WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 13, 2381-2395. Research Article ISSN 2277– 7105

EXPRESSION OF PROGESTERONE RECEPTORS ON BENIGN AND MALIGNANT SALIVARY GLAND TUMORS- AN IMMUNOHISTOCHEMICAL STUDY

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Article Received on
21 August 2022,

Revised on 11 Sept. 2022,
Accepted on 01 October 2022

DOI: 10.20959/wjpr202213-26042

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ABSTRACT

Salivary gland tumors are the most heterogenous group of tumors histologically with greatest diversity of morphologic features among their cells and tissues. As both the salivary gland and breast tissue share a common histological pattern of tubule-acinar unit, their similarity with certain breast tumors is possible. The importance of hormone receptor status in breast cancer has been pivotal in determining prognosis and likelihood of response to hormonal manipulation. Tumors which are both ER and PR positive are much more likely to respond to anti-ER and anti-PR therapy than ER and PR negative tumors. The expression of sex hormone receptors in some tumors suggests a role for these receptors in tumor pathogenesis and therapy. Hence an attempt has made to IHC expression of PR protein in benign and malignant salivary gland tumors and to compare the expression between benign and malignant SGTs.[1-2]

KEYWORDS: *Tumours, expression, response, IHC.*

INTRODUCTION

According to WHO, the global incidence of salivary gland tiumors varies between 0.4 to 13.5 cases per 100,000 population. In India, the overall incidence of salivary gland tumors are extracted from cancer registries established under National Cancer Registry Programme

(NCRP) by the Indian Council for Medical Research (ICMR).[17] The overall incidence of benign and malignant tumours is less than 5 per 100,000 head of population per year. Since about 80% of all tumours are benign it can be appreciated that salivary gland malignancies are very rare with reported incidences of only 1.2–1.3 cases per 100,000 and representing only around 3% of all cancers of the head and neck according to cancer research, UK.[18]

WHO has given its first edition of salivary gland tumor classification in 1972. 1991 was the year where the second edition of SGTs was published by WHO which were based almost exclusively on histomorphology and were essentially a simple list of lesions ordered by frequency of occurrence. Such a classification system has been criticized, especially by surgical oncologists, for being too complicated and for a lack of precision or applicability to modern oncological practice. Hence, a newer classification which included 37 entities were added in the third edition if SGT classification by WHO which was even reduced to 33 in the latest WHO classification of 2017.[18]

Few of the changes in the latest edition of WHO classification on SGTs (2017) include few rare malignant tumors which are now grouped together under "Adenocarcinoma NOS" and 2 separate entities known as intraductal and inverted ductal papillomas are now merged and named commonly as ductal papilloma. Few new entities are added in the current classification which include sclerosing polycystic adenosis and seceretory carcinoma. Formerly known as PLGA, a low grade indolent tumor, is now renamed as polymorphous adenocarcinoma as it can recur and metastasize. Inspite of the fact that the tumor has an overall survival rate of over 95%, still it required changes in its name because of its highly unpredictable behaviour. Removing the term 'low grade' also signifies that the lesion should be managed as a malignant neoplasm to avoid recurrence and further consequences.[18]

Progesterone is a C21 steroid secreted by the corpus luteum, the placenta, and (in small amounts) the follicle. It is an important intermediate in steroid biosynthesis in all tissues that secrete steroid hormones, and small amounts apparently enter the circulation from the testes and adrenal cortex. Plasma progesterone levels in men and women are 0.3ng/ml and 0.9ng/ml(follicular phase)- 18ng/ml(luteal phase) respectively. The principal target organs of progesterone are the uterus, the breasts, and the brain.[4] It is well known that hormonal therapy is very useful in the treatment of breast and prostate cancer. However, in spite of the confirmed expression of sex hormone receptors in other types of cancer such as endometrial carcinomas, carcinomas of the thyroid, renal cell carcinomas, malignant melanomas, and

meningiomas, the efficacy of hormonal therapy in these tumors has not yet been established.[4]

A number of studies have been carried out by research workers to observe the expression of PgR on benign and malignant salivary gland tumors. The most significant expression was seen in cases of AdCC, followed by MEC, PA and WT. It was also concluded that expression of PgR could be a prognostic factor in recurrent PA of parotid gland.[5,6,7,8,9,10] Hence, this study has been taken up to observe the immunohistochemical expression of progesterone receptor in benign and malignant salivary gland tumors and compare the same in both the groups.[3-5]

MATERIALS AND METHODOLOGY

The study group consisted of 15 benign and 15 malignant cases of salivary gland tumors retrieved from the archives of the Department of Oral and Maxillofacial Pathology, AECS Maaruti College of Dental Sciences and Research center, Bangalore and other Dental Colleges. 5 cases of ductal carcinoma of breast were taken as control tissues.

