BMC Cancer

RESEARCH

Open Access

Androgen receptor overexpression by immunohistochemistry in malignant salivary gland tumors in Tanzania

Subira Matiku[1,2,6*], Gad Murenzi[3,6], Eulade Rugengamanzi[3,4], Edwin Liwa[5,6], Idd Shaban[1,2,6], Aron Orgenes[1,2], Erick P. Magorosa[7], Amos Mwakigonja[6,7] and Edda Vuhahula[6,8]

Abstract

Background Malignant salivary gland tumors (SGTs) present diagnostic challenges and limited treatment options. This study aims to determine the proportion of malignant SGTs overexpressing the androgen receptor (AR) by immunohistochemistry (IHC) and its association to age, sex, anatomical site, histopathological subtype and grade which may inform customized treatment approaches.

Methodology This was a retrospective cross-sectional analytical study of archived paraffin embedded tissue blocks of malignant SGTs diagnosed at MNH Central Pathology Laboratory (CPL) from January 2019 to December 2022. IHC staining using a monoclonal Rabbit Anti-Human AR and interpretation was done using Allred score. The AR overexpression was assessed and compared by age, sex anatomical site, histological subtype and histological grade of the tumor.

Results Out of 158 (60%) malignant SGTs, 115 cases underwent AR IHC where, mean age was 49.7 ± 17.9, females were 61(53%). Major salivary gland involvement was (67)58.1%, predominantly parotid gland 35(52.2%), Adenoid cystic carcinoma and Mucoepidermoid carcinoma were the most common tumors accounting for 38(33%) and 22 (19%) respectively. High grade tumors were prevalent accounting for 53(46.1%). Androgen receptor overexpression was observed in 49(42.6%). A significant association was observed between AR and parotid gland anatomical location (aOR = 3.45, 95% CI = 1.1–10, $p = 0.027$) and high-grade tumors (aOR = 5.1, 95% CI = 1.4–19, $p = 0.014$). No significant association between AR overexpression and age (p-value 0.253), sex (p-value 0.708) and histological subtype (p-value 0.557), although highest proportion were seen in salivary duct carcinoma (71.4%).

Conclusion High-grade malignant SGTs and parotid gland location are associated with AR overexpression. This suggests that androgen deprivation therapy (ADT) has the potential to play a role in the management of advanced SGTs. However, large-scale studies that will include comprehensive molecular investigations and efficacy exploration of ADT are recommended to clarify our current findings and inform therapeutic options for patient with high grade and recurrent tumors.

Keywords Androgen receptor overexpression, Immunohistochemistry, Salivary gland tumors

*Correspondence:
Subira Matiku
subirahmatiku.sm@gmail.com

Full list of author information is available at the end of the article

Background

Salivary glands are exocrine glands that secrete saliva which moistens food and contains an enzyme amylase that is required for the breakdown of carbohydrates. There are three major paired salivary glands (the parotid, submandibular, and sublingual glands) and numerous minor salivary glands distributed throughout the mouth and extending into the tracheobronchial tree. It is estimated that there are also between 600 and 1000 minor salivary glands named for the sites which they occupy (i.e., labial, buccal, lingual, palatal, retromolar, etc.). Histologically, the major salivary glands are composed of acinar (secretory cells) and ductal cells arranged like a cluster of grapes on a stem. The clustered acinar cells (the "grapes") make up the secretory end pieces, while the ductal cells (the "stems") form an extensively branching system that modifies and transports the saliva from the acini into the oral cavity. There are three types of ductal cells: intercalated, striated, and interlobular. Despite their relatively simple morphology, the salivary glands give rise to 36 histologically distinct tumors described by WHO 5th Edition 2022 of which 21 are malignant [1].

Tumors arising from salivary glands constitute an uncommon heterogeneous group that vary considerably in their anatomic site of origin, histology, and biologic behavior. They constitute 3–10% of all head and neck tumors and less than 2% of all human tumors worldwide [2–5]. The global annual incidence for malignant salivary gland tumors is 0.3% and mortality 0.2% per 100,000 population [6].These tumors make up less than 3% of all neoplasms in the United States, and only 6% of all tumors in the head and neck [3, 7]. In Africa the incidence for both sexes is 9.2% of the population with mortality rate of 13% per 100,000 population [6]. In Tanzania the incidence is 0.48% and mortality 0.45% per 100,000 population from Globocan data 2020 and the prevalence ranges from 6.3 to 7.5% among of all head and neck tumors [8, 9]. Surgical management alone is curative for benign and low-grade cases of SGTs. The current management of malignant SGTs for loco-regional disease include surgical resection alone or with adjuvant radiotherapy for tumors with high-grade and those with positive margins or other high-risk features [10, 11]. For metastatic and recurrent tumors, radiation therapy is applied as a neo-adjuvant before surgical resection [10] however for most cases, the goal of treatment is palliation or lessening of symptoms severity [12, 13]. Local ablative treatments such as surgery or stereotactic body radiation therapy may be offered to delay local disease progression, but there is informal consensus, and it is weakly recommended [10].

Systemic drugs trials for advanced and metastatic disease are still ongoing. Few drugs have been proposed especially for SGTs overexpressing Her2, Herceptin as an example [10], but due to lack of randomized clinical trials and prospective studies their use is still in question [10]. Further trials on androgen deprivation therapy (ADT) have shown promising results in treatment of metastatic disease even though most research were on SDC [14], with complete resolution of the disease for tumors that overexpress AR. This treatment modality has no absolute contraindication and has less side effects as compared to chemotherapy [10]. Immunotherapy with pembrolizumab in patients with PDL1 receptor positive as well as molecular targeted therapy such as tyrosine kinase inhibitors and epidermal growth factor inhibitors are still also under clinical trial [15, 16]. In Tanzania, 76.5% present with stage III and IV disease of which 69.1% are not amenable to treatment by surgical and radiotherapy alone resulting in poor outcome [17]. Prostate cancer and hepatocellular carcinoma, which are both treated with androgen restriction treatment, are two tumor forms in which androgen receptor overexpression plays a significant role as an oncogenic driver [18, 19]. Even though, this modality of treatment is under clinical trials for salivary gland cancers several case reports have shown promising outcome and there are apparently, no absolute contraindications to this therapy [20, 21]. Thus, this study attempts to provide knowledge on AR overexpression in malignant SGTs as well as clinico-pathological associations amongst Tanzanian patients which may help inform targeted therapy including androgen deprivation therapy (ADT), particularly for unresectable, advanced and metastatic malignant tumors.

Methodology

Study design, population and settings

This retrospective study was a lab based cross-sectional analytical study of archival formalin fixed paraffin-embedded (FFPE) tissue blocks of malignant epithelial salivary gland tumours diagnosed at the Central Pathology Laboratory's (CPL), Department of Anatomical Pathology, MNH covering 4 years from 1st January 2019 to 31st December 2022.

Eligibility

We included all patients with available complete clinical records and tissue blocks with enough tissue to perform tissue microarray, diagnosed with malignant epithelial salivary gland tumors, patients with missing tissue blocks and small or scanty tissue for tissue microarray were excluded.

