

FOXO1 Expression and Its Prognostic Significance in Oral Squamous Cell Carcinoma: An Immunohistochemical Study

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Abstract

Background

OSCC is the most common and aggressive malignancy of the oral cavity. OSCC has unfavorable prognosis, a high rate of recurrence and a low rate of survival. The fork head box protein O1 (FOXO1) is a transcription factor involved in processes such as apoptosis, oxidative stress and cancer suppression, but its prognostic value for OSCC is not fully understood.

Aim

The present study aimed to investigate the expression of FOXO1 at various histological grades of OSCC and study its association with apoptosis, proliferation, oxidative stress and clinicopathological features

Materials and Methods

A total of eighty subjects were included in the study that were divided into four groups such as control (healthy) group, well-differentiated OSCC, moderately differentiated OSCC, poorly differentiated OSCC (n = 20 in each group). The immunohistochemical assessment was performed for FOXO1, Caspase-3, Ki-67, PCNA, and Bcl-2 expression levels. The oxidative stress markers such as MDA and SOD was also measured. Statistically evaluated correlations between FOXO1 expression and clinicopathological features were assessed.

Results

The expression of FOXO1 was decreased in OSCC tissues compared with controls and reduced significantly with increasing tumor grade ($P < 0.001$). Downregulated FOXO1 expression was correlated positively with reduced expression of Caspase-3 and SOD levels whereas negatively with expression levels of Ki-67, PCNA, Bcl-2, MDA, inflammation score, size of the tumor, status of the lymph nodes, recurrence and survival. Patients with high expression of FOXO1 had a lower recurrence and better survival rates than patients with low expression of FOXO1.

Conclusion

Downregulation of FOXO1 is correlated positively with the progression of OSCC, diminished apoptosis, accelerated proliferation, oxidative stress and poor outcome of the OSCC patients. FOXO1 is a reliable prognostic marker and could be the target for therapy for OSCC.

Introduction

Oral squamous cell carcinoma (OSCC) represents a considerable public health challenge, with more than 300,000 new diagnoses occurring globally each year and a five-year survival rate that falls below 50%. (Kumar, 2025) Etiology involves complex biological and external factors, notably tobacco, alcohol, and betel nut consumption combined with human papilloma virus (HPV) infection. Dysfunction of a number of genes and associated signaling pathways—e.g., p53, cyclin D1, UBE2C, COX-2, the PI3K/Akt/PTEN axis—has been implicated in OSCC pathogenesis (Cheng et al., 2022). Despite this knowledge, the mechanistic details of oral cancer development remain incompletely elucidated (Lu, J et al., 2021).

Forkhead box protein O1 (FOXO1) is a member of the forkhead family of transcription factors that regulates a diverse array of cellular processes, including cellular differentiation, metabolism, cell cycle progression, oxidative stress response, and apoptosis (Zhang et al., 2022). FOXO1 can function as an oncogene or tumor suppressor in a context-dependent manner (Chen et al., 2021). In OSCC, loss of FOXO1 expression has been correlated with aggressive tumor behavior and unfavorable prognosis. Various studies have indicated that FOXO1-mediated regulation of apoptosis may be relevant to oral carcinogenesis (Alonso-Juarranz et al., 2023). Nevertheless, the relationship between FOXO1 expression and apoptotic marker expression in OSCC has yet to be investigated through immunohistochemical (IHC) methods (Alshammari et al., 2025). Through IHC analysis of a tissue microarray composed of OSCC specimens, the present study aims to determine whether FOXO1 expression is associated with the expression of four apoptosis-related molecules: Bcl-2, cleaved caspase-3, p-Akt, and PTEN (El-Din et al., 2022).

Oral squamous cell carcinoma (OSCC) is the predominant head and neck squamous cell carcinoma, with an aggressive behavior leading to a poor five-year prognosis. Its multifactorial etiology incorporates tobacco, alcohol, betel quid, and human papillomavirus as risk factors. (Nokovitch et al.2023) Molecularly, OSCC arises through the gradual accumulation of genetic and epigenetic alterations affecting key oncogenic and tumor-suppressor pathways (Alaa El-din et al., 2022). The phosphoinositide 3-kinase/Akt and cyclooxygenase-2 pathways are particularly prominent during early tumourigenesis and angiogenesis (Lu, Y et al., 2021). Forkhead box O (FOXO) transcription factors, including FOXO1, constitute an important family of tumour-suppressor regulators that drive antioxidant defence, cell cycle arrest, and apoptosis; the tight control of FOXO activity is deregulated in OSCC tumours and cell lines. Early work in other types of cancer implicated FOXO1 in tumour suppression and pro-apoptotic transcriptional control (Chen et al., 2021). Subsequent studies demonstrated that FOXO1 also promotes OSCC growth and migration by enhancing the transcription of cyclin D1 (El-Din et al., 2022). Nonetheless, the influence of FOXO1 on OSCC apoptosis remains unexplored, even though activation increases pro-apoptotic Bim and decreases anti-apoptotic Bcl-2 expression during the epithelial-to-mesenchymal transition (Tan et al., 2023). The lack of agreement on correlates of FOXO1 expression and the absence of links between FOXO1 immunohistochemical results and apoptosis markers imposed the need for further investigation. (Maiese et al., 2023)

Diverse immunohistochemical studies have established significant associations between varying FOXO1 expression and OSCC parameters, including locoregional spread, nodal involvement, recurrence, and

prognosis (Alshammari et al., 2025). The investigation of FOXO1 expression and its relationships with established pro- and anti-apoptotic regulators is warranted as a means of disentangling these complexities and as a necessary first step in establishing FOXO1's role in OSCC apoptosis and the wider regulatory circuit involved. (El-Din et al., 2022). This study aims to investigate the FOXO1- expression in human oral Squamous cell carcinoma.

Materials and methods

This retrospective, observational study was carried out on 80 formalin fixed paraffin embedded (FFPE) oral squamous cell carcinoma (OSCC) specimens collected from the files of Department of Oral and Maxillofacial Pathology, Faculty of Dentistry,

The study group of subjects was divided into four groups (n = 20 in each group) on the basis of the histopathological grade of OSCC:

- Group I: Normal oral mucosal tissues (Control Group)
- Group II: Well differentiated OSCC
- Group III: Moderately differentiated OSCC
- Group IV: Poorly differentiated OSCC

Diagnosis of OSCC and the classification into different grades were confirmed individually by two oral pathologists based on World Health Organization (WHO) classification system.

Inclusion Criteria

- Histopathologically confirmed OSCC cases
- Adequate tissue specimens for histological and immunohistochemical evaluation
- Complete clinicopathological records

Exclusion Criteria

- Patients who received chemotherapy or radiotherapy before biopsy
- Recurrent OSCC cases
- Inadequate or damaged tissue samples

Histopathological Examination

Sections of 4–5 µm in thickness were prepared from FFPE tissue blocks and stained with H&E for conventional histopathological evaluation and confirmation of diagnosis.)Lu et al. 2021

Immunohistochemical Analysis

Immunohistochemistry was carried out by using the streptavidin-biotin peroxidase complex method. Sections were deparaffinized with xylene, and hydrated with ethanol of decreasing grade. Antigen retrieval was done with the use of citrate buffer (pH 6.0) under microwave conditions. Sections were incubated with specific primary antibodies (FOXO1, Caspase-3, Ki-67, PCNA and Bcl-2) overnight at 4°C. The secondary antibodies were applied with the use of diaminobenzidine (DAB) as chromogen, and subsequently with Mayer's hematoxylin as counter stain.

Positive immune reaction was defined as brown staining in nuclei and/or cytoplasm depending on the marker assessed.

Immunohistochemical Scoring

FOXO1, Ki-67, PCNA, Caspase-3 and Bcl-2 expressions by immunohistochemistry were assessed on five randomly selected high power fields (HPF) under light microscope (400x). Percentage of positively stained cells was determined using the formula:

$$\text{Positive cells (\%)} = (\text{Number of positive cells} / \text{Total count of cells}) \times 100$$

Scoring was done independently by two blinded observers, the values obtained from their evaluations were averaged and were recorded.

Assessment of Oxidative Stress

The levels of malondialdehyde (MDA) and superoxide dismutase (SOD), oxidative stress markers were analyzed by using ready-made assay kits according to manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed with the use of the SPSS statistical package, version [XX] (IBM Corp., Armonk, NY, USA). Data were expressed as mean $\pm$ standard deviation (SD). One-way ANOVA was followed by post hoc test of Tukey to compare multiple groups. Paired t-test was used for longitudinal comparisons. Pearson's correlation coefficient was used for the assessment of correlation between FOXO1 expression and the other biological parameters. Chi-square test was employed for categorical variable comparisons. A P value of < 0.05 was considered statistically significant.

