African Journal of Biological Sciences

Research Paper Open Access

X-Linked Inhibitor of Apoptosis Protein as A Potential Predictor for Patients` Survival and Treatment Response in Oral Squamous Cell Carcinoma Patients; A Retrospective Clinicopathological Study

Reham Elmogy[1], Heba A. Elhendawy[2], Dina Abdallah Ibrahim[3], Hala Elshenshawi[4], Azza El-Sissi[5]

[1]Oral pathology department, Faculty of dentistry, Mansoura University, Dentist at Egyptian ministry of health.
[2]Lecturer of Oral pathology department, Faculty of dentistry, Mansoura University.
[3]Assistant professor of pathology, Faculty of medicine, Mansoura University.
[4]Prof clinical oncology and nuclear medicine, Faculty of medicine, Mansoura University.
[5]Professor of Oral Pathology, Faculty of Dentistry, Mansoura University.

Corresponding author (*): Reham Elmogy
Email: rema.dentist88@gmail.com.

Article Info

Volume 6, Issue 8, April 2024
Received: 12 Feb 2024
Accepted: 23 March 2024
Published: 08 April 2024

Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common neoplasm of the oral cavity. Although the great advancement in treatment strategies of OSCC, the five-year survival rate has remained low due to the high resistance of cancer cells to the cancer-therapy mediated apoptosis. Chemo-radio resistance of cancer cells is the major obstacle in the treatment of OSCC cases. Defective apoptosis represents an important causative factor in the development and progression of cancer. The ability of tumor cells to evade apoptosis can play a significant role in resistance to chemotherapeutic and radiation-induced cell death. X-linked inhibitor of apoptosis protein (XIAP) can suppress the apoptotic process and confer resistance to anticancer therapy. **Aim**: The current study aimed to elucidate the benefits of utilizing XIAP immuno-expression as a predictor for the sensitivity of cancer cells to the received treatment modality in OSCC patients. Moreover, we hypothesized that XIAP expression could aid in customizing the preferred treatment modality (CTH, RTH, or CCRT) for OSCC patients. Finally, can XIAP expression predict DFS and OS survival rates in OSCC patients? **Methods:** The current retrospective study was carried out on 50 OSCC cases**.** Pearson's Chi-square test was used to correlate XIAP immuno-expression with all clincopathological parameters of the studied cases and the patient's clinical response to the different received treatment modalities. Clinical response was categorized into clinically responders, and non-responders following the WHO Criteria. Moreover, in univariate analysis, the Kaplan-Meier method was used to calculate OS and DFS using log-rank test to detect the effect of risk factors. Additionally, for multivariate analysis, the COX regression model was used to record the independent predictors for DFS and OS. A P-value of 0.05 or less was regarded as statistically significant. **Results:** High Significant XIAP expression were recorded concerning WHO histologic grades ($p<0.001$), TNM clinical stages ($p= 0.012$), mortality rates ($p=0.006^*$), incidence of recurrence ($p= 0.01$) in addition to clinical response to treatment ($p<0.001^*$). The univariate analysis revealed significantly reduced DFS and low OS rates in the cases that showed high XIAP expression ($p<0.001$ and $p= 0.005$ respectively), non-responsive cases reported significantly reduced DFS ($p=<0.001^*$) and OS ($p=<0.001^*$) than responsive cases. Furthermore, DFS was significantly reduced in cases that received CTH only than those receiving CCRTH or RTH only ($P=0.016^*$). In contradiction, there was no statistically significant difference in patients` OS considering the

received treatment type (P= 0.35). Additionally, the multivariate analysis revealed that treatment type (P= 0.02), patients` clinical response (P= 0.001), and XIAP expression score (P=0.003*) could be considered the independent predictors for DFS, while patients` clinical response (P=0.019*) could be considered the only independent predictors for OS in the worked OSCC cases.
Conclusion: XIAP immuno-expression could be considered a potential predictor for the patient's clinical response to the received treatment. Moreover, patients` clinical response and XIAP immuno-expression could be potentially used as survival predictors in OSCC patients.
Key words: Oral squamous cell carcinoma (OSCC), XIAP, Immunohistochemistry, Patients` clinical response, risk factors, prognostic indicators, Disease free survival (DFS), Overall survival (OS).

Introduction

Oral cancer, the sixth most common malignancy worldwide with an annual incidence of over 300,000 cases presents pre-dominantly as oral squamous cell carcinoma (OSCC)(1). Oral squamous cell carcinoma (OSCC) is the most common neoplasm of the oral cavity, which accounts for about 95% of all cases of head and neck cancers. This neoplasm has a multifactorial etiology and commonly affects men between the sixth and seventh decades of life with smoking and drinking habits(2,3).

Surgery, radiation (RT), and chemotherapy (CT) are the main treatment modalities that are used to treat oral cancer, either alone or in combination (4,5). The decision about the mode of OSCC treatment is determined by various factors associated with the tumor, the patient, and the medical crew (6). Tumor characteristics such as the site of occurrence, proximity to bone, the depth of invasion, and tumor stage including tumor size, lymph node involvement, and risk of metastasis are considered along with the patient`s age, co-morbidities, and compliance to treatment (6). Despite the great advancement in treatment strategies of OSCC, the five-year survival rate has remained low (approximately 50%) during the past 30 years due to the high resistance of cancer cells to cancer-therapy mediated apoptosis(7). Chemo-radio resistance of cancer cells is the major obstacle in the treatment of OSCC cases (8,9). Many studies reported a great number of OSCC patients had poor responses to chemotherapeutic drugs (10–12). The clinical drug response of a cancer sub-type seems to be mediated by several mechanisms. These drug response mechanisms include overexpression of membrane transporters effluxing anticancer drugs from the cells, activation of DNA repair enzymes, defects in proteins involved in cell cycle and apoptosis, and activation of cytosolic drug detoxification (13–16).

Apoptosis is an essential process to maintain a wide variety of physiological processes, such as embryonic development, tissue homeostasis, and immune defense, and its role is to remove harmful, damaged, or unwanted cells. Defective apoptosis represents an important causative factor in the development and progression of cancer. The ability of tumor cells to evade apoptosis can play a significant role in resistance to chemotherapeutic and radiation-induced cell death (17,18). Inhibitors of apoptosis proteins (IAPs) represent one set of potent endogenous modulators of apoptosis in mammalian cells (19). Among the two families of known apoptosis regulators (IAP and Bcl-2 families of proteins), The X-linked inhibitor of apoptosis protein (XIAP) is considered the most potent inhibitor of cell death and an attractive therapeutic target due to its ability to suppress caspase activation via both intrinsic and extrinsic pathways and its ability to act as a signaling intermediate in tumor cell survival, immune and inflammatory pathways (20,21).