Data collection methods

Data collection from records and archival tissues

Each case study was given a unique study number. From the histological request forms submitted at the Central Pathology Laboratory, demographic data was extracted which include age and sex. Histological diagnosis,

subtype and grade (Corresponding to known scheme for some subtypes) was determined from review of the H&E slides from the archive and finally the AR expression status was noted after immunohistochemical staining using tissue microarray (TMA) technique. Data was collected using a structured excel data collection sheet that included all the variables.

Tissue slides and block for IHC

All SGTs pathology reports from 1st January 2019 to 31st December 2022 were retrieved from the stored files. Eligible cases were selected. The corresponding H&E slides were reviewed by the primary investigator and the Pathologist (Oral Pathologist) for histological diagnosis and selection of tissue blocks for Tissue microarray (TMA). Tumors were classified according to Head and Neck Tumours, WHO Classification, 5th Edition, 2022. In case of lost tissue slides, preparation of H&E slide was done for histomorphological analysis. The tissue blocks for selected patients were retrieved and prepared for TMA-AR immunohistochemistry.

Tissue microarray

Manual tissue microarray (TMA) was done, where from each FFPE, H&E–stained sections were used to select representative areas, and a 4 mm core diameter, was made from each case and inserted in a grid pattern into a recipient paraffin block using a tissue microarray mold. Section (3 micrometers thick) containing 14 representative cores and 1 control were then cut from each TMA block and mounted on charged glass slides, 2 slides from each TMA block were made, to minimize tissue washout.

Androgen receptor (AR) immunohistochemistry

Micro-arrayed FFPE tissue sections 4 µm thick were mounted on charged slides, deparaffinized and rehydrated in descending grades of alcohol. Then, AR IHC was performed after heat induced epitope retrieval (HIER) by pressure cooker, for which the tissue sections were immersed in Coplin jars containing 0.01-mol citrate buffer, pH 6.0, and subjected to preboiled water in pressure cooker, further heating at full pressure for 3 min was followed. Nonspecific immunoreactivity was blocked appropriately by incubation with bovine serum albumin (BSA) for 20 min at room temperature then blocking of endogenous peroxidase activity by incubating the slides in a 0.3% solution hydrogen peroxide in methanol for 30 min in darkness. Following a rinse for 2 min each in running tap water and phosphate-buffered saline (PBS) at room temperature, tissue sections were stained manually using commercially available pre diluted and ready to use, Monoclonal Rabbit Anti-Human AR (clone MD124R catalogue number RM0004RTU7) followed by incubation with mouse Horseradish Peroxidase (HRP) conjugated with secondary antibody and 3'3-Diaminobenzidine (DAB) tetrahydrochloride as a chromogen. Stained tissue sections were dehydrated, and cover slipped. The AR IHC-stained slides were evaluated using light microscope, interpreted using Allred score for hormonal receptor and few images were saved by using the Motic slides scanner.

Interpretation of AR IHC was done using Allred score system for hormonal receptors (Fig. 1). This score takes into consideration both intensity and percentage of nuclear staining for positive tumours. A positive control from known AR positive prostate cancer was used. This approach was based on previous study done on AR expression in malignant SGTs [22, 23]. The slides were interpreted by the principal investigator and then reviewed by one senior pathologist.

Proportion score (%)		Intensity score		Total score	
0	0	0	Negative	0	Negative
1	1			1	
2	10	1	Weak	2	Weak
				3	
3	33	2	Moderate	4	Moderate
				5	
4	66	3	Strong	7	Strong
				8	
5	100				

Fig. 1 Allred score for AR IHC interpretation

Data analysis

The data was analyzed by using STATA data software version 15.0, descriptive statistics were summarized using proportions and frequency tables. Bivariate analysis to test for the association between each independent variable and the dependent was done using Chi square test or Fisher's exact test where appropriate. For the independent variable that was associated with the dependent variable, a multivariable logistic regression analysis was done. A p-value ≤ 0.05 was considered statistically significant.

Ethical consideration

The study proposal was submitted to the MUHAS ethical clearance committee for approval through Head of Department of Pathology and Dean, School of Diagnostic Medicine, and was granted with reference number **MUHAS-REC-10-2022-1430**. Following approval, permission to conduct the study at CPL, was sought from the MNH Management and the Head of Anatomical Pathology with reference number **MNH/TRCU/Perm/2022/083.**

Results

A total of 264 patients were recruited in our study (Fig. 2), where malignant SGTs accounted for 158(60%). One hundred and fifteen (115) patients with malignant SGTs were included in this study where the mean age was 49.7 years. Most tumors 73(63.5%) were prevalent between 25 and 60 years of age. Female patients were the majority and accounted for 61 (53%) cases. The majority 67(58.3%) of patients had major salivary gland involvement. Among the major salivary glands, parotid was the most affected [35 cases, (52.2%)]. In minor salivary glands, the gingiva and buccal mucosa were affected most with 17(35.4%) and 15(31.2%) cases respectively. Overall, the most common subtypes were adenoid cystic carcinoma and mucoepidermoid carcinoma and accounted for 38 (33%) and 22 (19.1%) cases, respectively (Fig. 3A and B). Amongst the malignant salivary gland tumors, high grade tumors were 53(46.1%) compared to 30(26.1%) low grade tumors (Table 1). Overall, among the 115 cases, 49(42.6%) were AR-positive (Fig. 4). Among AR-positive tumors, majority (42.9%) had weak staining followed by 34.7% moderate staining and strong staining accounted 22.4% (Fig. 5). Among the AR-positive, 38(77.6%) cases had patchy staining pattern while diffuse pattern was only seen in 11(22.4%) (Figs. 5 and 6). The AR status varied among different histological subtypes where the highest prevalence of AR overexpression was observed in SDC at 71.4%, followed by Polymorphous adenocarcinoma (PAC) and Salivary carcinoma not otherwise classified (SC NOS) at 55.6% and 54.6% respectively. Equal distribution of AR negative and positive was observed in Carcinoma expleomorphic adenoma (CXPA), Sclerosing microcystic adenocarcinoma (SMA) and Hyalinizing clear cell carcinoma (HCCC). Despite the high frequency of AdCC and MEC as the commonest histological subtypes, these subtypes had lower prevalence of AR overexpression which was 35.2% and 40.9% respectively (Table 2).

