RESEARCH ARTICLE

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Estrogen, Progesterone and Androgen Receptor in Primary and Recurrent Pleomorphic Salivary Adenomas

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Abstract

Objective: Our study aimed to characterize alteration in the immunohistochemical expression of estrogen, progesterone and androgen receptor in the tumour cells of primary pleomorphic adenomas and recurrent pleomorphic adenomas. **Methods:** A retrospective study of data including 30 cases of primary pleomorphic adenomas (PA) without recurrences and 15 cases of recurrent pleomorphic adenomas were examined (RPA). RPA included 8 males and 7 females. Immunohistochemical expression of estrogen, progesterone and androgen receptor was examined in the selected cases. The percentage of slides was semi-quantitatively assessed by two independent observers and scores were given. The statistical analysis included the use of descriptive statistics and proportional frequencies. **Results:** AR expression was identified in 12 (40. %) out of 30 cases of (PA) pleomorphic adenomas and 7 of 15 cases recurrent pleomorphic adenomas (RPA) (46 %). The results showed that ER and PR expression were negative in PA and RPA. **Conclusion:** Androgen receptors might have role in pathogenesis of PA and RPA. Estrogen and progesterone receptors have no role in development of recurrent pleomorphic salivary adenoma.

Keywords: Expression of estrogen- progesterone and androgen receptor- recurrent pleomorphic salivary adenoma

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Introduction

Pleomorphic adenoma (PA) is benign tumour and very common in salivary glands (Barnes et al., 2005) PA is classified as a benign tumor. The incidence of recurrence (RPA) after first operative treatment varies depending on differences in surgical technique (Riad et al., 2011; Andreasen at al., 2016). This risk of malignant transformation is rare as only 3% of salivary gland pleomorphic adenoma as recur at 12.5-year follow-up (Valstar et al., 2020) .

Some factors had played a main role in increase the recurrence of PA such as multinodularity and pseudopodia, the age of the patients, and incomplete surgical excision (Kanatas and Mücke, 2018; Ana et al., 2019; Abu-Ghanem et al.,2016). RPA has been associated with molecular changes (Ana et al.,2015). de Brito et al., (2016) have studied PLAG1 protein expression in 40 PAs and 36 RPAs by immunohistochemistry. PLAG1 expression was detected in 37 cases (93 %) of PA and in 34 RPA cases (94%). They indicated that PLAG1 might play a role in tumor recurrence. The p16, cyclin D1, and E2F proteins, which compound the retinoblastoma pathway that controls cell cycle phases, also showed strong expression in RPA. These results show that cell cycle-related changes in RPA are similar to changes in carcinoma ex pleomorphic adenoma CXPA (Ana et al., 2015). The treatment of RPA is complicated due to the multiple nodules that may add up to as many as 130 in a single patient (Soares et al., 2011). The development in molecular changes have helped in studying such this tumour. The use of antagonist hormones for patients with prostate cancer positive to androgen receptor (AR) has shown reduction of the recurrences and improvement of the survival rates (EBCTCG, 2015; Berthelet et al., 2005). The discovery of hormonal receptors Estrogen (ER) and progesterone receptors (PR) facilitated the identification of breast cancer patients who can get the benefit from hormone therapy (Ma, 2009). ER including ERα and ERβ, where the ERα is the most common isoform studied (Dimery et al.,1987). Many studies indicated that some tumours have hormone receptors e.g melanoma, carcinoid tumors, pancreatic, and renal cancers (Driscoll et al., 2009; Ripoll et al., 2008; Birsak et al.,1996; Mai et al., 2008). There is limited number of published studies concerning the existence of AR, ER, and PR in RPA. The purpose of this study was to determine, with the use of immunohistochemistry, if AR, ER and PR identified in formalin- fixed paraffin embedded tumour cells of recurrent pleomorphic adenomas and primary pleomorphic adenomas.

