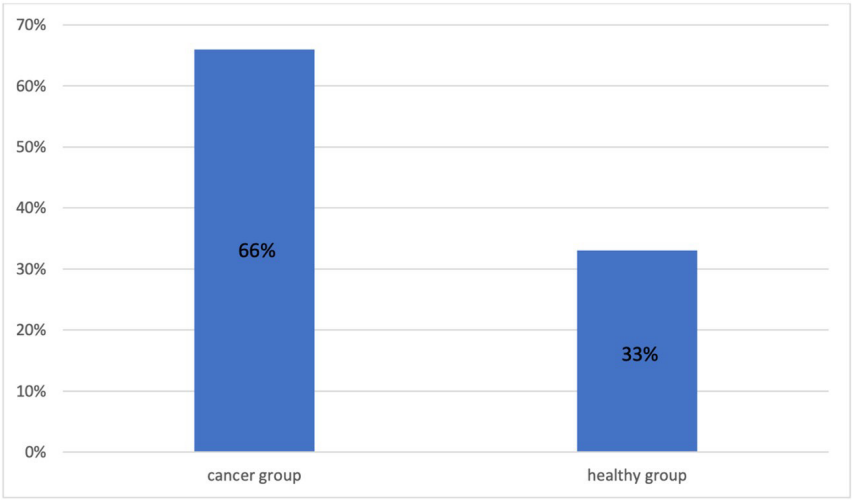

Figure 1. Percentage of NCBP2 mRNA-positive samples in the peripheral blood of OSCC cases and healthy individuals.

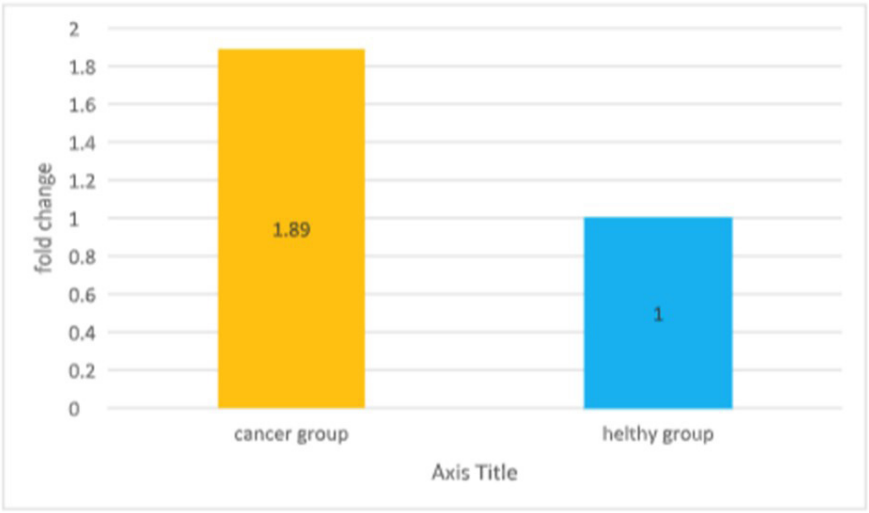

Figure 2. NCBP2 expression difference between OSCC cases and healthy individuals.

Discussion

A class of cancers affecting the oral cavity, nasal cavity, paranasal sinuses, pharynx, larynx, and salivary glands is known as squamous cell carcinoma of the head and neck (HNSCC). The most common kind of oral epithelial cancer among these is oral squamous cell carcinoma (OSCC), representing a significant health burden worldwide [5–6]. Tumor progression in OSCC is governed by complex molecular interactions involving mRNAs, miRNAs, and protein-level regulators [10–11,14–15]. In the present study, we investigated the expression of NCBP2 mRNA in peripheral blood samples of OSCC patients using Real-Time PCR, a sensitive and widely used technique in clinical molecular diagnostics [20]. Our findings revealed that NCBP2 was positively expressed in 10 out of 15 OSCC cases, indicating a potential role for this gene as a circulating biomarker in the detection of OSCC. These results support the hypothesis that NCBP2 overexpression is associated with oral cancer development and may be helpful for non-invasive diagnostic screening.

The implications of these findings are significant. Given that NCBP2 encodes a nuclear cap-binding protein involved in mRNA processing and stability, its dysregulation may contribute to the aberrant gene expression patterns observed in tumor cells. Detecting NCBP2 in peripheral blood also enhances its clinical utility, especially in early-stage diagnosis when tissue biopsies may not yet be feasible. Furthermore, its compatibility with Real-Time PCR platforms enables potential integration into routine clinical workflows. Our findings are consistent with previous studies that demonstrated the presence of OSCC-related molecular markers in various body fluids. For instance, Wong et al. detected miR-184 in the plasma of 80% of tongue OSCC patients, while only 13% of healthy controls showed detectable levels [22]. Similarly, Liu et al. and Lin et al. reported elevated levels of miR-31 and miR-24 in the saliva of OSCC patients [23–24]. Zheng et al. emphasized that tumor-associated miRNAs are selectively released into circulation and contribute to cancer progression [21].

Specifically regarding NCBP2, Kurokawa et al. evaluated its expression among six tumor markers and found it to be elevated in 34.5% of OSCC patients' plasma samples [25]. This aligns with our data, which also showed increased NCBP2 mRNA expression in a substantial proportion of OSCC patients. Furthermore, recent work by Arora et al. reinforced the prognostic potential of NCBP2 and TFRC in OSCC, supporting our conclusion that NCBP2 plays a relevant role in tumor biology [26]. However, this study has some limitations. The sample size was relatively small, limiting the statistical power and generalizability of the findings. Additionally, gene expression was only measured at the mRNA level, without further validation at the protein level or functional assays to confirm the causative mechanisms. The study also lacked long-term follow-up to assess the prognostic value of NCBP2 expression over time. Future research should address these limitations by including larger cohorts, longitudinal sampling, and mechanistic studies to better define the role of NCBP2 in OSCC pathogenesis. Based on our results and current literature, future research should investigate the co-expression patterns of NCBP2 with established oncogenic miRNAs and explore its involvement in cellular pathways, including proliferation, apoptosis, and immune evasion. Additionally, expanding this research to diverse patient populations may help determine

whether NCBP2 expression varies by tumor stage, site, or demographic factors.

Conclusion

In conclusion, this study identified significant overexpression of NCBP2 mRNA in the peripheral blood of OSCC patients, reinforcing its potential as a non-invasive biomarker for early detection. Our findings are consistent with previous studies and contribute to the growing understanding of circulating molecular markers in OSCC. While further investigation is needed, NCBP2 may hold promise for future diagnostic applications and therapeutic targeting in the management of oral cancer.

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Conflict of Interest

There is no conflict of interest to declare.

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