A significant association was observed between AR-overexpression and major salivary gland anatomical location (p-value=0.03) as well as high grade tumors (p-value=0.042 (Table 3). No association was found between sex (p-value=0.708), age (p-value=0.253) and histological subtype (p-value=0.557) (Table 4). However, it is worth noting that there were high proportions of AR-positive cases in salivary duct carcinoma and age above 60 years. Furthermore, the staining pattern and intensity of AR immunohistochemistry was not associated with histological grade (p-value 0.405 and 0.518 respectively) (Table 4) (Fig. 6).In logistic regression analysis using a bivariate model the odds of having AR-positive status were 3 times higher in parotid tumors compared to submandibular and sublingual tumors (cOR=3.0, 95% CI=1.1–8.4, p=0.033) and the association remained significant and higher when adjusted for confounders (aOR=3.45, 95% CI=1.1–10, p=0.027). Similarly, for high grade tumors a non-significant trend towards association with AR status was observed, with higher odds of AR-positive status compared to low-grade

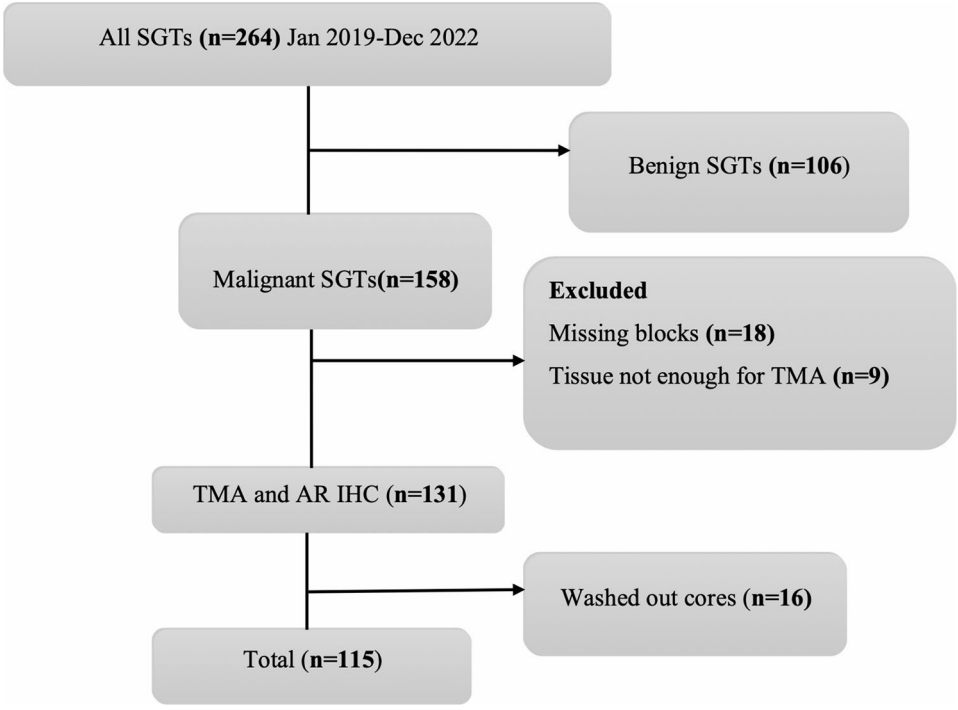

Fig. 2 Data flow chart. Key: SGTs-Salivary gland tumors, TMA-Tissue microarray, IHC-Immunohistochemistry. Jan 2019 to Dec 2022

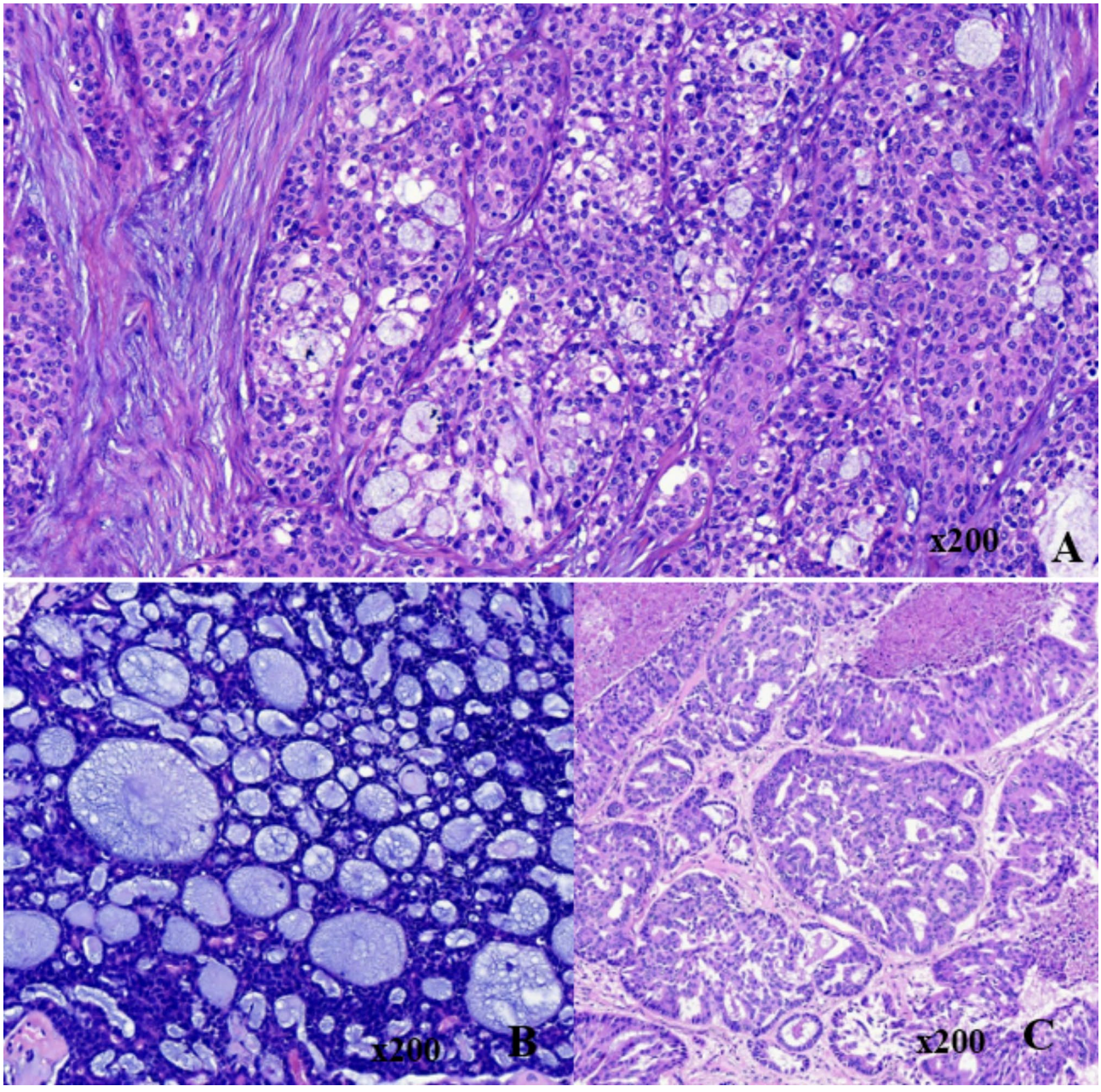

Fig. 3 Photomicrographs showing 1. Hematoxylin and eosin-stained sections of (**A**) Mucoepidermoid carcinoma, intermediate grade, (**B**) Adenoid cystic carcinoma, intermediate grade, (**C**) Salivary duct carcinoma, high grade

tumors (cOR=2.1, 95% CI=0.8-5, p=0.116). However, after adjusting for other variables, high-grade tumors exhibited a significant association with AR positive status, with the odds of AR-positive status being 5.1 times higher compared to low-grade tumors (aOR=5.1, 95% CI=1.4–19, p=0.014) (See Table 5).