Ethical consideration

The study protocol was reviewed and approved by the Institutional Review Board (IRB), Faculty of Medicine, Al-Azhar University (Assiut), Egypt, under Approval No.

RESEARCH/AZ.AST./PAT005/12/226/1/2024. Written informed consent was obtained from all

participants prior to inclusion in the study. All procedures involving human participants were performed in accordance with the ethical standards of the Institutional Research Committee and the 1964 Declaration of Helsinki and its subsequent amendments

Results

Table 1
Demographic and Clinical Characteristics of the Study Population

Variable	G1 Healthy (n = 20)	G2 OSCC Well (n = 20)	G3 OSCC Moderate (n = 20)	G4 OSCC Poor (n = 20)	P value
Age (years), Mean ± SD	42.3 ± 8.1	55.6 ± 7.9	59.8 ± 6.5	63.4 ± 5.8	< 0.001*
Age					
30–45 years, n (%)	12 (60%)	2 (10%)	1 (5%)	0 (0%)	
46–60 years, n (%)	6 (30%)	9 (45%)	6 (30%)	4 (20%)	
> 60 years, n (%)	2 (10%)	9 (45%)	13 (65%)	16 (80%)	< 0.001*
Sex					0.08
Male, n (%)	11 (55%)	14 (70%)	15 (75%)	16 (80%)	
Female, n (%)	9 (45%)	6 (30%)	5 (25%)	4 (20%)	
Clinical Manifestations					
Pain, n (%)	0 (0%)	6 (30%)	12 (60%)	17 (85%)	< 0.001*
Dysphagia, n (%)	0 (0%)	4 (20%)	9 (45%)	15 (75%)	< 0.001*
Local Bleeding, n (%)	0 (0%)	3 (15%)	7 (35%)	13 (65%)	< 0.001*
Weight Loss, n (%)	0 (0%)	2 (10%)	6 (30%)	11 (55%)	< 0.001*

- ANOVA was used for comparison of mean age between groups.
- Chi square test was used for categorical variables.
- P < 0.05 was considered statistically significant.

According Table 1, an age demographic analysis indicated that as the severity of the tumor increases, there is a corresponding increase in the average ages of the patients with oral squamous cell carcinoma (OSCC). Patients from the poorly differentiated (most severe) group were older on average than other

OSCC patients (63.4 years old, standard deviation = 5.8). Additionally, they were also significantly older than the age-matched healthy control group (42.3 years, standard deviation = 8.1) ($p < 0.001$). There were also marked changes in the distribution of individuals within the various age categories as the tumor severity increased. The majority of OSCC patients were male (80% among the poorly differentiated group), however, there was not a significant difference between males and females in OSCC groups ($p = 0.08$). Starting with the mildest grade (well differentiated) and progressing to the most severe grade (poorly differentiated), manifestations of clinical symptoms such as pain, dysphagia (difficulty swallowing), bleeding from the tumor site, and weight loss also increased significantly ($p < 0.001$). This was reflected by the fact that as the grades of differentiation increased, the grades of clinical aggressiveness also increased. The demographic and clinical characteristics of the study population are summarized in Table 1 and illustrated in Fig. 1

According to figure (1), shifted over time, with the younger individuals being most common in the healthy population (60% of participants aged 30–45), while those greater than 60 years have increased steadily from well-differentiated to poorly differentiated OSCC, with the majority of those in the OSCC poor group being 80% in this group. Therefore, there is a clear correlation between the increasing age of patients with cancer (tumor) and their tumor (clinic) grading (severity of malignancy). The male sex predominated in all OSCC tumor's grades but particularly in the poorly differentiated group, whereas there was a more equal distribution of males and females in the healthy group. Thus, the data reflect the male sex predominance and higher age of males with cancer (OSCC) as the level of tumor aggressiveness increases.

Table 2
(Pre and Post Comparison) for all groups

Variable	Time	G1 Healthy (n = 20)	G2 OSCC Well (n = 20)	G3 OSCC Moderate (n = 20)	G4 OSCC Poor (n = 20)	P value
FOXO1 Score (Mean $\pm$ SD)	Pre	10.4 $\pm$ 1.2	8.1 $\pm$ 1.5	5.2 $\pm$ 1.7	2.9 $\pm$ 1.4	< 0.001
	Post	10.3 $\pm$ 1.1	7.4 $\pm$ 1.4	4.3 $\pm$ 1.5	1.8 $\pm$ 1.2	< 0.001
Caspase-3 Positive %	Pre	9.8 $\pm$ 2.1	5.4 $\pm$ 1.8	3.2 $\pm$ 1.1	1.6 $\pm$ 0.9	< 0.001
	Post	9.7 $\pm$ 2.0	4.8 $\pm$ 1.6	2.4 $\pm$ 1.0	0.9 $\pm$ 0.6	< 0.001
PCNA Positive %	Pre	3.2 $\pm$ 1.4	28.6 $\pm$ 6.2	52.3 $\pm$ 8.5	71.4 $\pm$ 9.3	< 0.001
	Post	3.1 $\pm$ 1.3	32.1 $\pm$ 7.1	58.7 $\pm$ 9.4	79.5 $\pm$ 10.2	< 0.001
Inflammation Score	Pre	1.1 $\pm$ 0.6	3.4 $\pm$ 1.1	4.8 $\pm$ 1.3	5.9 $\pm$ 1.2	< 0.001
	Post	1.0 $\pm$ 0.5	3.8 $\pm$ 1.2	5.3 $\pm$ 1.4	6.4 $\pm$ 1.3	< 0.001
Mean MDA (nmol/mg)	Pre	1.1 $\pm$ 0.3	3.2 $\pm$ 0.8	4.7 $\pm$ 1.0	5.8 $\pm$ 1.1	< 0.001
	Post	1.0 $\pm$ 0.3	3.8 $\pm$ 0.9	5.5 $\pm$ 1.2	6.6 $\pm$ 1.3	< 0.001
SOD (U/mg protein)	Pre	10.1 $\pm$ 1.5	6.7 $\pm$ 1.4	4.2 $\pm$ 1.2	2.8 $\pm$ 1.0	< 0.001
	Post	10.0 $\pm$ 1.4	5.9 $\pm$ 1.3	3.3 $\pm$ 1.1	2.0 $\pm$ 0.9	< 0.001
Tumor Size (cm)	Pre	0	2.1 $\pm$ 0.6	3.8 $\pm$ 0.9	5.4 $\pm$ 1.2	< 0.001
	Post	0	1.6 $\pm$ 0.7	3.5 $\pm$ 1.1	5.9 $\pm$ 1.4	< 0.001
TNM Stage	Pre	—	I–II	II–III	III–IV	< 0.001
	Post	—	I–III	II–IV	III–IV	< 0.001
Lymph Node Positive (%)	Pre	0%	20%	45%	70%	< 0.001
	Post	0%	15%	55%	80%	< 0.001
Treatment Response (Complete %)	Pre	—	—	—	—	—
	Post	—	75%	48%	22%	< 0.001
Recurrence (%)	Pre	0%	15%	40%	65%	< 0.001
	Post	0%	20%	50%	75%	< 0.001
Survival (Alive %)	Pre	100%	100%	100%	100%	—
	Post	100%	90%	68%	50%	< 0.001
Ki-67%	Pre	2.8 $\pm$ 1.1	30.4 $\pm$ 6.5	55.6 $\pm$ 8.7	74.9 $\pm$ 9.5	** <0.001

Variable	Time	G1 Healthy (n = 20)	G2 OSCC Well (n = 20)	G3 OSCC Moderate (n = 20)	G4 OSCC Poor (n = 20)	P value
	Post	2.7 ± 1.0	35.8 ± 7.4	63.2 ± 9.3	82.6 ± 10.4	** <0.001
Bcl-2%	Pre	3.1 ± 1.2	25.7 ± 5.9	48.3 ± 7.6	69.5 ± 8.8	** <0.001
	Post	3.0 ± 1.1	30.2 ± 6.8	55.9 ± 8.4	78.1 ± 9.7	** <0.001

*ANOVA test used for comparison

P < 0.05 was considered statistically significant.

According to Table 2, Evidence of FOXO1 expression in human oral squamous cell carcinoma (OSCC) decreases with increasing grades of OSCC and over time. FOXO1 levels were consistently high in healthy individuals, with little change during the study period; however, FOXO1 levels were markedly reduced in all OSCC subjects, especially in poorly differentiated OSCC. Caspase-3 also displayed progressively decreasing levels consistent with increasing tumor grade, suggesting a decrease in apoptosis. While markers of cancer cell proliferation and oxidative stress (specifically PCNA and MDA) increased at one-year follow-up, patients with moderate and poorly differentiated OSCC had the greatest increase in these markers. Additionally, over time, antioxidant activity (as measured by SOD) displayed a continued decline with tumor progression. Overall, patients with greater tumor size, TNM (tumor-node-metastasis) stage, lymph node positivity, recurrence rate, and decreased survival were more prominent with increased tumor grade. Taken together, findings from this study provide evidence that FOXO1 may be useful as a prognostic biomarker related to apoptosis, tumor aggressiveness, and clinical outcome.