Apoptosis induced by radiation, death receptors, and several widely used chemotherapeutic compounds is mediated by activation of caspases. Inhibition of caspases 3, 7, and 9 by XIAP can suppress the apoptotic process and thereby may confer resistance to anticancer therapy(22).

Tumor cells adopt a variety of methods to avoid cell death via apoptosis. This anti-apoptotic pathway allows tumor cells to avoid host-immune destruction, progress, and metastasize (23). Studies on OSCC were few and deficient in information about the crucial contribution of XIAP in oral cancer. For that reason, the current study aimed to elucidate the benefits of utilizing XIAP immuno-expression as predictors for the sensitivity of cancer cells to the received treatment modality in OSCC patients. Moreover, we hypothesized that marker expression could aid in customizing the preferred treatment modality (CTH, RTH, or CCRTH) for OSCC patients. Finally, does XIAP expression could predict overall survival(OS) and disease free survival (DFS) in OSCC patients?.

Materials and Methods

Patients' selection and data retrieval

In a retrospective study, formalin-fixed paraffin-embedded tissue blocks from 50 patients were retrieved and thereby included in the present work. The inclusion criteria of the selected cases were as follows; 1. All patients had a confirmed diagnosis of primary OSCC according to WHO classification(24), 2. Availability of operable formalin fixed paraffin embedded tissue blocks for all the included cases, 3. All the cases selected for the study were surgically treated and then received adjuvant therapy either radiotherapy (RT), chemotherapy (CT), or concurrent chemoradiotherapy (CCRT), 4. None of the selected cases had received neoadjuvant therapy before their surgery, 5. All the enrolled cases were aroused from the oral cavity, 6. Completion of at least three years follow up in the Clinical Oncology and Nuclear Medicine Departments, Faculty of Medicine, Mansoura University, Exclusion of cases was made based on having insufficient or inoperable biopsy specimens, missing medical, clinical, and follow-up records, death due to OSCC nonrelated events, and unrespectable tumors hence all the included cases were completely resected. The studied cases were retrieved from archival files of the Pathology Laboratory, Faculty of Medicine, Mansoura University, from the years 2015 to 2018.

Clinicopathological, medical, and follow-up records were obtained for all patients. Following the completion of the treatment, patients were followed (every 3 months) with clinical examinations, abdomen ultrasonography, chest X-rays, and bone scans. Ultrasonography was performed when relapse was suspected. The medical reports included information about the patients` disease-free survival (DFS), and overall survival (OS) over the past three years that follow the treatment.

According to the clinical data of the patients, they were evaluated for their response one month after the completion of the treatment, according to the following WHO Criteria(25): Complete response (CR), Partial response (PR), Stable disease (SD) and Progressive disease (PD). Complete and partial responses were considered responsive cases while patients with stable or progressive disease were classified as clinically non responders.

The current study was approved by the Institutional Review Board and the National Research Ethics Committee in compliance with the 1964 Helsinki Declaration and its subsequent amendments (Faculty of Dentistry Ethical Committee, Mansoura University IRB Approval no A02030821).

The immunohistochemical technique:

The paraffin blocks of OSCC tissue were cut into 3 to 4 microns-thick sections and mounted on positively charged glass slides. One section was prepared for hematoxylin and eosin (H&E) staining for microscopic evaluation of the studied cases, and the other two sections were prepared for immunohistochemical (IHC) staining. IHC staining was carried out with the streptavidin-biotin complex technique and overnight incubation was used. After application of the tissue section on the coated slides deparaffinization was made, and then followed by rehydration using different concentrations of alcohol and water. Antigen retrieval was carried out using a 0.01 M citric acid buffer (pH = 6.0) heated in the microwave for ten minutes. Following a 15-minute incubation in methanol that contained 3% H2O2 to inactivate endogenous peroxidase, slices were then washed with distilled water. Using a rabbit polyclonal antibody against humans and the primary antibody to XIAP, the slides were incubated at 4 °C for an overnight period. XIAP was used in the optimal dilution of 1:100.

Immunohistochemical evaluation:

The evaluation of XIAP IHC was confirmed by recording both the intensity of staining and the percentage of positive cells under the optical microscope in five-degree magnification fields chosen at random, and the findings were evaluated as follows: The percentage of positive cells in 1% was counted as 0, the percentage of positive cells in 1-20% as 1, the percentage of positive cells in 21-50% as 2, the percentage of positive cells in 51-80% as 3, and the percentage of positive cells > 80% as 4. Furthermore, staining intensity was assigned a value of 0 for weak, 1 for moderate, and 2 for strong. The percentage of positive cells and the intensity values were added together to yield(26).

Statistical analysis: To detect the possible significant differences and correlations between the different markers` expressions concerning the different clinicopathologic parameters of the studied cases the Pearson's Chi-square test was used. Qualitative data were described using numbers and percentages. Quantitative data were described using mean± Standard deviation for normally distributed data after testing normality using the Kolmogrov-Smirnov test. The statistical significance of the obtained results was accepted at the (≤0.05) level. For univariate analysis, the Kaplan-Meier test was used to calculate overall survival (OS) and disease-free survival (DFS) using log-rank x^2 to detect an effect of risk factors affecting survival. Additionally, for multivariate analysis, the COX regression model was used to record the independent predictors for DFS and OS. The statistical analysis was done using the Statistical package of social science software version 22 (SPSS Inc., PASW Statistics for Windows version 22. Chicago: SPSS Inc.).

Results and Discussion

The present retrospective study was carried out on 50 OSCC cases, the clinicopathological characteristics of the considered cases, the current retrospective study was carried out on 50 OSCC cases were categorized following the criteria of (2017) WHO classification into well (20 cases, 40%), moderately (24 cases, 48%), and poorly differentiated carcinomas (6 cases, 12%). The study was applied to equal groups of different genders (25 cases). Patients` ages ranged from 25 to 76 years old with a range of 51 years, and mean 54.14±12.38. Among the 50 OSCC studied cases 9 cases (18%) were presented clinically as stage I, 13 cases (26%) were stage II, 11 cases (22%) were presented clinically as stage III, and 17 cases (34%) were presented clinically as stage IV. Regarding the recurrence of the 50 OSCC studied cases 31 cases (62%) showed recurrence. Regarding the death rate of the studied OSCC cases during the three-year follow-up period after surgery; 30 cases (60%) continued the 3 years of follow-up while 20 cases (40%) died during the follow-up period. Among the treatment types received after surgical removal of the studied OSCC cases; 5 cases (10%) had chemotherapy after surgery, 18 cases (36%) had concurrent chemo-radiotherapy and 27 cases (54%) resembling more than half of the cases had radiotherapy. Furthermore, after finishing the treatment 31 cases (62%) were responders to treatment while 19 cases (38%) were nonresponders.