Discussion

Salivary gland tumors are a broad and diverse category of lesions that differ in their morphology. Numerous epidemiologic studies of SGTs have been conducted in various countries, with varying results most likely reflecting differences in the study's origin, divergences in histologic classification, restriction to a specific population, anatomical location, or tumor type. Salivary glands tumors in Africa have not been studied extensively as compared to Western countries. The majority of the studies are only descriptive and are not recent. This current study, to the best of our knowledge is the first to be done in Tanzania.

In this study 60% of all SGTs were malignant tumors, which is consistent with other studies where malignancies were more frequent than benign tumors [24, 25]

Table 1 Characteristic of study population ($n=115$), demographics and histopathological feature

Variable	Malignant ($n=115$): n (%)	Variable	Malignant ($n=115$): n (%)
Age		Histological Subtype	
Mean (±SD)	**49.7 (±17.9)**	AdCC	38 (33.0)
Age group		MEC	22 (19.1)
≤24	8 (6.9)	SC NOS	11 (9.6)
25–60	73 (63.5)	PAC	9 (7.8)
>60	34 (29.6)	MECA	8 (7.0)
Sex		SDC	7 (6.1)
Male	54 (47.0)	AciCC	5 (4.3)
Female	**61 (53.0)**	HCCC	4 (3.5)
Site		MSA	4 (3.5)
Major	**67 (58.3)**	CXPA	2 (1.7)
Parotid	35 (52.2)	SMA	2 (1.7)
Submandible	31 (46.3)	BCAC	1 (0.9)
Sublingual	1 (1.5)	EMC	1 (0.9)
Minor	48 (41.7)	SC	1 (0.9)
Palate	13 (27.1)	**Histological grade**	
Buccal	15 (31.2)	Low grade	30 (26.1)
Gingiva	17 (35.4)	Intermediate	32 (27.8)
Tongue	3 (6.3)	High grade	53 (46.1)

Key AdCC, Adenoid cystic carcinoma, MEC, Mucoepidermoid carcinoma, PAC, Polymorphus adenocarcinoma, SC NOS, Salivary carcinoma not otherwise specified, MECA, Myoepithelial carcinoma, SDC, Salivary duct carcinoma, AciCC, Acinic cell carcinoma, HCCC, Hyalinizing clear cell carcinoma, MSA, Microsecretory carcinoma, CXPA, Carcinoma ex-pleomorphic adenoma, SMA, Sclerosing microcystic carcinoma, BCAC, Basal cell adenocarcinoma, EMC, Epithelial myoepithelial carcinoma, SC, Secretory carcinoma

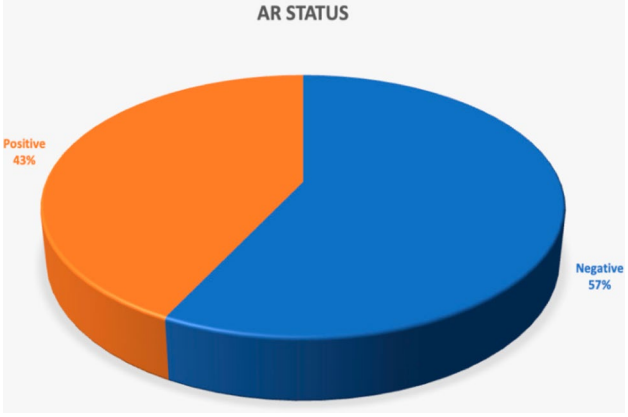

Fig. 4 Pie Chart diagram showing the proportion of AR status among the malignant SGTs

and contrasted with studies done by Masanja et al., 2003 and Vuhahula et al.., 2004 where benign proportion were higher than malignant [8, 26]. This can be due to the fact that Muhimbili is a national apical hospital where all difficult cases are referred to. The mean age for malignant salivary gland tumors in our current study was 49.7 and the peak age for occurrence was between 25 and 60 years

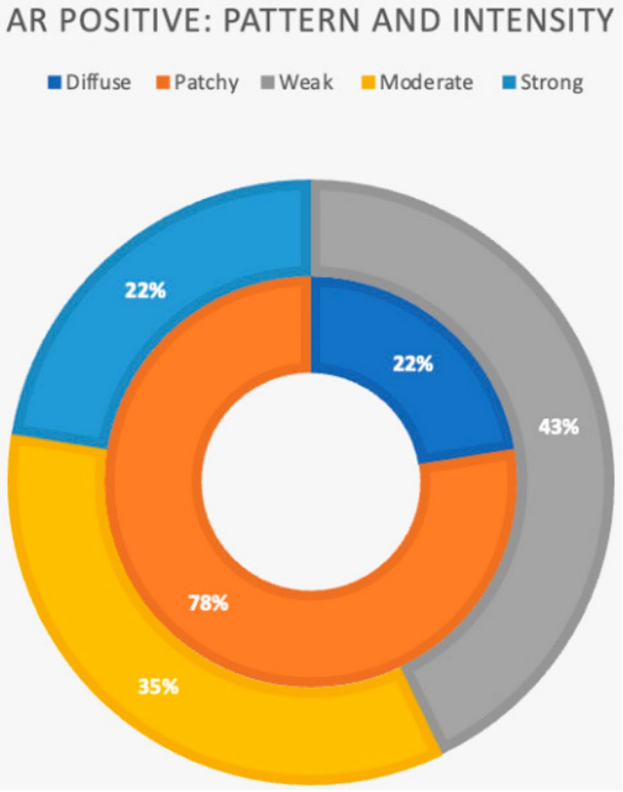

Fig. 5 A 2-tier pie chart diagram showing the proportions of both pattern and intensity staining score and among the AR-positive cases

of age similar to study done by Vuhahula et al.., in Uganda 2004, although the mean age in that study was 38.1 years [26]. These current results contrast with other studies where the mean age was reported to be either lower [8, 27] or higher [24] than that found in this study.

In addition to age, the current study also noted a preference for malignant SGTs in the female population accounting for 53%. This observation aligns with the findings of several other studies [24, 26–28]. In contrast to these findings, a previous study done by Masanja et al.., 2003 in Tanzania showed higher frequency of malignant salivary gland tumors in males [7, 8], this may be explained by different geographical location and hospital capacities where these studies were conducted. Equal distribution in sexes for SGTs was also reported in some studies [29].