Table 3
Group Longitudinal Changes

Variable	OSCC Well	Δ	OSCC Moderate	Δ	OSCC Poor	Δ	P value
FOXO1 Score	8.1 → 7.4	-0.7	5.2 → 4.3	-0.9	2.9 → 1.8	-1.1	< 0.01*
Caspase-3%	5.4 → 4.8	-0.6	3.2 → 2.4	-0.8	1.6 → 0.9	-0.7	< 0.01*
PCNA %	28.6 → 32.1	3.5	52.3 → 58.7	6.4	71.4 → 79.5	8.1	< 0.01*
Inflammation Score	3.2 → 3.8	0.6	4.7 → 5.5	0.8	5.8 → 6.6	0.8	< 0.01*
Mean MDA	3.2 → 3.8	0.6	4.7 → 5.5	0.8	5.8 → 6.6	0.8	< 0.01*
SOD	6.7 → 5.9	-0.8	4.2 → 3.3	-0.9	2.8 → 2.0	-0.8	< 0.01*
Tumor size (cm)	2.1 → 1.6	-0.5	3.8 → 3.5	-0.3	5.4 → 5.9	0.5	< 0.01*
Lymph node positive %	20 → 25	5%	45 → 55	10%	70 → 80	10%	< 0.01*
Recurrence %	15 → 20	5%	40 → 50	10%	65 → 75	10%	< 0.01*
Survival %	100 → 90	-10%	100 → 68	-32%	100 → 50	-50%	< 0.01*
Bcl-2%	25.7 → 30.2	4.5	48.3 → 55.9	7.6	69.5 → 78.1	8.6	< 0.01*
Ki-67%	30.4 → 35.8	5.4	55.6 → 63.2	7.6	74.9 → 82.6	7.7	< 0.01*

*Paired t-test used for longitudinal comparison

P < 0.05 was considered statistically significant.

According to Table 3. The longitudinal analysis revealed a gradual reduction of FOXO1 expression levels in all OSCC groups studied over one year. FOXO1 expression was further demonstrated to have decreased the most in the poorly differentiated OSCC group at the earliest points in time of data collection; this decrease in FOXO1 expression level coincided with a gradual decrease in the level of Caspase-3, suggesting there was a decrease in apoptotic activity. Conversely, tumor cell proliferation markers (PCNA and Ki-67) and the anti-apoptotic marker (Bcl-2) exhibited continual increases over time. Likewise, tumor markers of oxidative stress (MDA and inflammation score) were observed to increase, while antioxidant marker activity (SOD) decreased over time. Clinically, moderate and poorly

differentiated OSCC exhibited more rapid tumor size progression, higher TNM stage, higher incidence of lymph node positivity, higher rate of recurrences after treatment and lower survival after treatment than did well differentiated OSCC. Overall, FOXO1 continues to provide important prognostic information, as FOXO1 downregulation is highly associated with tumor progression and adverse clinical outcomes.

Figure 2: **the various classifications of the three groups of OSCC patients have changed over a one-year period**

Figure 2 displays how the various classifications of the three groups of OSCC patients have changed over a one-year period and shows that, similar to FOXO1, Caspase-3 has been downregulated, with the most significant downregulation occurring in poorly differentiated patients. Increased markers of proliferation (e.g., PCNA and Ki-67) and decreased markers of apoptosis (e.g., Bcl-2) are also present, with more pronounced increases in the higher-grade tumors. The clinical picture has also deteriorated, with increased lymph nodes positive for OSCC, increased recurrence rates of the OSCC, and decreased survival rates in OSCC patients with poorly differentiated tumors. Based on these findings, the greatest amount of change occurred in higher-grade patients, thereby validating the prognostic importance of FOXO1 being downregulated in tumor progression.

Figure 3, shows the reflects the longitudinal comparison of FOXO1 expression between the four study populations before and after one-year of follow-up. The results indicate that FOXO1 expression remains stable in healthy individuals and declines progressively in OSCC, particularly in poorly differentiated tumors, supporting the potential prognostic value of FOXO1.

Table 4

Correlation Between FOXO1 and Apoptotic Index

Variable Correlation	r value	P value
FOXO1 vs Caspase-3%	0.71	< 0.001*
FOXO1 vs PCNA %	-0.78	< 0.001*
FOXO1 vs MDA	-0.66	< 0.001*
FOXO1 vs SOD	0.69	< 0.001*
FOXO1 vs Inflammation Score	-0.73	< 0.001*

***Pearson correlation test used**

P < 0.05 was considered statistically significant.

According to Table 4. Correlation analysis of FOXO1 expression in relation to different tumor behavior-related biological markers demonstrated a strong, statistically significant correlation between FOXO1 and the five biological markers evaluated. Greater levels of FOXO1 expression were positively correlated to elevated levels of Caspase-3 and SOD protein (associated with apoptosis and antioxidant activity), meaning as FOXO1 level increases, so do levels of these two proteins. Conversely, FOXO1 had a strong,

negative correlation to the three biological markers of PCNA, inflammation score, and MDA (associated with cellular proliferation/inflammation and oxidative stress), indicating that decreased FOXO1 expression would be associated with increased cellular proliferation/inflammation and oxidative stress. Together, these results indicate that FOXO1 is likely to represent a marker of tumor suppression that is intimately associated with regulation of apoptosis and tumor aggressiveness.

Table 5
Prognostic Impact of FOXO1 Expression After 1 Year

FOXO1 Expression	Recurrence %	Survival Rate %	Complete Response %	P value
High FOXO1 (n = 30)	13%	90%	72%	< 0.01*
Low FOXO1 (n = 30)	58%	55%	28%	

FOXO1 Expression Physically Impacts Outcomes of Patients With OSCC

P < 0.05 was considered statistically significant.

According to Table 5 The prognostic evaluation of FOXO1 clearly demonstrates that FOXO1 expression has considerable effects on patient outcomes. Higher rates of recurrence and much higher rates of survival and complete response were demonstrated for high FOXO1 patients compared to low FOXO1 patients. Recurrence was much lower in high FOXO1 patients, with survival at 90% for high FOXO1 patients and only at 55% for low FOXO1 patients. Treatment response was much better in patients with increased levels of FOXO1 as well. These findings confirm the beneficial antioxidant properties of FOXO1 and the association of FOXO1 as a determining factor in therapeutic responses and overall survival amongst patients diagnosed with OSCC.

Figure 4 shows the demonstrates the prognostic significance of FOXO1 expression in patients with OSCC. Patients with high levels of FOXO1 expression demonstrated a much lower recurrence rate than patients with low levels of FOXO1 expression. Furthermore, the rates of survival and complete response were much higher in patients with high levels of FOXO1 expression than low levels of FOXO1 expression. Conversely, low levels of FOXO1 expression demonstrated increased rates of recurrence and poorer rates of survival. The findings presented in this figure further endorse FOXO1 as a favorable prognostic biomarker for better clinical outcomes and improvement in therapeutic response. Representative hematoxylin and eosin (H&E)-stained sections showing the histopathological features of the different study groups are presented in Fig. 5.

According to figure (5), (A) The Healthy Control group consists of stratified squamous epithelium (arrow), with underlying connective tissue of the lamina propria (star) shows loose connective tissue with small blood vessels (Control group, H&E staining × 100). Figure (B) moderately differentiated squamous cell carcinoma, showing focal keratinization (arrow), intercellular bridges, moderate pleomorphism, with intervening lymphocytic infiltrate (H&E staining, ×200). Figure (C) Well differrniated

squamous cell carcinoma, abundant keratinization (arrow), intercellular bridges invading the lamina propria, associated dense inflammatory lymphocytic infiltrate (H&E staining, $\times 200$). Figure (D) Invasive poorly differentiated squamous cell carcinoma shows invasive islands of squamous cells infiltrates the muscle fibers (arrow), (H&E staining X100). Representative immunohistochemical staining demonstrating FOXO1 expression in different histological grades of OSCC is shown in Fig. 6

According to figure (6), the Expression of FOXO1 in different grade of oral squamous cell carcinoma (OSCC), Figure (A, B) FOXO1 expression in well differentiated squamous cell carcinoma showing strong nuclear expression (X100 for A, X200 for B). Figure (C) FOXO1 expression in moderately differentiated squamous cell carcinoma(X100). Figure (D) FOXO1 expression in poorly differentiated squamous cell carcinoma showing decrease expression of FOXO1(X100).