XIAP immunohistochemical expression concerning the different clinicopathologic parameters:

Both nuclear and cytoplasmic XIAP immunoexpression were observed in the worked tissue sections of OSCC. XIAP demonstrated different levels of immuno-expression as low (13 cases, 26%), moderate (22 cases, 40%), and high (15 cases, 30%). Upon correlating XIAP immunoexpression with different clinicopathological parameters, no statistically significant difference was present concerning the following parameters; the age of the patients (p= 0.762), and genders (p= 0.231). Conversely, there was a high statistically significant difference in XIAP immune expression considering the WHO different histologic grades of the worked OSCC cases (p<0.001, **Table 1**); 12 cases (92.3%) that showed low XIAP expression were classified histologically as well differentiated OSCC, moderate XIAP expression was observed in seven cases (31.8%) of well-differentiated carcinoma, 14 cases (63.6%) of moderately differentiated carcinoma and one case (4.5%) of poorly differentiated carcinoma (Figures 1; A, B and C respectively). The majority of cases that showed high XIAP expression were moderately and poorly differentiated carcinomas (9 cases, 60%, and 5 cases, 33.3% respectively).

Moreover, there was a statistically significant difference in XIAP expression considering the different TNM clinical stages (p= 0.012). Most of the cases that showed low XIAP expression had early clinical stages (stages I and II), while the majority of cases that showed high XIAP expression had advanced clinical stages (stages III and IV, **Table 1**).

Furthermore, there was a statistically significant difference in XIAP expression concerning the recurrence prevalence (p= 0.01). Most of the cases that showed low XIAP expression were free from recurrence (9 cases, 69.2%), while high XIAP expression cases reported the presence of recurrence (13 cases, 86.7%, **Table 1**).

XIAP, the crucial inhibitor of apoptosis protein, revealed a statistically significant difference among the three expression scores (Low, Moderate, and High) concerning the patient's clinical response. Almost a high XIAP expression score (73.3%) presented nonresponder cases. On the contrary, the majority of low and moderate XIAP expression scores presented responder cases (100% and 63.6% respectively, Person chi-square test, P<0.001*, **table 1**). However, the type of adjuvant treatment that the patients received had no significant relation to XIAP expression. More than half of the cases showed low XIAP expression (7 cases, 53.8%) received radiotherapy treatment besides, the majority of the cases showed moderate XIAP expression (15 cases, 68.2%) also received radiotherapy treatment. However, the greatest ratio among the cases showed high XIAP expression (7 cases, 46.7%) received concurrent chemo-radiotherapy as a line of treatment after surgery.

Finally, the current study revealed a high statistically significant difference in XIAP expression considering the incidence of death (p= 0.006). Almost, all cases that demonstrated low XIAP expression had no deaths during the follow-up (12 cases, 92.3%). On the contrary, a great number of cases that showed high XIAP expression reported death during the follow-up (10 cases, 66.7%, **Table 1**).

Table (1): The different XIAP immunoexpression levels in correlation with different clinicopathological parameters of the studied OSCC cases.

Clinicopathological parameters		XIAP immunoexpression			Test of significance
	Groups	Low n=13 (%)	Moderate n=22 (%)	High n=15(%)	
Age groups	25- 45- 65-	2(15.4) 8(61.5) 3(23.1)	4(18.2) 14(63.6) 4(18.2)	5(33.3) 7(46.7) 3(20)	x^2=1.86 p=0.762
Gender	Male Female	8(61.5) 5(38.5)	8(36.4) 14(63.6)	9(60) 6(40)	x^2=2.93 p=0.231
WHO histologic grades	Poor Moderate Well	0 1(7.7) 12(92.3)	1(4.5) 14(63.6) 7(31.8)	5(33.3) 9(60) 1(6.7)	x^2=27.67 p<0.001
TNM Stage	I II III IV	5(38.5) 2(15.4) 6(46.2) 0	2(9.1) 7(31.8) 2(9.1) 11(50)	2(13.3) 4(26.7) 3(20) 6(40)	x^2=16.40 p=0.012*
Incidence of recurrence	No Yes	9(69.2) 4(30.8)	8(36.4) 14(63.6)	2(13.3) 13(86.7)	x^2=9.28 p=0.01*
Treatment type	CTH CCRT RTH	1(7.7) 5(38.5) 7(53.8)	1(4.5) 6(27.3) 15(68.2)	3(20) 7(46.7) 5(33.3)	x^2=5.19 p=0.268
Clinical response to treatment	Non responders. responders	0 13(100)	8(36.4) 14(63.6)	11(73.3) 4(26.7)	x^2=15.94 p<0.001*
Mortality rate	Dead Alive	1(7.7%) 12(92.3 %)	9(40.9%) 13(59.1%)	10(66.7%) 5(33.3%)	x^2=10.1 p=0.006*

Significance reached at level ≤ 0.05, Pearson chi-square test.