Materials and Methods

Thirty cases of primary pleomorphic adenomas (PA)

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without recurrence. 15 cases of recurrent pleomorphic adenoma (RPA) were included in this study (Table 1) from hospital of Aleppo university. PA included 18 females and 12 males. Mean age of PA was 45. PA identified in 7 cases in submandibular gland and 23 cases in parotid gland. RPA included 8 males and 7 females. The mean age of RPA was 50. RPA were identified in 2 cases in submandibular gland and 13 cases in parotid gland. Immunostaining techniques were applied to localize the AR, PR, and ER in the tissues. Nuclear staining of estrogen, progesterone and androgen receptor was considered only as a positive result indicating presence of receptor protein. Two independent examiners scored the sections for the presence of the mentioned receptors. Following examination, the entire section, five random areas were chosen from each slide. The scoring criteria was considered two categories: positive or negative nuclear staining. AR, PR and ER positive nuclei was assessed by two independent observers and scored as: negative is <75% staining and positive as greater than 75% of cells. Cytoplasmic staining was not considered. Positive and negative controls were included in all reactions. The Research Ethics approval was provided from Aleppo university.

Immunohistochemistry

Paraffin-embedded, 5-µm-thick tissue sections from 30 specimens of PA and 15 specimens of RPA were cut. The sections were deparaffinized in xylene and rehydrated through graded alcohols. Sections were processed used streptavidin-biotin-peroxidase method. Briefly, the endogenous peroxidase was blocked by 3 % hydrogen peroxidase for 5 min followed by TBS wash. Non-specific immunoreactivity was blocked by incubation with normal goat serum for 20 minutes. The sections were incubated with the following primary antibody: anti-Androgen receptor (clone AR441, Dako, USA) was diluted to 1: 25 (40 µL / ml) in tris buffer saline (TSA) containing 0.1 % bovine serum albumin for 10 minutes at the room temperature. Dako mouse anti-human Estrogens receptor Erα 1D5 was diluted to 1: 25 (40 µL /ml) in tris buffer saline (TSA) containing 0.1 % bovine serum albumin for 10 minutes at the room temperature. Dako mouse anti-human progesterone receptor PR 636 was diluted to 10 µ/ml in tris buffer saline (TSA) containing 0.1 % bovine serum albumin for 10 minutes at the room temperature. All sections were washed by TBS for 5 minutes. Sections were incubated with the biotinylated secondary antibody reagent for 10 minutes followed by (TBS) wash for 5 minutes. Slides were incubated with streptavidin and horseradish peroxidase for 10 minutes followed by (TBS) tris buffer saline wash for 5 minutes. Incubate with a prepared chromogenic substrate solution (Diaminobenizidine) for 15 minutes. Sections were counterstained with 0.25 % methyl green in distilled water for 5 minutes. Sections were dehydrated and mounted in Depax. Tissue blocks of breast carcinoma of known positive and negative estrogens and progesterone receptors and androgen receptor status were used as positive controls.

Statistical analysis

The data were described using frequency distribution (descriptive data). Also, the Mann Whitney test was used. Results with $P < 0.05$ were considered significant.

Results

Our results showed that ER was negative in all PA and RPA. There was no significant difference between expression of ER in PA and RPA ($p > 0.05$). Expression of PR was negative in all PA and RPA cases. There was no significant difference between expression of PR in PA and RPA ($p > 0.05$). AR expression was identified in 12 (40%) out of 30 cases of PA. AR expression was identified in 7 of 15 cases RPA (46%) had positive nuclear staining for androgen receptor. The clinical characterization and ER, PR, and AR immunostaining evaluation of each case of RPA is shown in Table 1. AR, ER and PR expression was

Table 1. shows the Clinical, and Immunohistochemical Characterization of AR, PR, and ER in Recurrent Pleomorphic Adenomas (RPA)