Discussion

This study aims to investigate the FOXO1- expression in human oral Squamous cell carcinoma.

The research analyzes the demographic and clinical attributes of oral squamous cell carcinoma (OSCC) patients, emphasizing notable alterations linked to tumor severity. The examination of age trends reveals that patients with higher-grade OSCC, encompassing moderate and poorly differentiated tumors, are often older, with mean ages of 59.8 ± 6.5 years and 63.4 ± 5.8 years, respectively. The ages are significantly elevated compared to healthy controls (42.3 ± 8.1 years) and well-differentiated patients (55.6 ± 7.9 years), with statistical significance ($p < 0.001$).

This study aligns with epidemiological patterns indicating a significant rise in OSCC incidence beyond age 50, commonly associated with cumulative tobacco and alcohol consumption, alongside field cancerization effects in older demographics. Nevertheless, certain studies indicate no substantial link between age and tumor grade, implying that discrepancies may stem from geographical variations, such as the earlier emergence of OSCC in high-risk Asian populations attributable to betel nut use (Harada, K., et al., 2019; Remadevi, V., et al., 2021).

The age distribution of OSCC patients transitions from a predominance of younger persons (60% aged 30–45 years in healthy controls) to a majority of older patients (over 60 years in 80% of cases with poor-grade tumors), which correlates with tumor severity ($p < 0.001$). Prior studies indicate that elderly patients, particularly those over 60, frequently exhibit advanced stages of OSCC, resulting in inferior outcomes associated with delayed diagnosis and comorbidities. In contrast, current research examining younger populations, especially those with HPV-related OSCC, indicate a diminished correlation between age and tumor grade, revealing severe tumors in non-smoking adults under 50 (Harada, K., et al., 2019; Kim YG, et al., 2023; Remadevi, V., et al., 2021).

Males are the predominant demographic of OSCC cases (80% in poor distinction), albeit this disparity lacks statistical significance ($p = 0.08$). This conclusion aligns with global meta-analytic data indicating a stable male-to-female ratio of roughly 2:1 in OSCC, primarily due to behavior-related factors such as

smoking. Nonetheless, the picture is evolving, as research reveals a surge in HPV-positive cases and a heightened occurrence of OSCC among females in non-traditional demographics (Harada, K., et al., 2019; Remadevi, V., et al., 2021).

Clinical symptoms linked with OSCC exhibit a significant increase in severity corresponding to tumor grade. Prominent symptoms encompass pain (85%), dysphagia (75%), hemorrhage (65%), and weight reduction (55%), all of which intensify in severity among patients with poor-grade OSCC (all $p < 0.001$). Studies have demonstrated associations between these symptoms and advanced tumor stages (T/N stages) together with perineural invasion, with substantial weight loss ($> 10\%$) serving as an indicator of poorer survival outcomes. However, conflicting clinicopathologic analyses indicate that symptom severity may not directly correspond with tumor grade in early submucosal lesions, as well-differentiated tumors might radiographically resemble the aggressive characteristics of higher-grade tumors (Remadevi, V., et al., 2021; Kim YG, et al., 2023).

Table 2 illustrates a significant downregulation of FOXO1 and Caspase-3 in oral squamous cell carcinoma (OSCC) across multiple grades, which correlates with heightened expression of proliferation markers (PCNA, Ki-67, Bcl-2), oxidative stress (MDA), inflammation, and negative clinical outcomes, including increased tumor size, advanced TNM stage, lymph node involvement, recurrence, and reduced survival rates, with statistical significance (all $p < 0.001$).

The expression levels of FOXO1 markedly diminish from healthy controls (10.4 pre-treatment to 10.3 post-treatment) to poor-grade OSCC (2.9 pre-treatment to 1.8 post-treatment), demonstrating a direct association with tumor grade and underscoring its function as a tumor suppressor. Prior research confirms that FOXO1 promotes apoptosis via the transcriptional control of pro-apoptotic genes, with diminished levels linked to more aggressive tumor characteristics. Conversely, certain studies on head and neck tumors indicate that FOXO1 may be increased in hypoxic conditions to augment survival signaling instead of facilitating apoptosis (Remadevi, V., et al., 2021; Kim YG, et al., 2023).

The positive rate of Caspase-3 markedly declines from 9.8% in controls to merely 0.9% following treatment in poor-grade OSCC cases. This pattern parallels that of FOXO1 and indicates a disturbance in the apoptotic mechanism. Evidence suggests that FOXO1 promotes the Caspase-3 and Caspase-9 pathways in OSCC cell lines, such as YD-9, where its overexpression results in restored apoptosis and suppressed tumor growth. Conversely, certain studies indicate increased Caspase-3 levels in early-stage OSCC, despite diminished FOXO1, which is ascribed to alternate activation pathways that are independent of p53 (Harada, K., et al., 2019; Kim, Y. G., et al., 2023)

In suboptimal post-treatment patients, proliferation measures such as PCNA (79.5%), Ki-67 (82.6%), and Bcl-2 (78.1%) are markedly enhanced, facilitating tumor growth. These results exhibit an inverse correlation with FOXO1 levels, consistent with observations showing FOXO1 knockdown elevates PCNA and Ki-67 expression via the PI3K/Akt pathway in OSCC. Contrarily, studies from docetaxel-treated cohorts demonstrate a transient reduction in Bcl-2 levels that does not correlate with FOXO1, but is ascribed to chemotherapy-induced feedback mechanisms (Harada, K., et al., 2019).

Oxidative Stress and Antioxidants: MDA levels rise to 6.6 nmol/mg in suboptimal post-treatment, while SOD levels decline to 2.0 U/mg, signifying a redox imbalance that worsens clinical outcomes. FOXO1's function in enhancing SOD expression through antioxidant response elements is emphasized, with research in OSCC confirming changes in MDA and SOD levels in advanced stages. Conversely, several studies indicate compensatory increases in SOD inside specific inflamed OSCC microenvironments, irrespective of FOXO1 activity (Kim, Y. G., 2023; Remadevi, V., et al., 2021).

Patients with poor-grade OSCC exhibit substantial tumor growth (averaging 5.9 cm post-treatment), an 80% incidence of nodal positive, a 75% recurrence rate, a 50% survival rate, and merely 22% achieving a full response, with these trends deteriorating over time. FOXO1 is positioned as a potential prognostic marker, corroborated by meta-analyses demonstrating an association between diminished levels of FOXO1 and Caspase-3 and adverse survival outcomes in OSCC patients. Counter views from HPV-positive OSCC subgroups suggest enhanced treatment responses, despite analogous marker profiles, potentially affected by viral oncogene (Harada, K., et al., 2019; Kim, Y. G., 2023).

The results in Table 3 are consistent with current literature on oral squamous cell carcinoma (OSCC), identifying FOXO1 as a tumor-suppressive, pro-apoptotic element. The downregulation of FOXO1 is associated with enhanced tumor growth, oxidative stress, and unfavorable prognostic outcomes in high-grade malignancies. The results on FOX family members, apoptotic markers, and predictive behavior exhibit complexities and conflicting interpretations that warrant consideration.

Multiple studies validate that FOXO proteins, such as FOXO1, function as tumor suppressors by facilitating cell-cycle arrest and death. In OSCC models, the overexpression of FOXO1 and FOXO3A leads to reduced proliferation and enhanced expression of apoptotic markers. In contrast, the inhibition of FOXO activity promotes tumor proliferation. Experiments demonstrate that increased expression levels of FOXO1/FOXO3A—attainable via retinoids—decrease oncogenic FOXM1 and its downstream cell-cycle regulators, thereby clarifying the association between diminished FOXO levels and aggressive tumor characteristics. These findings align with the recorded increasing reduction of FOXO1 concurrent with increases in markers such as PCNA/Ki-67 and Bcl-2 over time (Osei-Sarfo, K., & Gudas, L. J., 2019).

In contrast, several research offer more complex accounts concerning FOX family members. FOXM1, which possesses an oncogenic function, is frequently overexpressed in OSCC and is associated with unfavorable treatment results. This highlights the intricacy of reading FOX-related research, as not all FOX factors serve identical functions. Furthermore, in certain instances of head and neck cancers, FOXO expression does not consistently correlate with patient survival outcomes, indicating that prognostic implications may be affected by upstream signals such as PI3K/AKT activation, challenging the simplistic assumption that elevated FOXO levels ensure favorable prognoses (Harada, K., et al., 2019; Steigen, S. E., et al., 2020).