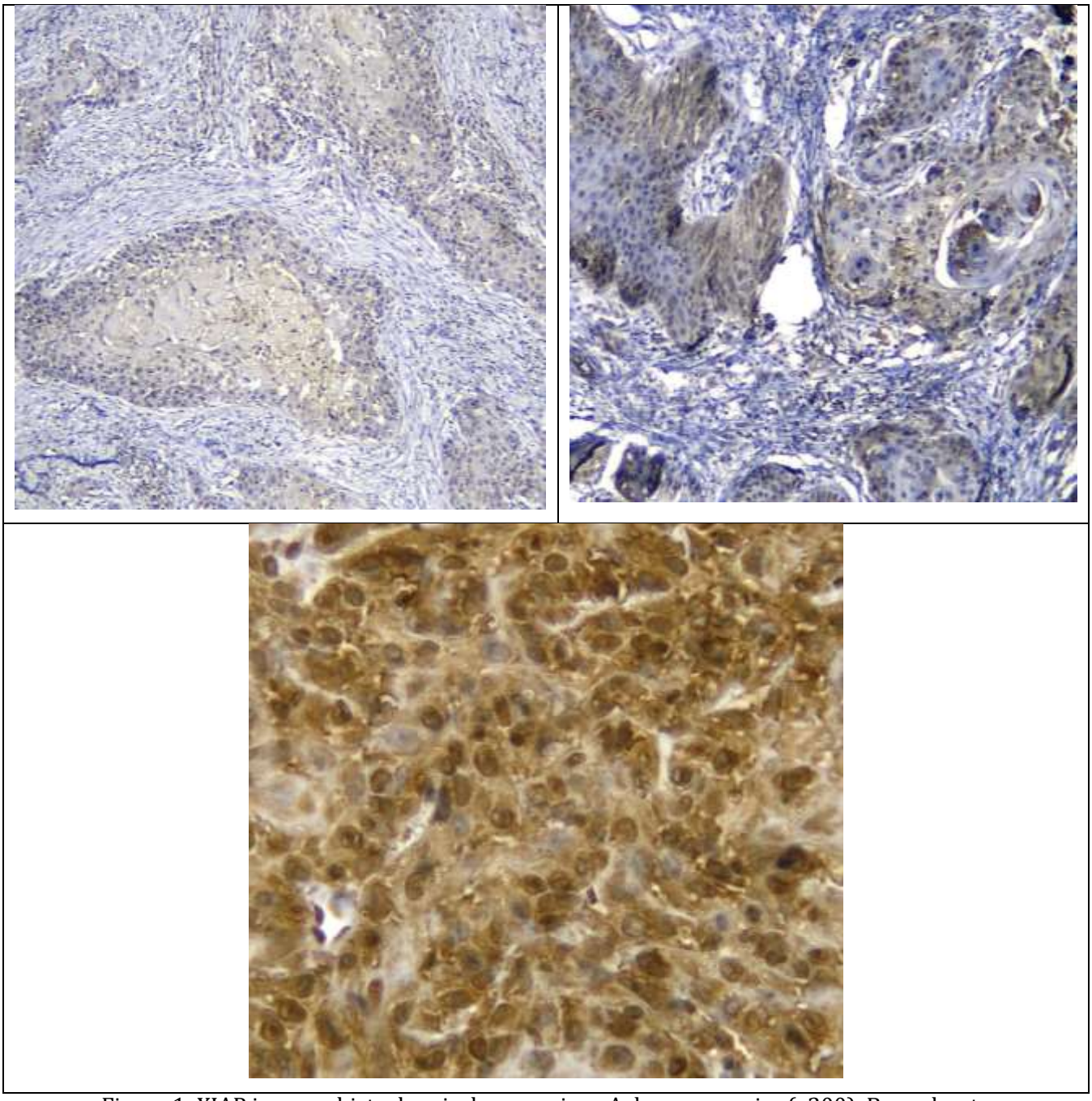

Figure 1: XIAP immunohistochemical expressions A; low expression(x200), B; moderate expression(x200) and C; high expression(x400).

Table (2): Detection of risk factors for disease free survival and overall survival rates

Clinical parameters		Univariate analysis Of DFS			Univariate analysis Of OS		
		Median DFS (95% CI) Months	Log rank x^2	p value	Median DFS (95% CI) Months	Log rank x^2	p value
treatment types	Chemotherapy CRT Radio therapy	0.933(0.002-1.99) 1.95(1.40-2.51) 1.74(1.33-2.16)	8.23	**0.016***	1.79(0.858-2.72) 2.27(1.83-2.71) 2.54(2.27-2.80)	2.10	0.350
response	Non responders responders	0.575(0.445-0.704) 2.45(2.13-2.77)	47.37	<**0.001***	1.56(1.18-1.93) 2.86(2.73-2.99)	33.38	**<0.001***
XIAP SCORE	Low Moderate High	2.71(2.33-3.09) 1.75(1.28-2.23) 0.875(0.426-1.32)	16.95	**<0.001***	2.88(2.66-3.10) 2.38(2.03-2.74) 1.89(1.41-2.36)	10.59	**0.005***

#If one of more categories is censored, *statistically significant, CI: Confidence interval

Table (3): Cox regression for predictors of disease-free survival among studied cases

	β	p value	Hazard ratio (95%CI)
TNM Stage			
I (r)			1
II	0.481	0.540	1.62(0.346-7.56)
III	0.316	0.692	1.37(0.287-6.55)
IV	0.813	0.323	2.25(0.450-11.29)
treatment types			
Chemotherapy	1.68	.01*	5.42(1.48-19.88)
Concurrent chemo-radio therapy	-1.25	0.02*	0.287(0.103-.796)
Radio therapy (r)			1
response			
progressive	3.34	<0.001*	28.19(6.01-132.26)
Complete response (r)			1
XIAP SCORE			
Low (r)			1
Moderate	1.15	0.069	3.18(0.915-11.06)
High	2.09	0.003*	8.04(2.07-31.24)

r: reference group

Table (4): Cox regression for predictors of overall survival among studied cases

		β	p value	Hazard ratio (95%CI)
WHO grading system	Poor (r) Moderate Well	r 0.527 0.003	 0.417 0.997	1 1.69(0.474-6.06) 1.003(0.169-5.94)
TNM Stage	I (r) II III IV	r 8.15 9.39 9.94	 0.935 0.926 0.921	 Undefined Undefined Undefined
Recurrence	No (r)			1

	yes	10.11	0.897	24.56(0.002-36.58)
response	Non responder responder (r	1.48	0.019*	4.39(1.27-15.17) 1
XIAP SCORE	Low (r) Moderate High	1.21 1.83	0.275 0.105	1 3.34(0.383-29.15) 6.21(0.682-56.53)

r: reference group

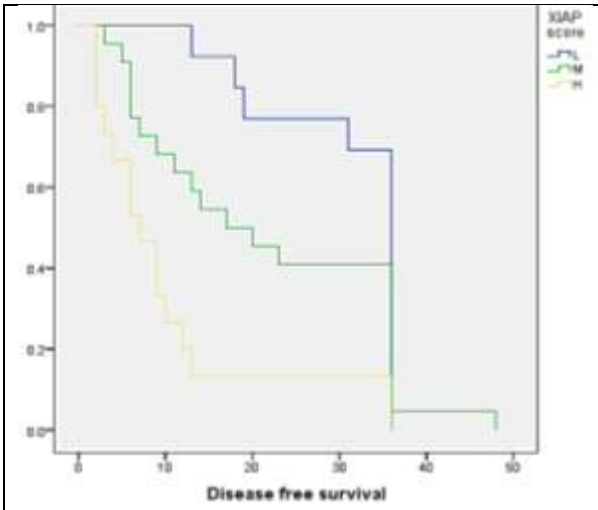

Chart 1: The univariate analysis using Kaplan Meier method revealed that DFS was significantly reduced in high XIAP expression cases (p<0.001).