The parotid and the gingiva were the most common sites of the major and minor salivary gland respectively in the current study. These current findings were different from other studies conducted in Tanzania and Uganda [8, 26] and Western countries where the palate followed by lip was the most affected site among the minor salivary glands [24, 29–31]. A study by Onyango et al.., 1992 reported that of the major salivary glands, submandibular glands were affected more in contrast to this study [32]. It was also noted in this study that minor salivary

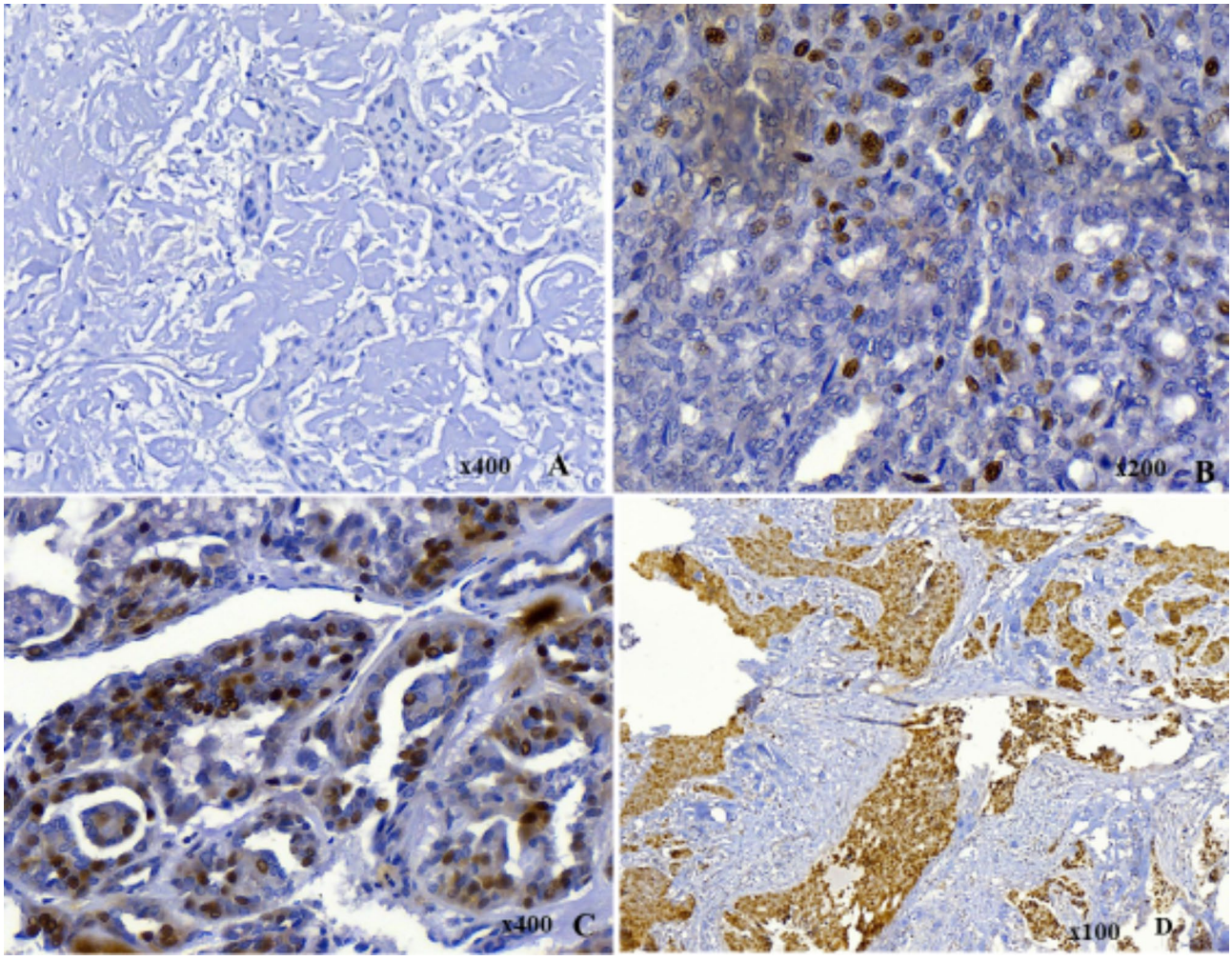

Fig. 6 Photomicrographs showing immunohistochemical (IHC) sections **A**. Negative AR in Secretory carcinoma, (**B**) Patchy strong positive AR in Acinic cell carcinoma (**C**) and Microsecretory adenocarcinoma respectively (**D**) Diffuse strong positive high-grade Adenoid cystic carcinoma

glands had more malignant tumors than benign, similar to studies done in Uganda, Nigeria and Cameroon [25, 26, 28, 33].

In this study Adenoid cystic carcinoma (AdCC) was the commonest histological subtype followed by Mucoepidermoid carcinoma (MEC) for malignant tumors which is similar to many studies in Africa including Uganda, Nigeria, Cameroon [8, 26, 27], in contrast to a study done in Kenya and most Western countries where MEC is the commonest malignant subtypes [7, 24, 30, 31, 34]. The differences in these results could partly be attributed to the different geographical location and associated environmental factors, however further studies are recommended to clarify this.

In this study, it was found that the overall androgen receptor overexpression among malignant salivary gland tumors was 42.6% although the frequency varied among different subtypes. Previous studies conducted by Dalin et al., 2017 and Nasser et al., 2003 reported overall frequencies of 31% and 54%, respectively, with a range of 20–100% depending on the specific subtype of the tumor [22, 23, 35, 36]. The higher proportion in our study may have been contributed by the fact that the proportions of malignant salivary gland tumors of high grade were also high. Androgen receptors have been studied widely in SDC and this has been shown to have the highest proportions of AR overexpression ranging from 61 to 89% by IHC to 100% when molecular studies are employed [21, 22, 36–39] and AR gene amplification have been observed in SDC [40]. Similarly in our current study the highest proportions of AR overexpression were seen in SDC where 71.4% showed AR overexpression. This was followed by Polymorphus adenocarcinoma (PAC) and Salivary carcinoma not otherwise specified (SC NOS) which contrasts with other studies where Carcinoma ex-pleomorphic adenoma (CXPA), Basal cell adenocarcinoma (BCAC) and high grade MEC were the second common to overexpress AR [14, 35, 41, 42].

Table 2 Frequency of AR overexpression by IHC among various histological subtypes of malignant SGTs diagnosed at MNH

Histological subtypes	AR status *n* = 115(%)		Total
	Positive	Negative	
AdCC	13 (34.2)	25 (65.8)	38 (33.0)
MEC	9 (40.9)	13 (59.1)	22 (19.1)
SC NOS	6 (54.6)	5 (45.6)	11 (9.6)
PAC	5 (55.6)	4 (44.4)	9 (7.8)
MECA	3 (37.5)	5 (62.5)	8 (7.0)
SDC	**5 (71.4)**	**2 (28.6)**	**7 (6.1)**
AciCC	2 (40)	3 (60)	5 (4.3)
HCCC	2 (50)	2 (50)	4 (3.5)
MSA	1 (25)	3 (75)	4 (3.5)
CXPA	1 (50)	1 (50)	2 (1.7)
SMA	1 (50)	1 (50)	2 (1.7))
BCAC	1 (100)	0 (0)	1 (0.9)
EMC	0 (0)	1 (100)	1 (0.9)
SC	0 (0)	1 (100)	1 (0.9)
Total	49 (42.6)	66 (57.4)	115 (100)

Key:AdCC, Adenoid cystic carcinoma, MEC, Mucoepidermoid carcinoma, PAC, Polymorphus adenocarcinoma, SC NOS, Salivary carcinoma not otherwise specified, MECA, Myoepithelial carcinoma, SDC, Salivary duct carcinoma, AciCC, Acinic cell carcinoma, HCCC, Hyalinizing clear cell carcinoma, MSA, Microsecretory carcinoma, CXPA, Carcinoma ex-pleomorphic adenoma, SMA, Sclerosingmicrocystic carcinoma, BCAC, Basal cell adenocarcinoma, EMC, Epithelial myoepithelial carcinoma, SC, Secretory carcinoma

Interestingly, in this study, only one patient with BCAC and two patients with CXPA were included, and the AR overexpression rates were 100% and 50%, respectively suggesting that this receptor plays a role in malignant transformation or high-grade transformation. Even though the AdCC and MEC were the commonest tumors in this population, they had low proportion of AR overexpression. The disparity in these studies could be attributed to different scoring systems used, whether tissue microarray (TMA) or whole slide IHC staining was performed, pre analytical factors as well as the type of AR antibody used (monoclonal or polyclonal). Overall, these results highlight the variability in AR overexpression among malignant salivary gland tumors and suggest that further research is needed to elucidate the specific roles and implications of AR expression in different subtypes of these tumors.