The literature consistently indicates that the proliferation markers Ki-67 and PCNA escalate progressively from well-differentiated to poorly differentiated cancers, corresponding with advanced stages, heightened nodal metastasis, and increased recurrence rates. This corroborates the recorded

observations of increasing Ki-67 and PCNA levels in association with tumor grades over time. Elevated Ki-67 indices are associated with reduced overall and disease-free survival in OSCC, a trend observed throughout higher-grade categories in this study (Steigen, S. E., et al., 2020; Xie, S., et al., 2016).

A study by Montebugnoli, L., et al., 2009, identified methodological concerns in the evaluation of OSCC Ki-67, indicating that discrepancies between visual scoring and digital image processing result in differing cutoff points (e.g., > 20%). The research revealed significant heterogeneity in raised Ki-67 levels in "normal" mucosa (spanning from 2% to 41%), hence complicating the comparison of longitudinal or multi-site results. A 2016 meta-analysis by Zheng identified inconsistencies in scoring methodologies, including discrepancies in antibody clones, hot-spot selections, and cutoffs (ranging from 10% to 50%), indicating that these factors substantially contribute to heterogeneity and undermine the reliability of percentage shift interpretations in related analyses (Montebugnoli, L., et al., 2009; Xie, S., et al., 2016).

Comprehensive clinical data demonstrates that poorly differentiated OSCCs have advanced stages, heightened nodal involvement, elevated recurrence rates, and inferior survival outcomes relative to well-differentiated tumors. This aligns with the study's examination of tumor size, nodal positivity, and survival rates (Lin, N. C., et al., 2020; Steigen, S. E., et al., 2020; Sufiawati, I., et al., 2024).

Nonetheless, new grading systems that emphasize factors like invasive depth may undermine the independent prognostic significance historically associated with histological grading. Particularly, some smaller studies imply that there are no substantial survival differences between well and moderately differentiated cases when stages are nearly matched, indicating that grade alone may not fully account for the observed outcome variances (Sharma, P., & Wadhwan, V., 2023).

A 2020 review by Almangush et al. indicates that POI and DOI are superior predictors of survival compared to WHO grading in early-stage OSCC cohorts (Almangush, A., et al., 2020). Sharma et al. (2023) performed a study evaluating the predictive significance of Bryne's invasive front scores in comparison to WHO grades for predicting 5-year survival rates. Patients with Bryne's scores over 12 had a markedly better survival rate of 60.6%, in contrast to 17.6% for those with lower scores. The WHO classification revealed a significant disparity in survival rates: 48.9% for grades I and II combined, whereas grade III patients had a 0% survival rate. This study demonstrates that Bryne's intrusive front scores surpass WHO grades in evaluating patient prognosis.

Table 4 demonstrates that elevated FOXO1 expression is favorably associated with pro-apoptotic and antioxidant markers, including Caspase-3 and SOD, while exhibiting a negative correlation with indicators of proliferation, oxidative stress, and inflammation, such as PCNA, MDA, and inflammation score. This indicates that FOXO1 acts as a tumor-suppressive transcription factor, facilitating apoptosis and enhancing antioxidant defenses while restraining tumor aggressiveness associated with inflammation and proliferation.)Mohamed & Mansour 2018(.

Research indicates that elevated FOXO1 levels in granulosa-cell and ovarian models increase Caspase-3 expression, consistent with its positive association with pro-apoptotic genes. Furthermore, FOXO1 is

recognized for enhancing antioxidant enzymes, thereby diminishing oxidative stress indicators, which substantiates the inverse relationship with MDA and the direct relationship with SOD. Moreover, the knockdown of FOXO1 in cancer cell lines demonstrates elevated proliferation markers, substantiating the inverse relationship with PCNA and strengthening the role of FOXO1 in suppressing tumor cell proliferation. In models of chronic stress and reproductive diseases, FOXO1 activation is associated with the inhibition of inflammation and oxidative stress pathways, consistent with its negative connection to the inflammatory score (Han, Q., et al., 2020; Zhang, J. Q., et al., 2016; Shen, M., et al., 2012; Ponugoti, B., et al., 2013; Lu, J., et al., 2021;

Conversely, certain evidence indicates that FOXO1 may display multiple or carcinogenic functions contingent upon cellular environment, tissue type, and mutational backgrounds. In specific wound-healing and epithelial-repair models, FOXO1 acts as an antioxidant to promote cell survival, contrasting its pro-apoptotic action in tumor environments. In hormone-responsive or stromal contexts, FOXO1 may facilitate differentiation or survival, indicating that elevated FOXO1 levels may not inherently signify improved tumor suppression. Furthermore, in particular cancer subtypes, FOXO1 may be incorporated into pathways that promote survival, potentially facilitating tumor development in conditions of chronic inflammation or elevated stress. These data suggest that FOXO1's function as a tumor suppressor is contingent upon specific contexts, differing according to cell types and signaling settings, and is not universally applicable in all instances (Lu, J., et al., 2021; Han, Q., et al., 2020; Zhang, J. Q., et al., 2016; Ponugoti, B., et al., 2013).

Elevated FOXO1 expression in oral squamous cell carcinoma (OSCC) is associated with markedly reduced recurrence (13% compared to 58%), enhanced survival rates (90% versus 55%), and superior complete-response rates (72% against 28%). This suggests that FOXO1-high tumors exhibit reduced aggressiveness and demonstrate improved therapeutic responses, reinforcing the function of FOXO1 as a tumor-suppressor transcription factor that modulates apoptosis and affects recurrence and survival rates. Research from numerous studies substantiates this optimistic prognosis for elevated FOXO1 levels, indicating improved overall survival and reduced recurrence across various malignancies, including colorectal cancer (Kim, Y. G., 2023; Zhang, G., et al., 2025). Nonetheless, certain studies suggest that FOXO1 may exert detrimental effects contingent upon the kind of cancer, underscoring its inconsistent prognostic significance shaped by the tumor microenvironment and signaling pathways that may diminish its efficacy. Consequently, whereas elevated FOXO1 expression often indicates a favorable prognosis in OSCC, its impact may differ depending on particular circumstances (Ide, H., et al., 2019; Li, J., et al., 2024).

The healthy control has stratified squamous epithelium with intact maturation and a loosely organized, vascular lamina propria, signifying a baseline mucosa marked by tightly regulated epithelial proliferation and death. Under homeostatic settings, FOXO1 is essential for sustaining cell-cycle arrest and promoting physiological apoptosis. The standard architectural arrangement, characterized by ordered layers devoid of irregular alterations, highlights a harmonic interaction between cellular proliferation and FOXO1-

mediated pro-apoptotic signaling. This equilibrium efficiently inhibits the clonal proliferation of compromised keratinocytes, thus preserving skin integrity and functionality (Remadevi, V., et al., 2021).

Figures B–D illustrate a discernible trend of architectural and cytological disarray in the carcinoma sections, frequently associated with the disruption of apoptotic regulating mechanisms, notably the inactivation or downregulation of FOXO1. Research demonstrates that the progression of (OSCC) results in the diminished transcriptional activity of FOXO1, which subsequently reduces the production of pro-apoptotic mediators such as Bim, FasL, and TRAIL-related components. This reduction aids in the survival of dysplastic and malignant keratinocytes within tumor nests, promoting disease development (Min, F., et al., 2021).

The moderately differentiated oral squamous cell carcinoma (OSCC) has focal keratinization, intercellular bridges, and mild pleomorphism accompanied by a lymphocytic infiltration, signifying partial retention of squamous traits yet clear malignancy. This pattern indicates a moderate degree of apoptotic dysregulation, wherein FOXO1-mediated differentiation is partially active, whereas survival signaling (e.g., PI3K/AKT) may suppress FOXO1 and enhance tumor cell viability. The lymphocytic infiltrate signifies an immune response, and research reveals that more differentiated tumor regions demonstrate elevated expression of pro-apoptotic factors, implicating FOXO1 in the apoptotic mechanisms within these intermediate-grade lesions. Within the Al-Azhar framework, moderately differentiated OSCC is regarded as a molecular intermediary between well-differentiated, apoptosis-competent tumors and poorly differentiated, apoptosis-resistant tumors, necessitating an examination of FOXO1 immunoreactivity across several grades (Habba, D., et al., 2000; Kim, Y. G., et al., 2023).

The well-differentiated oral squamous cell carcinoma (OSCC) demonstrates considerable keratinization and intercellular connections infiltrating the lamina propria, accompanied by substantial lymphocytic infiltration, signifying pronounced squamous differentiation. Research indicates that these tumors exhibit a greater presence of terminal differentiation markers and an elevated apoptotic index compared to higher-grade tumors, possibly associated with maintained FOXO1 activity. Conversely, poorly differentiated OSCC has invasive clusters of atypical squamous cells characterized by low keratinization and architectural disarray, which correlates with aggressive behavior and unfavorable prognostic outcomes. This indicates that FOXO1-mediated apoptotic signaling is inhibited in high-grade lesions due to hyperactivation of upstream kinases, highlighting FOXO1's potential as a prognostic marker in OSCC (Elzarka, A., & Abd Al-Wahab, A. S., 2025; M Mohamed, A., & M Mansour, A., 2018).