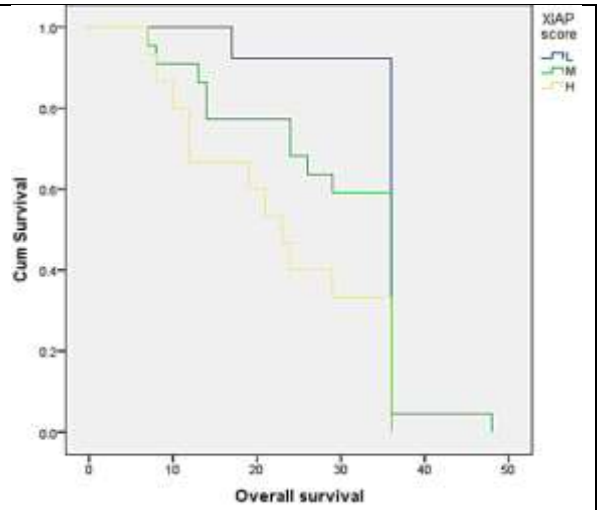

Chart 2: Reduced overall survival was significantly associated with high XIAP expression (P= 0.005), Kaplan Meier survival plots for univariate analysis).

Disease Free survival (DFS):

Moreover, the univariate analysis using the Kaplan-Meier method revealed that DFS was significantly reduced in non-responder cases (0.575 months) than in responder cases (2.45 months, Log Rank test, P= 0.001*). Furthermore, DFS was significantly reduced in cases that received CTH only (0.933 months) than those receiving CCRTH (1.95 months) or RTH only (1.74 months, Log Rank test, P= 0.016*). **High XIAP expression** was observed in cases that had significantly lower disease-free survival rates (DFS) (P<0.001*)(Table 2, Chart 1), moreover, the multivariate Cox regression model for prognosis prediction revealed that **treatment type (p=**0.02***), response to treatment (p**<0.001*) and **XIAP final score** (p<0.003*) could be considered the independent predictors for DFS (Table 3).

Overall survival (OS):

Furthermore, the univariate analysis using the Kaplan-Meier method revealed that OS was significantly reduced in non-responsive cases (1.56 months) than in responsive cases (2.86 months, Log Rank test, P= 0.001*). In addition, the cases showed **high XIAP expression** had significantly **low overall survival** rates during the 3-year follow-up (Log Rank test, p =0.005*)(Table 2, Chart 2). Furthermore, in contradiction to DFS, there were no statistically significant differences in patients` OS considering the received treatment type; CTH (1.79 months), CCRT (2.27 months), and RTH (2.54 months, Log Rank test, P=0.350). The multivariate Cox regression model for prognosis prediction revealed that patients` response to treatment (P= 0.019*)**,** remained as the only independent predictor for OS of the studied OSCC cases (Table 4).

Discussion:

Squamous cell carcinoma of the oral cavity is characterized by its strong local invasiveness and easy metastasis to the cervical lymph nodes, which in turn affects the prognosis of patients(27). The incidence of OSCC is a complex process, involving abnormal changes in various genes, proteins, and signaling pathways(28,29). In cancer cells, the cell cycle and apoptosis regulatory systems almost always fail, resulting in uncontrolled cell proliferation and tumor formation, the damage to the signaling pathways in cancer cells promotes the growth of cancer

cells and inhibits apoptosis(30). Based on strong evidence that the inhibitor of apoptosis protein XIAP expression in cancer cells promotes resistance to chemotherapy and radiation as well as elicits anti-cancer immune responses, the past decade has seen the development of XIAP-specific targeting using RNA approaches (31–35).

The present work was established to elucidate the possible utility of XIAP immunohistochemical expression as a potential predictor for OSCC cancer progression, and patients` survival. Using the Pearson chi-square test to correlate the various levels of XIAP with the examined clinical data, it was shown that there was no statistically significant difference between the levels of expression of XIAP considering the age and gender of the studied cases, however, OSCC cases presented with advanced TNM clinical stages showed significantly high XIAP expression (stage III and stage IV, P= 0.012) in the current study. Similar to our findings both Werner et al., 2016 and Hussain et al., 2015 found a strong positive correlation between high XIAP immunoexpression levels and the advanced tumor stages (UICC III/IV) of medullary thyroid carcinoma ($p<0.001$) and papillary thyroid carcinoma (P <0.0001), respectively (36,37). In contrast, Dizdar et al., 2017 reported high XIAP expression associated with less advanced UICC stages(38).

Regarding different histological grades, high XIAP expression levels were highly significant among moderately and poorly differentiated histological grades while well-differentiated histological grades showed marked depression in XIAP expression levels ($p<0.001$). This finding was consistent with Tamatani et al., 2012 study, which reported significant extensive XIAP expression in poorly differentiated OSCC, while well differentiated carcinomas demonstrated weak XIAP expression (39). However, in contrast to our results, Dizdar et al., 2017 and Kim et al, 2011 recorded high XIAP expression associated with well-differentiated adenocarcinomas and well-differentiated gastric carcinoma respectively (38,40).

Considering the incidence of recurrence during the follow up, there were significantly high XIAP expression levels associated with the presence of recurrence ($p=0.01$). Evans et al., 2018, stated the same results in aggressive breast cancer regarding XIAP expression levels among cases that showed recurrence ($P < 0.001$)(41). In addition, M. Li et al, 2007, also found an increased XIAP expression significantly associated with the recurrence of non-muscular invasive bladder cancer(42).

In the current study, treatment response had a highly significant relation with XIAP expression (<0.001*), all the cases showed low XIAP expression were responders to treatment while the majority of the cases showed high XIAP expression were nonresponders to treatment. Miyamoto et al., 2014, reported that cases with high XIAP expression had lower response rates to primary platinum-based chemotherapy (P=0.02*), so they concluded that XIAP plays a role in chemoresistance in clear cell carcinoma of the ovary (43). Consequently, XIAP has become a promising target for research into novel antitumor drugs (44). Concomitant with our study, Yang et al., 2012, found a strong relationship between the expression level of XIAP and the clinical response in addition to the prognosis of patients with advanced HNSCC. Low XIAP expression was closely correlated with chemotherapy response and favorable prognosis, whereas high XIAP expression may predict chemotherapy failure and poor outcomes (45). The results are consistent with previous reports showing that the down-regulation of XIAP sensitizes cancer cells to therapeutic drugs in lung cancer(46), prostate cancer(31), and pancreatic cancer (47). Fulda et al., 2014 and Lacasse et al., 2008, concluded that increased XIAP expression has been proposed to be associated with tumor development, treatment resistance, and poor prognosis(48,49). Therefore, XIAP is considered to be an oncogenic protein in various human malignancies, and targeting XIAP may constitute an attractive strategy for the development of new therapies for cancer (48).