Case	Age	Gender	Gland	Diagnosis	Nuclear PR staining	Nuclear AR staining	Nuclear ER staining
1	55	M	Parotid	RPA	-	+	-
2	49	M	Parotid	RPA	-	+	-
3	56	F	Parotid	RPA	-	+	-
4	55	F	Parotid	RPA	-	+	-
5	57	F	Parotid	RPA	-	+	-
6	51	F	Parotid	RPA	-	+	-
7	48	F	Parotid	RPA	-	+	-
8	47	F	Parotid	RPA	-	-	-
9	52	F	Parotid	RPA	-	-	-
10	50	M	Submandibular	RPA	-	-	-
11	48	M	Parotid	RPA	-	-	-
12	50	M	Parotid	RPA	-	-	-
13	47	M	Parotid	RPA	-	-	-
14	51	M	Parotid	RPA	-	-	-
15	45	M	Submandibular	RPA	-	-	-

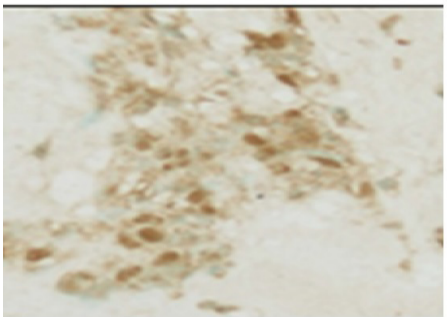

Figure 1. Shows AR Positive Expression in Recurrent Pleomorphic Adenoma

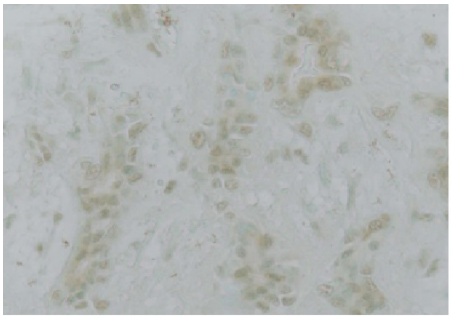

Figure 2. Shows ER Negative Expression in Recurrent Pleomorphic Adenoma

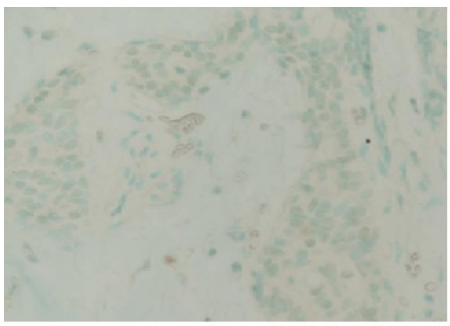

Figure 3. Shows PR Negative Expression in Recurrent Pleomorphic Adenoma

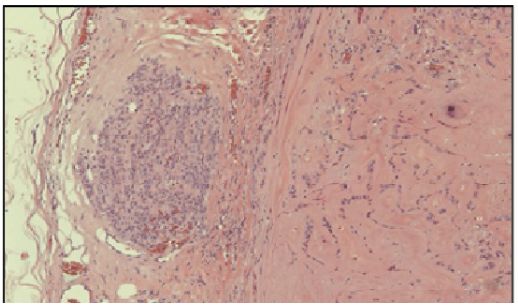

Figure 4. Showing Extra-Capsular Extension in Pleomorphic Salivary Adenoma.

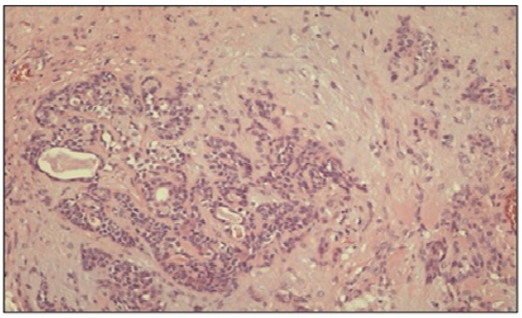

Figure 5. Islands and Strands in a Myxoid Stroma in Pleomorphic Salivary Adenoma

shown in PRA Figures 1-3. Figure 4 shows extra-capsular extension in pleomorphic salivary adenoma and Figure 5 shows islands and strands in a myxoid stroma in pleomorphic salivary adenoma.