Table 3 Association between AR overexpression and clinical pathological features (age, sex, anatomical site, and histological subtype) of malignant SGTs at MNH

Characteristic	Frequency *n* = 115(%)	AR status		*p*-value
		AR+ (%)	AR- (%)	
Sex				
M	54 (47.0)	24 (44.4)	30 (55.6)	***0.708*** **
F	61 (53.0)	25 (40.1)	36 (59.9)	
Age groups				
≤ 24	8 (6.9)	2 (25)	6 (75)	***0.253*** **
25–60	73 (63.5)	29 (39.7)	44 (60.3)	
> 60	34 (29.6)	18 (52.9)	16 (47.1)	
Site				
Major	67 (58.3)	28 (41.8)	39 (58.2)	
Parotid	**35 (52.2)**	**19 (54.3)**	**16 (45.7)**	***0.03*** **
Submandibular	31 (46.3)	9 (29)	22 (71)	
Sublingual	1 (1.5)	0 (0)	1 (100)	
Minor	48 (41.7)	21 (43.8)	27 (56.3)	
Palate	13 (27.1)	5 (38.5)	8 (61.5)	***0.621****
Buccal	15 (31.2)	8 (53.3)	7 (46.7)	
Gingiva	17 (35.4)	6 (35.3)	11 (64.7)	
Tongue	3 (6.3)	2 (66.7)	1 (33.3)	
Histological subtype				
AdCC	38 (33.0)	13 (34.2)	25 (65.8)	***0.557*** **
MEC	22 (19.1)	9 (40.9)	13 (59.1)	
SC NOS	11 (9.6)	6 (54.5)	5 (45.5)	
PAC	9 (7.8)	5 (55.6)	4 (44.4)	
MECA	8 (7)	3 (37.5)	5 (62.5)	
SDC	7 (6.1)	5 (71.4)	2 (28.6)	
Others	20 (17.4)	8 (40)	12 (60)	

*p-value from Fisher Exact test, **p-value from Chi2-test, AdCC, Adenoid cystic carcinoma, MEC, Mucoepidermoid carcinoma, PAC, Polymorphus adenocarcinoma, SC NOS, Salivary carcinoma not otherwise specified, MECA, Myoepithelial carcinoma, SDC, Salivary duct carcinoma

Furthermore, AR overexpression has been reported in patient with advanced age and in male populations although there was no significant association between these variables [42, 43]. Similarly, to the current study, there was no significant association between AR overexpression with sex and age. Notably 52.9% of those above 60 years of age had AR overexpression and there was comparably lower proportion of AR overexpression

Table 4 Association between AR overexpression and histological grade of malignant SGT at MNH

		Histological grade				*p value*
		Low *n* = 30 (%)	Intermediate *n* = 32 (%)	High *n* = 53 (%)	Total	
AR status	AR+	11 (36.7)	9 (28.1)	29 (54.7)	49 (42.6)	0.042**
	AR-	19 (63.3)	23 (71.9)	24 (45.3)	66 (57.4)	
Pattern (n = 49)	Patchy	7 (63.6)	7 (77.8)	24 (82.8)	38 (77.6)	***0.405****
	Diffuse	4 (36.4)	2 (22.2)	5 (17.2)	11 (22.4)	
Intensity (n = 49)	Weak	4 (36.4)	2 (22.2)	15 (51.7)	21 (48.9	***0.518****
	Moderate	5 (45.5)	4 (44.4)	8 (27.6)	17 (34.7)	
	Strong	2 (18.2)	3 (33.33)	6 (20.7)	11 (22.4)	

*p-value from Fisher Exact test, **p-value from Chi2-test

Table 5 Logistic regression analysis showing independent factors associated with androgen receptor overexpression

AR Status						
Variable	**cOR**	**95% CI**	***p*-value**	**aOR**	**95% CI**	***p*-value**
Site (Major)						
SM/SL	Ref					
Parotid	3.0	1.1–8.4	***0.033***	3.45	1.1–10	***0.027***
Grade						
Low	Ref					
Intermediate	0.6	0.2-2	*0.473*	1.2	0.2-6	*0.809*
High	2.1	0.8-5	*0.116*	5.1	1.4–19	***0.014***

SM, Submandibular glands, SL, Sublingual glands

below 60 years of age. Nasser et al., 2003 found nearly equal proportion of AR in both sexes similarly in this study [23]. In contrast, a study by Szewczyk et al., 2019 reported a significant association between male sex and AR overexpression [36]. The sample size in this study was similar to the current study, but differences in ethnicity and genetic profile may have contributed to the disparate findings.

Most studies have shown strong association for sex in SDC however, it's important to note that SDC is known to be more prevalent in males, which may explain the stronger association. Even though there was no statistically significant difference between histological subtypes and AR overexpression like in other studies, it is worth noticing that high proportions of SDC had AR overexpression in this study as reported in many other studies [22, 39].

In addition, Rahim et al.,2017 found the association between parotid gland involvement and AR overexpression similar to this study [43], again this may be explained by the fact that majority of the tumors occurred in parotid gland. Further research with larger sample sizes and inclusion of other subtypes is needed to better understand the relationship between AR overexpression, sex, anatomical site and histological subtypes. Additionally, studying the genetic profile of SDC in relation to sex and other subtypes could provide valuable insights.

Majority of our patients had high grade tumors and was significantly associated with AR overexpression [*p*-value 0.014, 95% CI=1.4–19], this finding is consistent with previous studies conducted by Rahim et al.., 2017 and Szewczyk et al., 2019 which also reported nodal metastasis and negative impacts on disease-free survival and overall survival [36, 43].Aquino et al.., 2008 nevertheless had a contrasting result where in his study there was no association between AR overexpression and grade or survival [42].

Although the staining pattern and intensity have been widely studied in SDC and high grade MEC and have been reported to be diffuse and strong [23, 35, 44] in this study however, the staining pattern and intensity of AR IHC was not significantly associated with the tumor grade, in fact highest proportion of diffuse and strong were seen in low grade and intermediate grade tumors as compared to high grade. These findings are not surprising considering that in most studies different scores were used [23, 42, 45]. Different pre-analytical factors, IHC techniques and methods may contribute to this disparity. However, to further elucidate these differences, molecular techniques should be employed ascertain the true association between the pattern and intensity of AR expression in different malignant subtypes and their grades.