In a univariate analysis using the Kaplan-Meier method to determine the risk factors for disease-free survival; high XIAP expression had a significantly lower disease-free survival rate (DFS) (P value <0.001). On the same line as our findings, Yang et al., 2019 revealed that the head and neck cancer patients whose tumors expressed high levels of XIAP had poorer disease-free survival rates than those whose tumors expressed low levels of XIAP ($P < 0.001$*)(50). Moreover, the multivariate Cox regression model done on our studied cases revealed that the XIAP final score ($p<0.003$*) remained an independent predictor for DFS. Similarly, Gao et al., 2019 study investigated the prognostic role of XIAP expression for DFS, they observed that elevated XIAP levels predicted shorter disease-free survival (DFS) ($P <0.001$*)(51).

Furthermore, cases treated with postoperative chemotherapy (CT) only or radiotherapy (RT) only had lower disease-free survival than cases treated by concurrent chemo-radiation (CCRT) ($p<0.016$*) in the univariate analysis, in addition, the multivariate Cox regression model revealed

that treatment type (p<0.01*) remained as independent predictors for DFS. K. H. Fan et al., 2017 reported findings parallel to ours, they observed a significantly higher 5-year recurrence-free survival rate among patients who received postoperative CCRT than the group of patients who received postoperative RT only (75.4% vs. 42.6%, p< 0.009*) in their univariate analysis, moreover, in their multivariate analysis concurrent chemotherapy was independent prognostic factors for recurrence-free survival (p= 0.002*)(52). Pignon et al., 2009 observed an improvement in treatment outcomes of OSCC patients using CCRT and they found that CCRT reduces the risk of death from head and neck cancer (53). On the other hand, Akula et al., 2021 found that in their study the administration of adjuvant therapy did not seem to predict disease-free survival, the p-value for disease-free survival in the Kaplan-Meier analysis was 0.098 and was statistically not significant, the reason behind the difference between our findings and Akula et al., 2021 findings is that 54.73% out of their studied cases which resemble more than half of the studied OSCC cases had not received any postoperative treatment, however, 45.26% patients only received radiotherapy and chemotherapy as adjuvant therapy (54).

The univariate analysis in our study revealed that the response to treatment was a risk factor for DFS hence non-responders to treatment among studied OSCC cases had significantly lower disease-free survival rates (DFS) (P <0.001*) than cases showed complete response to treatment and also the multivariate Cox regression model revealed that response to treatment (<0.001*) remained as independent predictors for DFS. This was similar to the findings reported by Zhong et al., 2015, they reported that Patients with favorable pathologic responses had a better DFS than those without favorable pathologic responses (p = 0.006)(55). Moreover, Zhang et al., 2023 observed similar results, they reported that more favorable recurrence-free survival (RFS) was observed among the group that showed favorable pathologic response to induction chemotherapy compared with the cases that showed resistance to induction chemotherapy (P < 0.001)(56). Finally, Zhong et al., 2013 reported that pathologic response was an independent risk factor for DFS on multivariate Cox model analysis (p=0.017) and they stated that a favorable pathologic response or clinical response predicted a better outcome with regard to DFS (57).

Regarding the correlation between the overall survival of the studied cases and the treatment type received after surgery, there was no significant difference in the univariate analysis (p=0.35). This was similar to the results reported by Asio et al., 2018 hence the univariate analysis showed that the 2-year and 5-year survival rates were no different among the different treatment groups (p=0.103)(58). On the other hand, response to treatment was observed to be significantly high among OSCC studied cases that showed high overall survival while nonresponders to treatment had significantly low overall survival rates during the 3 years follow up (p< 0.001*). Abdelmeguid et al., 2021 showed results consistent with ours, they reported that the Patients who had at least a partial response had better 5-year overall survival (60.1%) compared with non-responders (log-rank test; P = 0.004)(59).

Furthermore, the current study revealed a statistically significant relation of XIAP expression to the death rate (p= 0.006) hence; high XIAP expression levels were observed in cases that showed high mortality rates moreover, univariate analysis revealed that XIAP is an independent risk factor for overall survival hence; the studied cases showed high XIAP expression had significantly low overall survival rates during the 3 years follow up (p< 0.005*). Moreover, Yang et al., 2019 found similar results they revealed that the Head and neck cancer studied cases that showed high levels of XIAP presented poorer overall survival than cases that showed low levels of XIAP (P < 0.001*)(50). Correspondingly, Gao et al., 2019 results suggested that over-expression of XIAP correlated with poor OS of cancer patients (*P*<0.001*), there subgroup analyses showed that higher XIAP detection was related to worse OS in gastric cancer and head and neck cancer (HNC) however, surprisingly their meta-analysis showed that the non small cell lung cancer (NSCLC) patients that expressed higher levels of XIAP achieved a significantly longer OS compared with patients having lower expression of XIAP(51). They suggested that the reason behind the discrepancies in their study may be due to the different roles of XIAP, which might depend on the type of cancer(51).

Finally, the multivariate Cox regression model for prognosis predictors revealed that response to treatment (p=0.019) remained the only independent predictor for OS of the studied OSCC cases. This finding was similar to Zhang et al., 2023 study, they also observed that response to induction chemotherapy (IC) represents an independent predictor of 3-year OS (P = 0.004) among oropharyngeal squamous cell carcinoma (OPSCC) cases in the multivariate Cox regression analysis (56). More evidence revealed that a good response to IC can point to improved survival. Results demonstrated by Bossi et al., 2014 and Zhong et al., 2013 revealed that the patients with oral cancer with clinical response to IC had better prognoses than non-responders (57,60).

Conclusion

XIAP immuno-expression could be considered potential predictors for the patients` clinical response to the received treatment. Moreover, patients` clinical response, treatment type, and XIAP immuno-expression could be potentially used as survival`s predictors in OSCC patients.