Discussion

The study of ER, PR and AR in pleomorphic adenomas and recurrent pleomorphic adenomas were limited in literature. Only few publications were found in literature. (Malard et al., 2021) indicated that the most common causes of parotid RPA including enucleation; tumor rupture, or incomplete resection, and incomplete capsule with tumour extension in the form of pseudopods or remote satellite nodules (50% of cases). Hypocellularity or intermediate-level cellularity was also reported to be a risk factor. Rates of malignant transformation of RPA range from 2% to 25% (Niparko.,1986; Phillips.,1995). (Sarah et al., 2021) indicated that the rate of recurrence after first operation is less than 5% if the tumor is completely removed with intact capsule. If this tumour recurs, the rate of subsequent recurrence is approximately 50%. The surgical treatment of RPA is increased difficult with each recurrence due to preservation of facial nerve, risk of transformation into carcinoma ex pleomorphic adenoma (Sarah et al., 2021; Myung et al., 2009). Adjuvant radiotherapy for RPA can cause such as xerostomia, mucositis, and radiation-induced malignancy (Sarah et al., 2021), therefore endocrine treatment might be a treatment option of PRA if ER, PR, and AR can be detected in PRA.

The detection of ER, PR in breast cancers have improved prognosis of these patients. Also, the positive expression of AR in prostate cancers had improved the survival rates (EBCTCG, 2002; Berthel et., 2005). Glas et al., (2002) reported that there was no significant difference in ER expression in cases of PA and RPA. They concluded no role of ER in development PA or RPA. Glas et al., (2002) reported positive expression PR in recurrent tumours. Of the cases examined only 2 were negative and about 27% of cases showed overexpression, with significant difference between cases of PA and RPA. They considered that PR is a prognostic factor in the occurrence of recurrent pleomorphic adenoma. Our results were very similar to (Ana et al., 2019) who indicated that ER was negative in 19 cases of PA, whereas 1 case showed more than 10% positive cells. For RPA, 18 cases were negative for ER expression. PR expression in PA and RPA showed less than 10% positive staining.

Tom et al., (2008) indicated that 3 cases out of 41 of PA showed positive AR staining. This is similar to our results. Nasser et al., (2003) found weak AR expression (less than 3% of cells) in 4 of 10 pleomorphic adenomas

but its expression was detected in two out of ten mucoepidermoid carcinomas, and two out of ten adenoid cystic carcinomas, Fan et al., (2000) and Fan et al., (2001) suggested that AR may have a role in the pathogenesis of salivary duct carcinoma through the mediation of an epidermal growth factor receptor and transforming growth factor–α autocrine pathway similar to that seen in prostatic carcinoma. It is difficult to directly compare our study to the other studies, as each study used a different scoring system and different antibody clones. Though reported rates of ER and PR expression in salivary gland tumors are somewhat variable, the data suggest that only a minority of tumors express ER or PR in a predominantly weak to moderate pattern. Our study showed that AR expression was detected in (40%) PA and (46%) of RPA. PA and RPA are uncommon compared to other tumours. Unfortunately, only 15 limited cases of RPA were included in this study. This number of PRA cases is similar to (Glas et al., 2002) who reported 18 cases of RPA.

We believe that many difficulties are still present regarding the evaluation of effect of ER, PR and AR in PA and RPA. These difficulties included limited number of cases, and differences in scoring system of immunostaining technique and several steps in tissue processing that may influence staining patterns and intensity.