INCLUSION CRITERIA- Histopathologically diagnosed benign and malignant salivary gland tumors.

EXCLUSION CRITERIA- Non- neoplastic lesions of salivary gland.

REAGENTS USED- Antibodies used in the study were: **1. Monoclonal anti-human antibody PATHNSITU biotechnologies-** PR068-6ml ready to use) **2. PolyExcel HRP/DAB detection system-** Secondary antibody PATHNSITUIndia, 6ml-ready to use) Anhydrous citric acid• Sodium hydroxide• Tri sodium citrate purified• Sodium chloride• Hydrochloric acid•

EQUIPMENTS USED- Silane coated microscope slides• Water bath• Electronic weighing machine• Digital pH meter• Pressure cooker• Electric stove• Glass slides and cover slips• Mounting media expression• Research microscope (Olympus BX 41)

Immunohistochemical procedure for PR staining was performed under all aseptic conditions. The sections so stained were then viewed under the microscope and assessed for the staining characteristics.

INTERPRETATION OF THE STAINING- Presence of brown color end product was indicative of positive immunoreactivity. The distribution of stain in each case of salivary gland tumor was observed in the following areas- ductal and non- ductal cells of the lesional tissue. The results were subjected to appropriate statistical analysis.

RESULTS

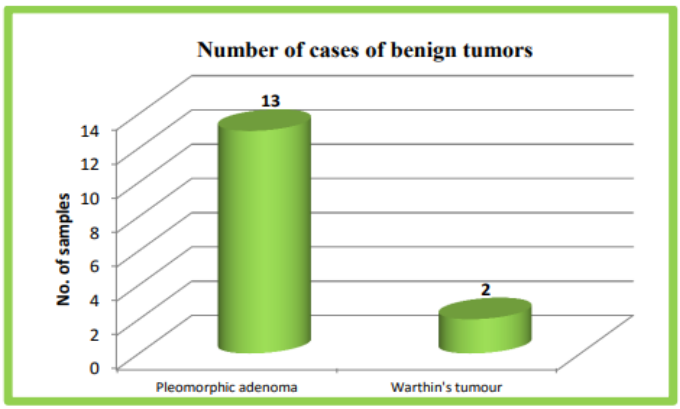
The present study included the following two groups: GROUP-1: 15 diagnosed cases of benign SGTs which included 13 cases of PA and 2 cases of WT (GRAPH-I) GROUP-2: 15 diagnosed cases of malignant SGTs which included 8 cases of MEC, 3 cases of Ca ex PA, 3 cases of AdCC and 1 case of PLGA. (GRAPH-II) POSITIVE CONTROL- 5 cases of ductal carcinoma of the breast. All 30 cases of SGTs and 5 control cases were subjected to immunohistochemistry using PR antibody. No expression of PR was seen in the nucleus of cells in all cases of SGTs. The nuclear positivity for PR protein in the control group was Case 1 = 97% Case 2 = 67% Case 3 = 62% Case 4 = 64% Case 5 = 61%

Table 1: Total no. of SGTs included in the study.

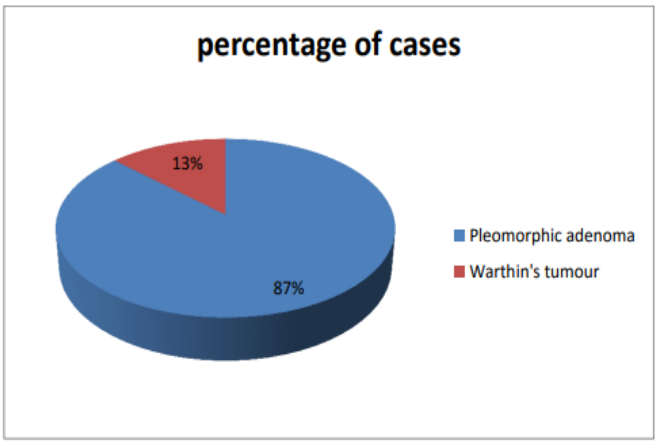
Total Number of Cases	No. of Positive Cases	No. of Negative Cases	No. of Cases with Faint Cytoplasmic Positivity
30	0	30	6

Table 2: Total no. of Benign and Malignant SGTs showing cytoplasmic positivity for PR protein.