Conclusion

This work demonstrates that FOXO1 expression is significantly diminished in oral squamous cell carcinoma (OSCC), especially in advanced instances, which correlates with reduced apoptosis, enhanced cell proliferation, increased oxidative stress, and elevated inflammation. Reduced FOXO1 levels are associated with bigger tumors, lymph node involvement, and a worse prognosis.

Declarations

Conflict of Interest

The authors declare that they have no known financial or personal relationships that could have appeared to influence the work reported in this manuscript. No competing interests exist.

Ethics approval and consent to participate

The study protocol was approved by the Research Ethics Committee of [University Name] under approval number [IRB Number]. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

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Authors' contributions

Conceptualization: All authors.

Methodology: All authors.

Data Curation: All authors.

Investigation: All authors.

Formal Analysis: All authors.

Validation: All authors.

Visualization: All authors.

Writing – Original Draft: All authors.

Writing – Review & Editing: All authors.

Supervision: The corresponding author supervised all stages of the study.

All authors have read and approved the final version of the manuscript.

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Data Availability

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

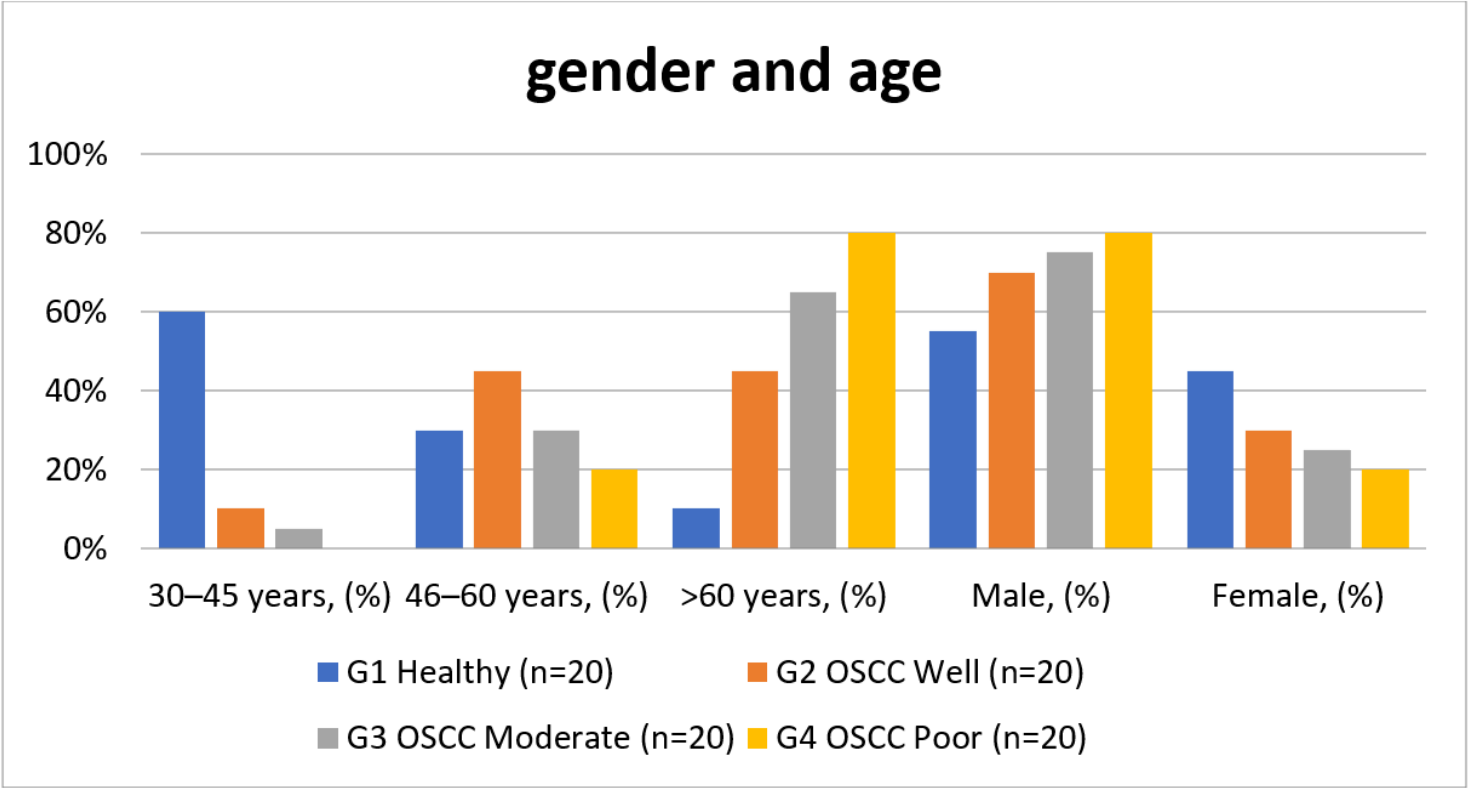


Figure 1

shows Democratic characteristics remain relative to age and gender.

According to figure (1), shifted over time, with the younger individuals being most common in the healthy population (60% of participants aged 30–45), while those greater than 60 years have increased steadily from well-differentiated to poorly differentiated OSCC, with the majority of those in the OSCC poor group being 80% in this group. Therefore, there is a clear correlation between the increasing age of patients with cancer (tumor) and their tumor (clinic) grading (severity of malignancy). The male sex predominated in all OSCC tumor’s grades but particularly in the poorly differentiated group, whereas there was a more equal distribution of males and females in the healthy group. Thus, the data reflect the male sex predominance and higher age of males with cancer (OSCC) as the level of tumor aggressiveness increases.

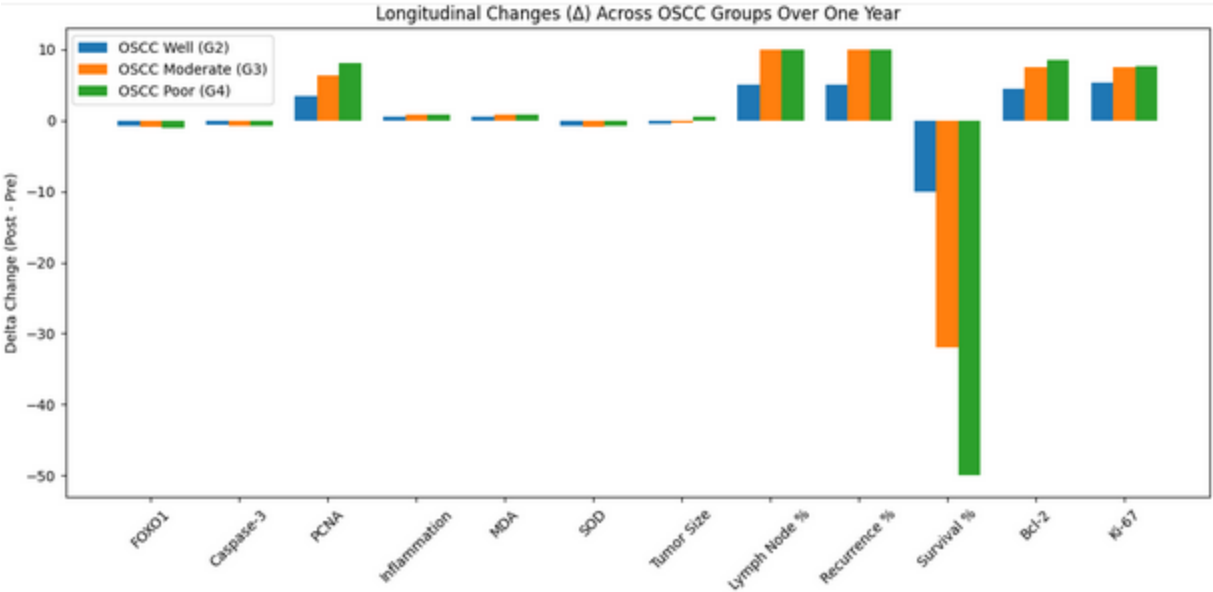


Figure 2

the various classifications of the three groups of OSCC patients have changed over a one-year period

displays how the various classifications of the three groups of OSCC patients have changed over a one-year period and shows that, similar to FOXO1, Caspase-3 has been downregulated, with the most significant downregulation occurring in poorly differentiated patients. Increased markers of proliferation (e.g., PCNA and Ki-67) and decreased markers of apoptosis (e.g., Bcl-2) are also present, with more pronounced increases in the higher-grade tumors. The clinical picture has also deteriorated, with increased lymph nodes positive for OSCC, increased recurrence rates of the OSCC, and decreased survival rates in OSCC patients with poorly differentiated tumors. Based on these findings, the greatest amount of change occurred in higher-grade patients, thereby validating the prognostic importance of FOXO1 being downregulated in tumor progression.