We recommend high cooperation from different hospitals over the world to look for large number of cases of RPA using one scoring criteria of evaluation ER, PR and AR in PA and specially RPA. The detection of AR in large number of cases of recurrent pleomorphic adenomas might help the patients to avoid malignant transformation of recurrent pleomorphic adenomas. There is still no significant evidence to support using endocrine treatment in recurrent pleomorphic adenomas.

In conclusion, the results suggested that recurrent pleomorphic adenoma was not dependent on endocrine function specially for estrogen and progesterone receptors. The detection of AR in 40 % cases of PA and in 46% of cases of RPA. This indicates that AR might have role in pathogenesis of PA and RPA. It is recommended that further work involves a large series of recurrent pleomorphic adenomas to determine if estrogens and progesterone, and androgen receptors using sensitive and specific biochemical methods can be detected in those tumours.

List of abbreviations

PA = Pleomorphic adenoma

RPA = recurrent pleomorphic adenoma

Author Contribution Statement

Tarakji B and Alali F had equally contributed for the conceptualization, design, drafting, writing and editing of the manuscript.

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Ethics approval and consent to participate

The Research Ethics approval was provided from Aleppo university, Faculty of dentistry.

Consent for publication

Written consent was signed by all patients.

Availability of data and materials

The data supporting the findings of the article is available within the article.

Conflict of interest

The authors declare no conflict of interest.

References

Andreasen S, Therkildsen MH, Bjørndal K, Homøe P (2016). Pleomorphic adenoma of the parotid gland 1985-2010: a Danish nationwide study of incidence, recurrence rate, and malignant transformation. *Head Neck*, **38**, 1364-9.

Ana Amélia dS, Albina A, Ney Soares dA, et al (2019). Estrogen Receptor, Progesterone Receptor, and HER-2 Expression in Recurrent Pleomorphic Adenoma. *Clin Pathol Clin Pathol*, **26**, 2632010X19873384.

Abu-Ghanem Y, Mizrachi A, Popovtzer A, et al (2016). Recurrent pleomorphic adenoma of the parotid gland: Institutional experience and review of the literature. *J Surg Oncol*, **114**, 714-8.

Ana Amélia dS, Albina A, Fabricio PS (2015). Dysregulation of the Rb pathway in recurrent pleomorphic adenoma of the salivary glands. *Virchows Arch*, **467**, 295-301.

Barnes L, Eveson JW, Reichart P, Sidransky D (2005). Pathology and Genetics Head and Neck Tumors: World Health Organization Classification of Tumors. Lyon, France: IARC Press, pp 254-62.

Beatriz Samara dB, Natália GG, Erika Said AE, et al (2016). PLAG1 expression is maintained in recurrent pleomorphic adenoma. *Virchows Arch*, **469**, 477-81.

Berthelet E, Pickles T, Lee KW, Liu M, Truong PT (2005). Prostate Cancer Outcomes Initiative. Long-term androgen deprivation therapy improves survival in prostate cancer patients presenting with prostate-specific antigen levels > 20 ng/mL. *Int J Radiat Oncol Biol Phys*, **63**, 781-7.

Birsak CA, Janssen PJ, van Vroonhoven CC, et al (1996). Sex steroid receptor expression in 'carcinoid' tumours of the breast. *Breast Cancer Res Treat*, **40**, 243-9.

Dimery IW, Jones LA, Verjan RP, et al (1987). Estrogen receptors in normal salivary gland and salivary gland carcinoma. *Arch Otolaryngol Head Neck Surg*, **113**, 1082-5.

Driscoll MS, Grant-Kels JM (2009). Estrogen receptor expression in cutaneous melanoma. *Arch Dermatol*, **145**, 73-5.

Early Breast Cancer Trialists' Collaborative Group (EBCTCG, 2005). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet*, **365**, 1687-717.

Fan CY, Wang J, Barnes EL (2000). Expression of androgen receptor and prostatic specific markers immunohistochemical analysis of 13 cases and review of the literature. *Am J Surg Pathol*, **24**, 579-86.