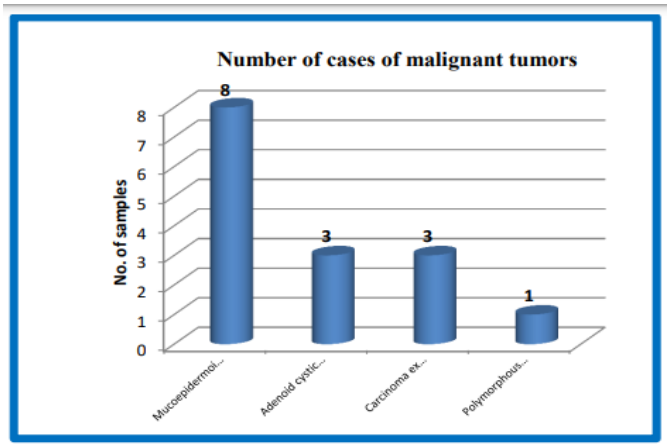
Sr No	Sgts Showing Cytoplasmic Positivity(Benign) (Graph-III)	SGTs Showing Cytoplasmic Positivity (Malignant) (Graph-Iv)
	PA	Ca ex PA
	PA	Ca ex PA
	PA	
	WT	



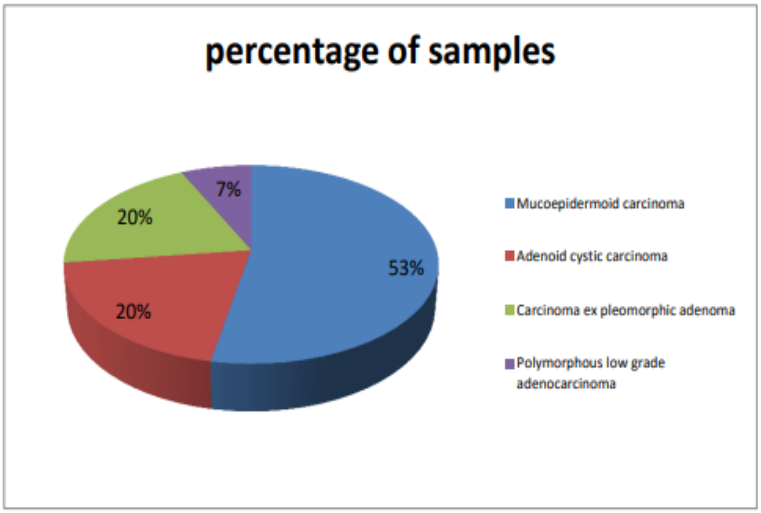
Graph- I- No. and distribution of benign SGTs included in the study.



GRAPH-II.



GRAPH-III- No. and distribution of malignant SGTs included in the study.



GRAPH-IV

Table-3: Benign SGTs which showed cytoplasmic positivity for PR protein.

Decision Criteria: The decision criteria compare the p-value with the level of significance. Though there were 4 benign tumours' and 2 malignant tumors showing positive PR expression in the cytoplasm, the p value was found to more than 0.05. Hence, Null Hypothesis was accepted and it was concluded that there is no significance difference in PR expression between the benign and malignant SGTs.

DISCUSSION

Salivary gland tumors constitute an important area in the field of oral and maxillofacial pathology. Although such tumors are uncommon, they are by no means rare. The annual incidence of salivary gland tumors around the world ranges from about 1.0 to 6.5cases per 100,000 people. The most common site for salivary gland tumors is the parotid gland, accounting for 64% to 80% of all cases. Fortunately, a relatively low percentage of parotid tumors are malignant, ranging from 15% to 32%. About 8% -11% of all salivary tumors occur in the submandibular gland, but the frequency of malignancy in this gland is almost double that of the parotid gland, ranging from 37 to 45%. Tumors of the sublingual gland are rare, comprising no more than 1% of all salivary neoplasms. However, 70% to 90% of sublingual tumors are malignant.Tumors of the minor salivary glands make up 9% to 23% of all tumors, which makesthis group the second most common site for salivary neoplasia. The palate is the most frequent site for minor salivary gland tumors, with 42% to 54% of all cases found there, followed by the lips and buccal mucosa. Morphologic mimicry and similarity in the expression of steroid hormone receptors between salivary gland tumors and breast tumors are well known phenomena and are occasionally debated in the field of surgical pathology. The expression of sex hormone receptors in some tumors suggests a role of these receptors in tumor pathogenesis and therapy. It is well known that hormonal therapy is very useful in the treatment of breast and prostate cancer. However, in spite of the confirmed expression of sex hormone receptors in other types of cancer such as endometrial carcinomas, carcinomas of the thyroid, renal cell carcinomas, malignant melanomas, and meningiomas, the efficacy of hormonal therapy in these tumors has not yet been established.