Strength and limitation of the study

This current study has several strengths apparently including a comparatively higher sample size than in other studies, which enhances the statistical power and generalizability of the findings. Additionally, the use of a monoclonal antibody for immunohistochemistry (IHC) adds specificity to the analysis, making it more appropriate to conclude that there is a promising future for targeted therapy with androgen deprivation therapy (ADT). Our current study also employed the widely used Allred score for interpreting sex hormone expression, facilitating easy understanding and comparability of the results among reader. However, the study did encounter certain limitations. The major limitation of this index study was the missing data and some technical issues, including missing or inadequate blocks as well as tissue washout from the slides during staining, which may have impacted data collection and potentially introducing bias.

Conclusion

This study reveals a significant prevalence of AR overexpression in high-grade salivary gland tumors (SGTs), particularly in the parotid gland, with equal distribution among male and female patients. This finding aligns with prior research but offers new insights into AR expression across tumor grades and sites. While our findings suggest ADT's potential for AR-positive high-grade SGTs, large-scale studies with molecular profiling and clinical trials are needed to confirm its therapeutic role and guide future management strategies.

Abbreviations

ACiCC	Acinic cell Carcinoma
AdCC	Adenoid cystic carcinoma
ADT	Androgen Deprivation Therapy
AJCC	American Joint Commission on Cancer
AR	Androgen receptor
BCAC	Basal Cell Adenocarcinoma
CPL	Central Pathology Laboratory
CXPA	Carcinoma ex pleomorphic adenoma
DAB	3'3 Diaminobenzidine
DNA	Deoxyribonucleic acid
DPX	Distyrene Plasticizer Xylene
EMC	Epithelial Myoepithelia Carcinoma
FFPE	Formalin fixed paraffin embedded
HCCC	Hyalinizing Clear Cell Carcinom
HRP	Horseradish Peroxidase
IHC	Immunohistochemistry
MEC	Mucoepidermoid carcinoma
MEC	Mucoepidermoid carcinoma
MECA	Myoepithelial carcinoma
MNH	Muhimbili National Hospital
MSA	Microsecretory carcinoma
MUHAS	Muhimbili University of Health and Allied Sciences
ORCI	Ocean Road Cancer Institute
PA	Pleomorphic adenoma
PAC	Polymorphous adenocarcinoma
SC	Secretory carcinoma
SC NOS	Salivary Carcinoma not otherwise classified
SDC	Salivary Duct Carcinoma
SGC	Salivary gland cancer
SGT	Salivary gland tumor
SMA	Sclerosing Microcystic Carcinoma
TBS	Tris-Buffered Saline
TMA	Tissue Microarray

Acknowledgements
We acknowledge all the patients whose medical records and tissue blocks and slides were used for this study. We also acknowledge the support of the technical staff at Muhimbili National Hospital and faculty at Muhimbili University of Health and Allied Sciences.

Author contributions
S.M. conceived and led the study and drafted the initial manuscript; E.V. and A.M. supervised the study and provided initial input to the study design; A.O. and E.P.M contributed and supported laboratory testing; E.L. and G.M. contributed and supported in data analysis; E.R. and I.S. read and contributed to the manuscript and provided significant input to the presentation and discussion of the results. All authors read and approved the manuscript for submission.

Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was reviewed and approved by the Muhimbili University of Health and Allied Science's institutional review board and informed consent was waived given that no contact was made with participants with the use of medical records and archived tissue blocks and slides.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Catholic University of Health and Allied Sciences, Mwanza, Tanzania
[2]Bugando Medical Center, Mwanza, Tanzania
[3]Research for Development, Kigali, Rwanda
[4]Butaro Cancer Center of Excellence, Burera, Rwanda
[5]Arusha Lutheran Medical Centre, Arusha, Tanzania
[6]Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[7]Muhimbili National Hospital, Dar es Salaam, Tanzania
[8]Hubert Kairuki Memorial University, Dar Es Salaam, Tanzania

Received: 11 August 2024 / Accepted: 19 December 2024
Published online: 07 January 2025

References

1. Skálová A, Hyrcza MD, Leivo I. Update from the 5th Edition of the World Health Organization Classification of Head and Neck tumors: salivary glands. Head Neck Pathol. 2022;16(1):40–53.
2. Jones AV, Craig GT, Speight PM, Franklin CD. The range and demographics of salivary gland tumours diagnosed in a UK population. Oral Oncol. 2008;44(4):407–17.
3. Ansari MH. Salivary gland tumors in an Iranian Population: a retrospective study of 130 cases. J Oral Maxillofac Surg. 2007;65(11):2187–94.
4. Guzzo M, Locati LD, Prott FJ, Gatta G, McGurk M, Licitra L. Major and minor salivary gland tumors. Crit Rev Oncol Hematol. 2010;74(2):134–48.
5. Laishram RS, AK K, Pukhrambam GD, Laishram S, Debnath K. Pattern of salivary gland tumors in Manipur, India: a 10 year study. South Asian J Cancer. 2013;02(04):250–3.
6. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer statistics 2020: GLOBOCAN estimates of incidence and Mortality Worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49.
7. Helen Lin H, Limesand KH, Ann DK. Current state of knowledge on salivary gland cancers. Crit Rev Oncog. 2018;23(3–4):139–51.
8. Masanja MI, Kalyanyama BM, Simon M. EN. SALIVARY GLAND TUMOURS IN TANZANIA. Vol. 80, EAST AFRICAN MEDICAL JOURNAL 429 East African Medical Journal. 2003.
9. Gilyoma JM, Rambau PF, Masalu N, Kayange NM, Chalya PL. Head and neck cancers: a clinico-pathological profile and management challenges in a resource-limited setting. BMC Res Notes. 2015;8(1).
10. Geiger JL, Ismaila N, Beadle B, Caudell JJ, Chau N, Deschler D et al. Management of Salivary Gland Malignancy: ASCO Guideline [Internet]. Vol. 39, J Clin Oncol. 2021. Available from: www.asco.org/head-neck-cancer-guidelines
11. Elkrief A, Saleh R. Androgen deprivation therapy for metastatic salivary gland cancer. Can Med Assoc J. 2018;190(33):E985–7.
12. Chintakuntlawar AV, Okuno SH, Price KA. Systemic therapy for recurrent or metastatic salivary gland malignancies. Cancers Head Neck. 2016;1(1).
13. Tanzania. United Republic of.
14. Boon E, van Boxtel W, Buter J, Baatenburg de Jong RJ, van Es RJJ, Bel M, et al. Androgen deprivation therapy for androgen receptor-positive advanced salivary duct carcinoma: a nationwide case series of 35 patients in the Netherlands. Head Neck. 2018;40(3):605–13.
15. Cohen RB, Delord JP, Doi T, Piha-Paul SA, Liu SV, Gilbert J, et al. Pembrolizumab for the Treatment of Advanced Salivary Gland Carcinoma. Am J Clin Oncol. 2018;41(11):1083–8.
16. Hitre E, Budai B, Takácsi-Nagy Z, Rubovszky G, Tóth E, Remenár É, et al. Cetuximab and platinum-based chemoradio- or chemotherapy of patients with epidermal growth factor receptor expressing adenoid cystic carcinoma: a phase II trial. Br J Cancer. 2013;109(5):1117–22.
17. Adatia A. Clinicopathological features and treatment outcomes in parotid malignancies at Ocean Road Cancer Institute, Dar-es-Salaam, Tanzania. J Glob Oncol. 2018;4(Supplement 2):s192–192.
18. Fujita K, Nonomura N. Role of androgen receptor in prostate Cancer: a review. World J Mens Health. 2019;37(3):288.
19. Munoz J, Wheler JJ, Kurzrock R. Androgen receptors beyond prostate cancer: an old marker as a new target. Oncotarget. 2015;6(2):592–603.
20. Locati LD, Quattrone P, Bossi P, Marchianò AV, Cantù G, Licitra L. A complete remission with androgen-deprivation therapy in a recurrent androgen receptor-expressing adenocarcinoma of the parotid gland. Ann Oncol. 2003;14(8):1327–8.
21. van Boxtel W, Locati LD, van Engen-van Grunsven ACH, Bergamini C, Jonker MA, Fiets E, et al. Adjuvant androgen deprivation therapy for poor-risk, androgen receptor–positive salivary duct carcinoma. Eur J Cancer. 2019;110:62–70.