Fan CY, Melhem MF, Hosal AS, Grandis JR, Barnes EL (2001).

Expression of androgen receptor, epidermal growth factor receptor, and transforming growth factor alpha in salivary duct carcinoma. *Arch Otolaryngol Head Neck Surg*, **127**, 1075-9.

Glas AS, Hollema H, Nap RE, Plukker JT (2002). Expression of estrogen receptor, progesterone receptor, and insulin-like growth factor receptor-1 and of Mib-1 in patients with recurrent pleomorphic adenoma of the parotid gland. *Cancer*, **1594**, 2211-6.

Kanatas A, Ho MWS, Mücke T (2018). Current thinking about the management of recurrent pleomorphic adenoma of the parotid: a structured review. *Br J Oral Maxillofac Surg*, **56**, 243-8.

Ma CX, Sanchez CG, Ellis MJ (2009). Predicting endocrine therapy responsiveness in breast cancer. *Oncology (Williston Park)*, **23**, 133-42.

Mai KT, Teo I, Belanger EC, et al (2008). Progesterone receptor reactivity in renal oncocytoma and chromophobe renal cell carcinoma. *Histopathology*, **52**, 277-82.

Malard O, Thariat J, Cartier C, et al (2021). Guidelines of the French Society of Otorhinolaryngology-Head and Neck Surgery (SFORL), part II: Management of recurrent pleomorphic adenoma of the parotid gland. *Eur Ann Otorhinolaryngol Head Neck Dis*, **138**, 45-9.

Myung-Whan S, J Hun H, Seong Keun K, et al (2009). Clinical manifestations of recurrent parotid pleomorphic adenoma. *Clin Exp Otorhinolaryngol*, **2**, 193-7.

Nasser SM, Faquin WC, Dayal Y (2003). Expression of androgen, estrogen, and progesterone receptors in salivary gland tumors. Frequent expression of androgen receptor in a subset of malignant salivary gland tumors. *Am J Clin Pathol*, **119**, 801–6.

Niparko KJ, Beauchamp ML, Krause CJ, et al (1986). Surgical treatment of recurrent pleomorphic adenoma of the parotid gland. *Arch Otolaryngol Head Neck Surg*, **112**, 1180–4.

Phillips PP, Olsen KD (1995). Recurrent pleomorphic adenoma of the parotid gland: report of 126 cases and a review of the literature. *Ann Otol Rhinol Laryngol*, **104**, 100–4.

Riad MA, Abdel-Rahman H, Ezzat WF, el al (2011). Variables related to recurrence of pleomorphic adenomas: outcome of parotid surgery in 182 cases. *Laryngoscope*, **121**, 1467-72.

Ripoll C, Ropero AB, Alonso-Magdalena P, et al (2008). Rapid regulation of pancreatic alpha- and beta- cell signalling systems by estrogens. *Infect Disord Drug Targets*, **8**, 61-4.

Soares AB, Altemani A, Araújo VC (2011). Study of histopathological, morphological and immunohistochemical features of recurrent pleomorphic adenoma: an attempt to predict recurrence of pleomorphic adenoma. *J Oral Pathol Med*, **40**, 352-8.

Sarah EN, Wei Fu, Angela LL, et al (2021). Regina DeLuna, Luka Vujaskovic, Justin Bishop, Brandi R Page, Harry Quon , et al. Radiation Therapy After Surgical Resection Improves Outcomes for Patients With Recurrent Pleomorphic Adenoma. *Adv Radiat Oncol*, **6**, 100674.

Tom CD, Aaron PH, Jennifer LH (2008). Immunohistochemical evaluation of androgen receptor, HER-2/neu, and p53 in benign pleomorphic adenomas. *Arch Pathol Lab Med*, **132**, 1907-11.

Valstar MH, Andreasen S, Bhairosing PA, McGurk A (2020). Natural history of recurrent pleomorphic adenoma: implications on management. *Head Neck*, **42**, 2058-66.