List of abreviations

OSCC Oral squamous cell carcinoma
XIAP X-linked inhibitor of apoptosis protein
CTH Chemotherapy
RTH Radiotherapy
CCRT concurrent chemo-radiation
DFS Disease free survival
OS Overall survival
IC induction chemotherapy

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
2. De Paz D, Kao HK, Huang Y, Chang KP. Prognostic Stratification of Patients With Advanced Oral Cavity Squamous Cell Carcinoma. Vol. 19, Current Oncology Reports. 2017.
3. Bosetti C, Carioli G, Santucci C, Bertuccio P, Gallus S, Garavello W, et al. Global trends in oral and pharyngeal cancer incidence and mortality. Int J Cancer. 2020;147(4):1040–9.
4. Deng H, Sambrook PJ, Logan RM. The treatment of oral cancer: An overview for dental professionals. Vol. 56, Australian Dental Journal. 2011. p. 244–52.
5. Haddad R, Annino D, Tishler RB. Multidisciplinary Approach to Cancer Treatment: Focus on Head and Neck Cancer. Vol. 52, Dental Clinics of North America. 2008. p. 1–17.
6. Shah JP, Gil Z. Current concepts in management of oral cancer - Surgery. Vol. 45, Oral Oncology. 2009. p. 394–401.
7. D'Silva NJ, Ward BB. Tissue Biomarkers for Diagnosis & Management of Oral Squamous Cell Carcinoma. Alpha Omegan. 2007;100(4):182–9.
8. Crowe DL, Sinha UK. p53 apoptotic response to DNA damage dependent on BCL2 but not bax in head and neck squamous cell carcinoma lines. Head Neck. 2006;28(1):15–23.
9. Tong D, Poot M, Hu D, Oda D. 5-Fluorouracil-induced apoptosis in cultured oral cancer cells. Oral Oncol. 2000;36(2):236–41.
10. da Silva SD, Hier M, Mlynarek A, Kowalski LP, Alaoui-Jamali MA. Recurrent oral cancer: Current and emerging therapeutic approaches. Front Pharmacol. 2012;3 JUL.
11. Jain V, Das SN, Luthra K, Shukla NK, Ralhan R. Differential expression of multidrug resistance gene product, P-glycoprotein, in normal, dysplastic and malignant oral mucosa in India. Int J Cancer. 1997;74(1):128–33.
12. Garraway LA, Jänne PA. Circumventing cancer drug resistance in the era of personalized medicine. Vol. 2, Cancer Discovery. 2012. p. 214–26.
13. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, et al. Drug resistance in cancer: An overview. Vol. 6, Cancers. 2014. p. 1769–92.
14. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: Role of ATP-dependent transporters. Vol. 2, Nature Reviews Cancer. 2002. p. 48–58.
15. Dasari S, Bernard Tchounwou P. Cisplatin in cancer therapy: Molecular mechanisms of action. Vol. 740, European Journal of Pharmacology. 2014. p. 364–78.
16. Florea AM, Büsselberg D. Cisplatin as an anti-tumor drug: Cellular mechanisms of activity, drug resistance and induced side effects. Vol. 3, Cancers. 2011. p. 1351–71.
17. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Vol. 144, Cell. 2011. p. 646–74.

18. Lo Muzio L, Sartini D, Santarelli A, Rocchetti R, Morganti S, Pozzi V, et al. Expression and prognostic significance of apoptotic genes in oral squamous cell carcinoma. Mol Carcinog. 2014;53(4):264–71.
19. Reed JC. The survivin saga goes in vivo. Vol. 108, Journal of Clinical Investigation. 2001. p. 965–9.
20. Abbas R, Larisch S. Targeting XIAP for Promoting Cancer Cell Death-The Story of ARTS and SMAC. Vol. 9, Cells. 2020.
21. Michie J, Kearney CJ, Hawkins ED, Silke J, Oliaro J. The Immuno-Modulatory Effects of Inhibitor of Apoptosis Protein Antagonists in Cancer Immunotherapy. Vol. 9, Cells. 2020.
22. Schimmer AD, Dalili S, Batey RA, Riedl SJ. Targeting XIAP for the treatment of malignancy. Vol. 13, Cell Death and Differentiation. 2006. p. 179–88.
23. Mizutani Y, Nakanishi H, Li YN, Matsubara H, Yamamoto K, Sato N, et al. Overexpression of XIAP expression in renal cell carcinoma predicts a worse prognosis. Int J Oncol. 2007;30(4):919–25.
24. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ. WHO Classification of Head and Neck Tumours-WHO/IARC Classification of Tumours. World Health Organization. 2017. p. 215–20.
25. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours - Revised RECIST Guideline New response evaluation criteria in solid tumours : Revised RECIST guideline (version 1 . 1). Eur J Cancer [Internet]. 2009;45(2):228–47. Available from: http://dx.doi.org/10.1016/j.ejca.2008.10.026
26. Pluta P, Jesionek-kupnicka D, Pluta A, Brzozowski K, Braun M. Prognostic value of XIAP and survivin expression in locally advanced breast cancer patients treated with anthracycline-based neoadjuvant chemotherapy. 2019;1–12.
27. Ma Y, Wang H. Clinical significance of Annexin A2 expression in oral squamous cell carcinoma and its influence on cell proliferation, migration and invasion. Sci Rep. 2021;11(1).
28. Al Rawi N, Elmabrouk N, Abu Kou R, Mkadmi S, Rizvi Z, Hamdoon Z. The role of differentially expressed salivary microRNA in oral squamous cell carcinoma. A systematic review. Vol. 125, Archives of Oral Biology. 2021.
29. Starzyńska A, Sejda A, Adamska P, Marvaso G, Sakowicz-Burkiewicz M, Adamski Ł, et al. Prognostic value of the PIK3CA, AKT, and PTEN mutations in oral squamous cell carcinoma: Literature review. Vol. 17, Archives of Medical Science. 2021. p. 207–17.
30. Rahman MM, Sarker MT, Alam Tumpa MA, Yamin M, Islam T, Park MN, et al. Exploring the recent trends in perturbing the cellular signaling pathways in cancer by natural products. Vol. 13, Frontiers in Pharmacology. 2022.
31. Amantana A, London CA, Iversen PL, Devi GR. X-linked inhibitor of apoptosis protein inhibition induces apoptosis and enhances chemotherapy sensitivity in human prostate cancer cells. Mol Cancer Ther. 2004;3(6):699–707.
32. LaCasse EC. Pulling the plug on a cancer cell by eliminating XIAP with AEG35156. Vol. 332, Cancer Letters. 2013. p. 215–24.
33. Cao C, Mu Y, Hallahan DE, Lu B. Erratum: XIAP and survivin as therapeutic targets for radiation sensitization in preclinical models of lung cancer. Oncogene. 2004;23(58):9448–9448.
34. Devi GR, Beer TM, Corless CL, Arora V, Weller DL, Iversen PL. In vivo bioavailability and pharmacokinetics of a c-MYC antisense phosphorodiamidate morpholino oligomer, AVI-4126, in solid tumors. Clin Cancer Res. 2005;11(10):3930–8.
35. Arora V, Devi G, Iversen P. Neutrally Charged Phosphorodiamidate Morpholino Antisense Oligomers: Uptake, Efficacy and Pharmacokinetics. Curr Pharm Biotechnol. 2005;5(5):431–9.
36. Werner TA, Tamkan-Ölcek Y, Dizdar L, Riemer JC, Wolf A, Cupisti K, et al. Survivin and XIAP: Two valuable biomarkers in medullary thyroid carcinoma. Br J Cancer. 2016;114(4):427–34.
37. Hussain AR, Bu R, Ahmed M, Jehan Z, Beg S, Al-Sobhi S, et al. Role of X-linked inhibitor of apoptosis as a prognostic marker and therapeutic target in papillary thyroid carcinoma. J Clin Endocrinol Metab. 2015;100(7):E974–85.