22. Xu B, Dogan S, Haroon Al Rasheed MR, Ghossein R, Katabi N. Androgen receptor immunohistochemistry in salivary duct carcinoma: a retrospective study of 188 cases focusing on tumoral heterogeneity and temporal concordance. Hum Pathol. 2019;93:30–6.
23. Nasser SM, Faquin WC, Dayal Y. Expression of Androgen, Estrogen, and progesterone receptors in salivary gland tumors. Am J Clin Pathol. 2003;119(6):801–6.
24. Fonseca FP, de Vasconcelos Carvalho M, de Almeida OP, Rangel ALCA, Takizawa MCH, Bueno AG, et al. Clinicopathologic analysis of 493 cases of salivary gland tumors in a Southern Brazilian population. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(2):230–9.
25. Adeyemi BF, Ogun GO, Akang EE. Retrospective analysis of intra-oral salivary gland tumours in Ibadan, Nigeria. West Afr J Med. 2010;29(2):98–103.
26. Vuhahula EAM. Salivary gland tumors in Uganda: clinical pathological study. Volume 4. African Health Sciences; 2004.
27. Sando Z, Fokouo JVF, Mebada AO, Djomou F, Ndjolo A, Oyono JLE. Epidemiological and histopathological patterns of salivary gland tumors in Cameroon. Pan Afr Med J. 2016;23.
28. Omitola OG, Soyele OO, Butali A, Akinshipo AO, Okoh D, Sigbeku O, et al. Descriptive epidemiology of salivary gland neoplasms in Nigeria: an AOPRC multicenter tertiary hospital study. Oral Dis. 2019;25(1):142–9.
29. Speight PM, Barrett AW. Salivary gland tumours: diagnostic challenges and an update on the latest WHO classification. Vol. 26, diagnostic histopathology. Elsevier Ltd; 2020. pp. 147–58.
30. Sentani K, Ogawa I, Ozasa K, Sadakane A, Utada M, Tsuya T, et al. Characteristics of 5015 salivary gland neoplasms registered in the Hiroshima Tumor Tissue Registry over a period of 39 years. J Clin Med. 2019;8(5):566.
31. Mengi E. Salivary gland tumors: a 15-year experience of a universıty hospital in Turkey. North Clin Istanb. 2020.
32. Onyango JF, Awange DO, Muthamia JM, Muga BI. Salivary gland tumours in Kenya. East Afr Med J. 1992;69(9):525–30.
33. Gbotolorun OM, Arotiba GT, Effiom OA, Omitola OG. Minor salivary gland tumours in a Nigerian hospital: a retrospective review of 146 cases. Odontostomatol Trop. 2008;31(123):17–23.
34. Hill AG. Major salivary gland tumors in a rural Kenyan Hospital. East Afr Med J. 2002;79(1).
35. Dalin MG, Watson PA, Ho AL, Morris LGT. Androgen receptor signaling in salivary gland cancer. Volume 9. Cancers: MDPI AG; 2017.
36. Szewczyk M, Marszałek A, Sygut J, Golusiński P, Golusiński W. Prognostic markers in salivary gland cancer and their impact on survival. Head Neck. 2019;41(9):3338–47.
37. Boon E, Bel M, van Boxtel W, van der Graaf WTA, van Es RJJ, Eerenstein SEJ, et al. A clinicopathological study and prognostic factor analysis of 177 salivary duct carcinoma patients from the Netherlands. Int J Cancer. 2018;143(4):758–66.
38. Fan CY, Melhem MF, Hosal AS, Grandis JR, Barnes EL. Expression of androgen receptor, Epidermal Growth Factor Receptor, and transforming growth factor α in Salivary Duct Carcinoma. Arch Otolaryngol Head Neck Surg. 2001;127(9):1075.
39. Williams L, Thompson LDR, Seethala RR, Weinreb I, Assaad AM, Tuluc M, et al. Salivary Duct Carcinoma. Am J Surg Pathol. 2015;39(5):705–13.
40. Meyer MT, Watermann C, Dreyer T, Ergün S, Karnati S. 2021 update on diagnostic markers and translocation in salivary gland tumors. Int J Mol Sci. 2021;22(13):6771.
41. Yeoh CC, Dabab N, Rigby E, Chhikara R, Akaev I, Gomez RS, et al. Androgen receptor in salivary gland carcinoma: a review of an old marker as a possible new target. J Oral Pathol Med. 2018;47(7):691–5.
42. Aquino G, Collina F, Sabatino R, Cerrone M, Longo F, Ionna F, et al. Sex hormone receptors in Benign and malignant salivary gland tumors: prognostic and predictive role. Int J Mol Sci. 2018;19(2):399.
43. Rahimi S, Bradley KL, Akaev I, Marani C, Yeoh CC, Brennan PA. Androgen receptor expression in primary nonsquamous cell rare-variant carcinomas of the Head and Neck. OTO Open. 2017;1(2):2473974X1771563.
44. Ishibashi K, Ito Y, Fujii K, Masaki A, Beppu S, Kawakita D, et al. Androgen receptor–positive Mucoepidermoid Carcinoma. Int J Surg Pathol. 2015;23(3):243–7.
45. Ito FA, Ito K, Coletta R, Della, Vargas PA, Lopes MA. Immunohistochemical study of androgen, estrogen and progesterone receptors in salivary gland tumors. Braz Oral Res. 2009;23(4):393–8.
46. Locati LD, Perrone F, Cortelazzi B, Lo Vullo S, Bossi P, Dagrada G, et al. Clinical activity of androgen deprivation therapy in patients with metastatic/relapsed androgen receptor-positive salivary gland cancers. Head Neck. 2016;38(5):724–31.
47. You Y, Wang P, Wan X, Xu L, Gong Y, Zhang W. Androgen Deprivation Therapy for patients with androgen-receptor-positive metastatic salivary Duct Carcinoma: a Case Report and Review of the literature. Onco Targets Ther. 2021;14:3481–6.

Publisher's note