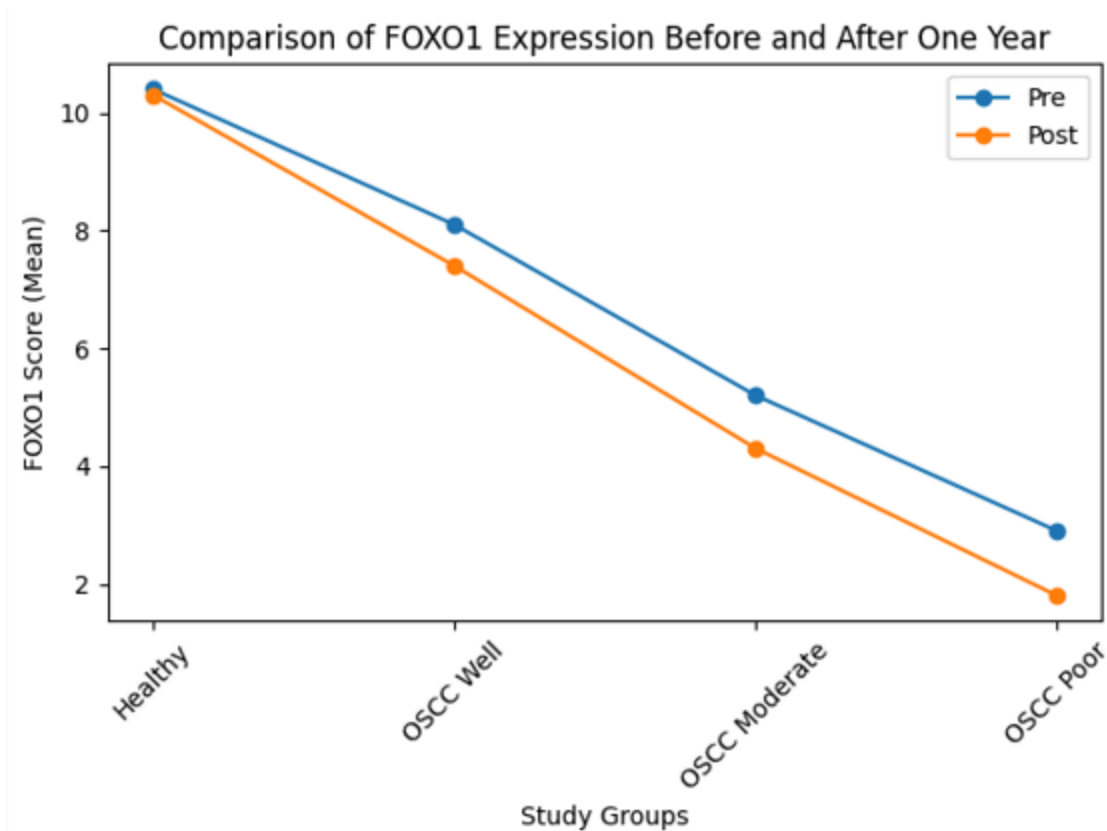


Figure 3

shows the reflects the longitudinal comparison of FOXO1 before and after one-year of follow-up

shows the reflects the longitudinal comparison of FOXO1 expression between the four study populations before and after one-year of follow-up. The results indicate that FOXO1 expression remains stable in healthy individuals and declines progressively in OSCC, particularly in poorly differentiated tumors, supporting the potential prognostic value of FOXO1.

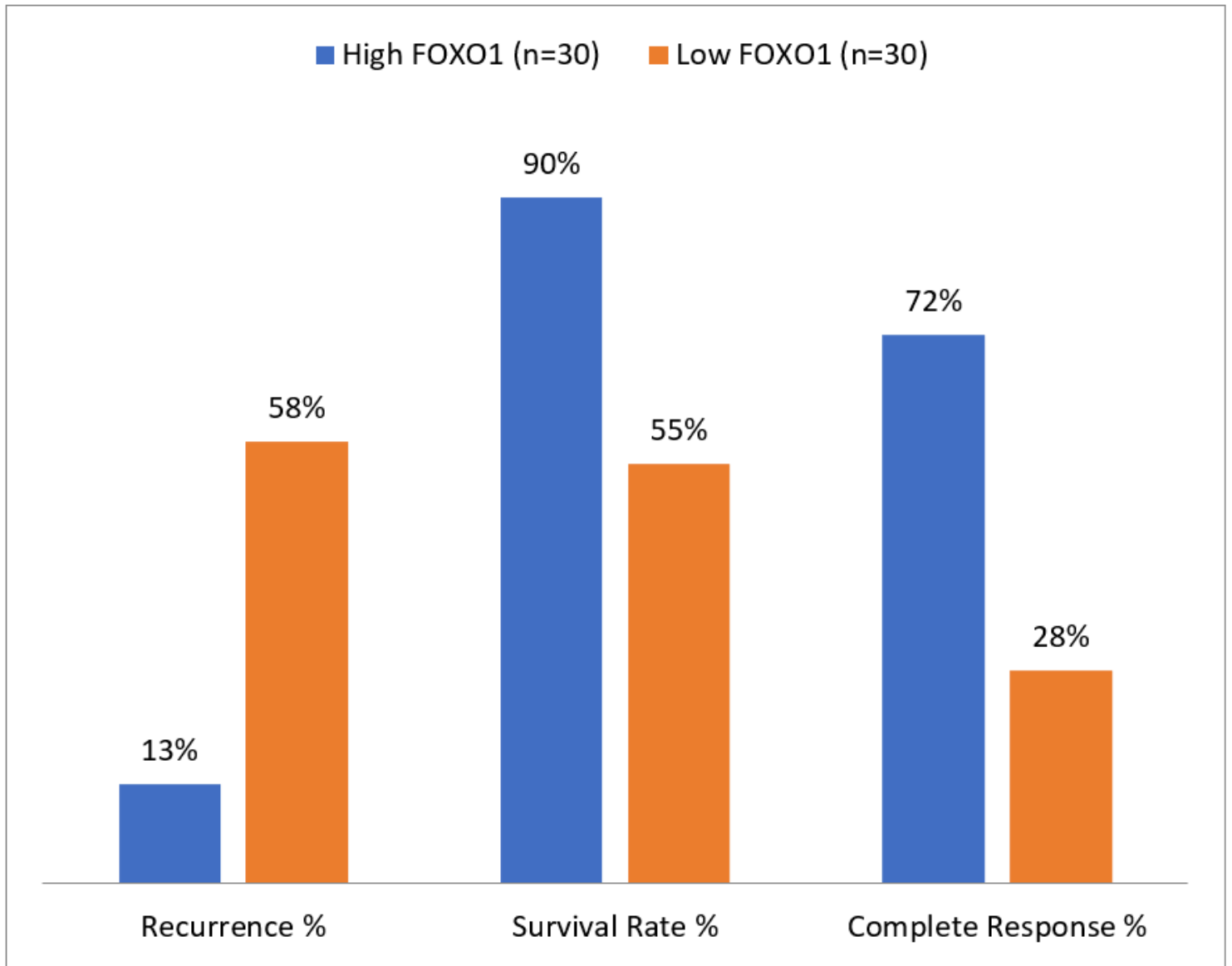


Figure 4

shows the demonstrates the prognostic significance of FOXO1 expression in patients with OSCC

shows the demonstrates the prognostic significance of FOXO1 expression in patients with OSCC. Patients with high levels of FOXO1 expression demonstrated a much lower recurrence rate than patients with low levels of FOXO1 expression. Furthermore, the rates of survival and complete response were much higher in patients with high levels of FOXO1 expression than low levels of FOXO1 expression. Conversely, low levels of FOXO1 expression demonstrated increased rates of recurrence and poorer rates of survival. The findings presented in this figure further endorse FOXO1 as a favorable prognostic biomarker for better clinical outcomes and improvement in therapeutic response. Representative hematoxylin and eosin (H&E)-stained sections showing the histopathological features of the different study groups are presented in Fig. 5.