38. Dizdar L, Tomczak M, Werner TA, Safi SA, Riemer JC, Verde PE, et al. Survivin and XIAP expression in distinct tumor compartments of surgically resected gastric cancer: XIAP as a prognostic marker in diffuse and mixed type adenocarcinomas. Oncol Lett. 2017;14(6):6847–56.
39. Tamatani T, Takamaru N, Uchida D, Nagai H, Fujisawa K, Miyamoto Y. Abstract 4954: The expression of X-linked inhibitor of apoptosis in human oral squamous cell carcinoma and its relationship with clinical factors. Cancer Res. 2012;72(8_Supplement):4954–4954.
40. Kim MA, Lee HE, Lee HS, Yang HK, Kim WH. Expression of apoptosis-related proteins and its clinical implication in surgically resected gastric carcinoma. Virchows Arch. 2011;459(5):503–10.
41. Evans MK, Brown MC, Geradts J, Bao X, Robinson TJ, Jolly MK, et al. XIAP regulation by MNK links MAPK and NFkB signaling to determine an aggressive breast cancer phenotype. Cancer Res. 2018;78(7):1726–38.
42. Li M, Song T, Yin ZF, Na YQ. XIAP as a prognostic marker of early recurrence of nonmuscular invasive bladder cancer. Chin Med J (Engl). 2007;120(6):469–73.
43. Miyamoto M, Takano M, Iwaya K, Shinomiya N, Kato M, Aoyama T, et al. X-chromosome-linked inhibitor of apoptosis as a key factor for chemoresistance in clear cell carcinoma of the ovary. Br J Cancer. 2014;110(12):2881–6.
44. Li X, Guo S, Xiong XK, Peng BY, Huang JM, Chen MF, et al. Combination of quercetin and cisplatin enhances apoptosis in OSCC cells by downregulating xIAP through the NF-kB pathway. J Cancer. 2019;10(19):4509–21.
45. Yang XH, Feng ZE, Yan M, Hanada S, Zuo H, Yang CZ, et al. Xiap is a predictor of Cisplatin-based chemotherapy response and prognosis for patients with advanced head and neck cancer. PLoS One. 2012;7(3):1–8.
46. Hu YP, Cherton-Horvat G, Dragowska V, Baird S, Korneluk RG, Durkin JP, et al. Antisense oligonucleotides targeting XIAP induce apoptosis and enhance chemotherapeutic activity against human lung cancer cells in vitro and in vivo. Clin Cancer Res. 2003;9(7):2826–36.
47. Li Y, Jian Z, Xia K, Li X, Lv X, Pei H, et al. XIAP is related to the chemoresistance and inhibited its expression by RNA interference sensitize pancreatic carcinoma cells to chemotherapeutics. Pancreas. 2006;32(3):288–96.
48. Fulda S. Molecular pathways: Targeting inhibitor of apoptosis proteins in cancer-from molecular mechanism to therapeutic application. Clin Cancer Res. 2014;20(2):289–95.
49. LaCasse EC, Mahoney DJ, Cheung HH, Plenchette S, Baird S, Korneluk RG. IAP-targeted therapies for cancer. Vol. 27, Oncogene. 2008. p. 6252–75.
50. Yang XH, Liu L, Hu YJ, Zhang P, Hu QG. Co-expression of XIAP and CIAP1 Play Synergistic Effect on Patient's Prognosis in Head and Neck Cancer. Pathol Oncol Res. 2019;25(3):1111–6.
51. Gao X, Zhang L, Wei Y, Yang Y, Li J, Wu H, et al. Prognostic value of XIAP level in patients with various cancers: A systematic review and meta-analysis. J Cancer. 2019;10(6):1528–37.
52. Fan KH, Chen YC, Lin CY, Kang CJ, Lee LY, Huang SF, et al. Postoperative radiotherapy with or without concurrent chemotherapy for oral squamous cell carcinoma in patients with three or more minor risk factors: A propensity score matching analysis. Radiat Oncol. 2017;12(1).
53. Pignon JP, Maître A le, Maillard E, Bourhis J. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): An update on 93 randomised trials and 17,346 patients. Radiother Oncol. 2009;92(1):4–14.
54. Akula S, Neralla M, George R. Influence of clinical and pathological characteristics of OSCC on disease free and overall survival - A single institute study. Int J Dent Oral Sci. 2021;8(4):2205–13.
55. Zhong LP, Zhang CP, Ren GX, Guo W, William WN, Hong CS, et al. Long-term results of a randomized phase III trial of TPF induction chemotherapy followed by surgery and radiation in locally advanced oral squamous cell carcinoma. Oncotarget. 2015;6(21):18707–14.

56. Zhang Q, Xu T, Shen C, Qian W, Ying H, He X, et al. Response to induction chemotherapy predicts survival outcomes in oropharyngeal cancer. Cancer Med. 2023;12(8):9175–85.
57. Zhong LP, Zhang CP, Ren GX, Guo W, William WN, Sun J, et al. Randomized phase III trial of induction chemotherapy with docetaxel, cisplatin, and fluorouracil followed by surgery versus up-front surgery in locally advanced resectable oral squamous cell carcinoma. J Clin Oncol. 2013;31(6):744–51.
58. Asio J, Kamulegeya A, Banura C. Survival and associated factors among patients with oral squamous cell carcinoma (OSCC) in Mulago hospital, Kampala, Uganda. Cancers Head Neck. 2018;3(1).
59. Abdelmeguid AS, Silver NL, Boonsripitayanon M, Glisson BS, Ferrarotto R, Gunn GB, et al. Role of induction chemotherapy for oral cavity squamous cell carcinoma. Cancer. 2021;127(17):3107–12.
60. Bossi P, Lo Vullo S, Guzzo M, Mariani L, Granata R, Orlandi E, et al. Preoperative chemotherapy in advanced resectable OCSCC: Long-term results of a randomized phase III trial. Ann Oncol. 2014;25(2):462–6