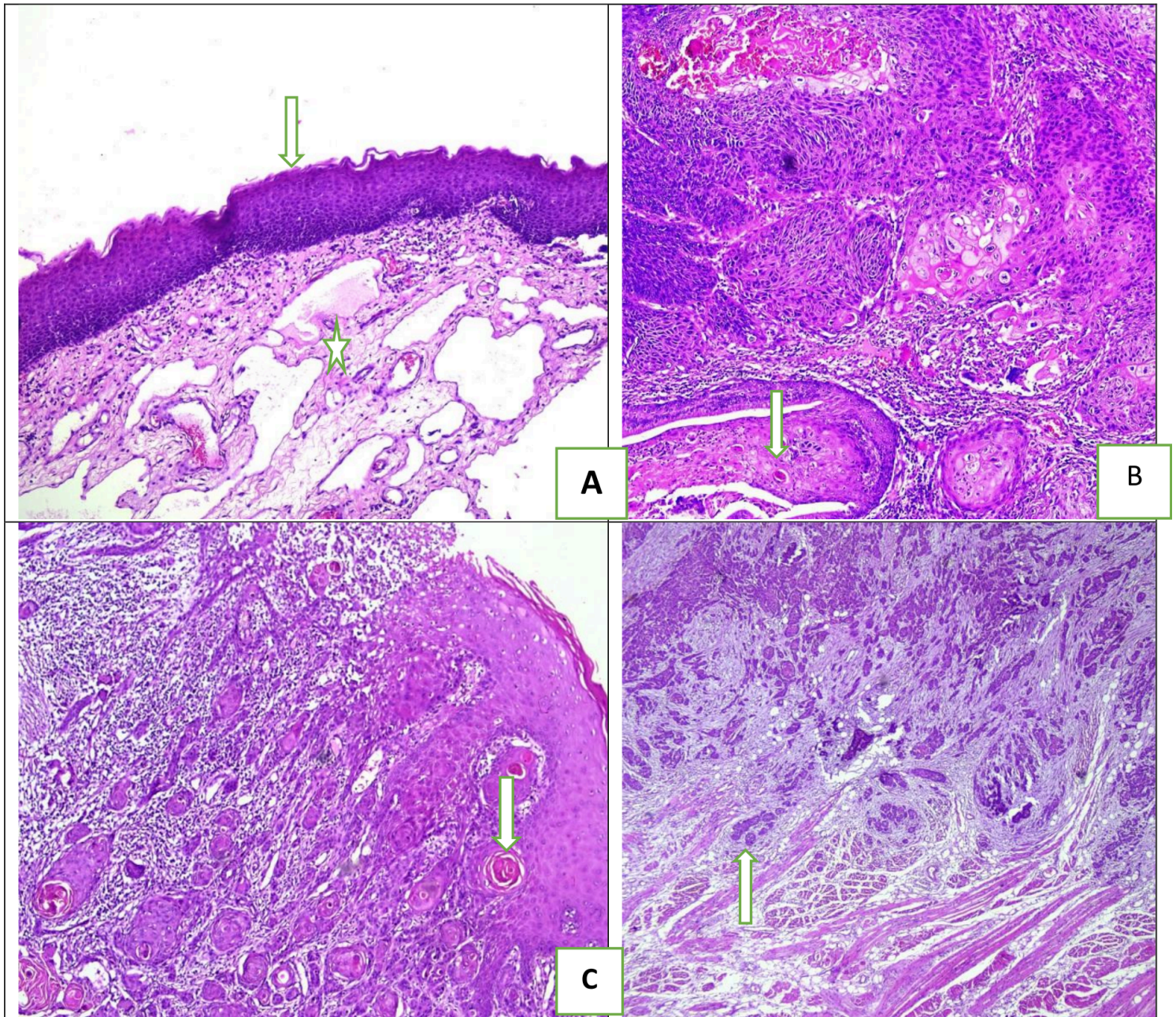


Figure 5

Comparison of the three groups in terms of stratified squamous cell carcinoma:

According to figure (5), (A) The Healthy Control group consists of stratified squamous epithelium (arrow), with underlying connective tissue of the lamina propria (star) shows loose connective tissue with small blood vessels (Control group, H&E staining $\times 100$). Figure (B) moderately differentiated squamous cell carcinoma, showing focal keratinization (arrow), intercellular bridges, moderate pleomorphism, with intervening lymphocytic infiltrate (H&E staining, $\times 200$). Figure (C) Well differentiated squamous cell carcinoma, abundant keratinization (arrow), intercellular bridges invading the lamina propria, associated dense inflammatory lymphocytic infiltrate (H&E staining, $\times 200$). Figure (D) Invasive poorly differentiated squamous cell carcinoma shows invasive islands of squamous cells infiltrates the

muscle fibers (arrow), (H&E staining X100). Representative immunohistochemical staining demonstrating FOXO1 expression in different histological grades of OSCC is shown in Fig. 6

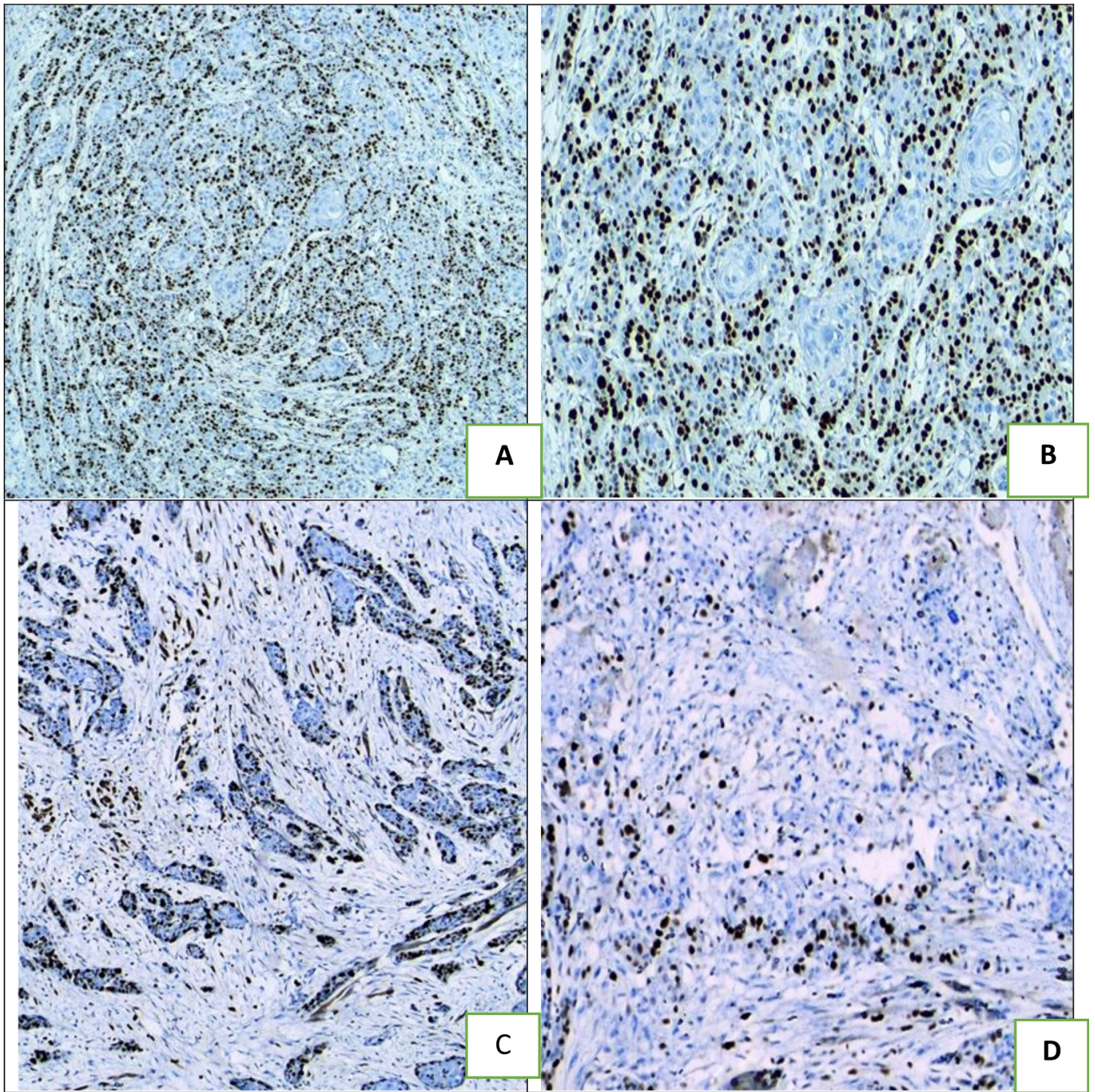


Figure 6

shows. the Expression of FOXO1 in different grade of oral squamous cell carcinoma (OSCC),

According to figure (6), the Expression of FOXO1 in different grade of oral squamous cell carcinoma (OSCC), Figure (A, B) FOXO1 expression in well differentiated squamous cell carcinoma showing strong

nuclear expression (X100 for A, X200 for B). Figure (C) FOXO1 expression in moderately differentiated squamous cell carcinoma(X100). Figure (D) FOXO1 expression in poorly differentiated squamous cell carcinoma showing decrease expression of FOXO1(X100).