Various studies have been carried out by research workers in the field of immunohistochemistry to observe the expression of PR on benign and malignantsalivary gland tumors. The most significant expression was seen in cases of AdCC, followed by MEC, PA and WT. It was also concluded that expression of PgR couldbe a prognostic factor in recurrent PA of parotid gland.[5,6,7,8,9,10]

A total of 30 salivary gland tumor cases were taken up for the study which were divided into 2 groups-

Group I- 15 Benign Salivary Gland Tumors (Graph-I and Graph-II)

Group II- 15 Malignant Salivary Gland Tumors (Graph-III and Graph-IV)

Control Group-5 cases of ductal carcinoma of the breast.

IHC study was performed on formalin fixed and paraffin embedded tissue sections using PR antibody. Each case was assessed on the basis of nuclear expression of PR in ductal/ non-ductal cells.

- In control group, PR was constantly expressed in the nuclei of ductal cells. (PMG-1 and PMG-2)
- No expression of PR was observed in the nuclei of lesional cells in all the cases of Benign and Malignant SGTs. (Table-1; PMG-3 to PMG-12)
- 6 cases (4 benign and 2 malignant) showed cytoplasmic positivity in the lesional cells. (Graph-V and Graph-VI; Table-1,2,3 and 4; PMG-3 to PMG-7)

In our study, 13 cases of PA were included and none of the cases showed nuclear expression of PR. This was in accordance with the previous studies by Lamey PJ, Leake RE, Cowan SK, Soutar DS, McGregor IA, McGregor FM (1987)12, Nasser MS, Faquin WC, Yogeshwar P (2003)6, Ito FA, Ito K, Coletta RD, Vargas PA, Lopes MA (2009)16 and Safoura S, Mohamad E, Nosrat AE, Fereshte E (2011)20, which concluded that neither PR nor ER has any role in the tumorigenesis of PA.

In SGTs, ER, PR and AR expression has been described in several studies which are not in accordance with our study. They include-Afina S Glas, Harry Hollema, Raoul E Nap, John T Plukker (2001) 21 and Kolude B, Adisa A, Adeyemi B, Lawal A (2013)22 Their study pointed towards the fact that PR is a prognostic factor in occurrence of recurrent PA and a potential target for hormone treatment. Hhowever, due to the small number of cases taken and rarity of some histological variants of SGTs, a definitive pattern of PR expression could not be drawn.

We subjected 2 cases of WT for PR expression and found that both the cases showed negative nuclear staining. This is in accordance with the studies by-Nasser MS, Faquin WC, Yogeshwar P (2003)6 and Ito FA, Ito K, Coletta RD, Vargas PA, Lopes MA (2009)16 who

did not relate the role of PR in histogenesis of SGTs and hence, they were of the opinion that the clinical management of such tumors on the basis of hormonal therapy is doubtful.

Out of the two WT cases included in our study, one showed cytoplasmic positivity in the cells of oncocytic epithelium. In our literature review, we found that none of the studies have mentioned about the cytoplasmic positivity with respect to cases of WT subjected to PR expression.

In our study, the malignant SGTs included 8 cases of MEC and all were found to be negative for PR. This is in accordance with the studies conducted by Ito FA, Ito K, Coletta RD, Vargas PA, Lopes MA (2009)[16], Kolude B, Adisa A, Adeyemi B, Lawal A (2013)[22] and Omar TA, Eldidi F, Nawar WM (2013).[21] The authors concluded that their results do not support the role of either estrogen or progesterone in the tumorigenesis of MEC.

Our results for MEC were not in accordance with the study conducted by-Nasser MS, Faquin WC, Yogeshwar P (2003).[6] In their cases, they found weak nuclear staining in 1 case of MEC which was inconclusive and hence, the role of hormones in the causation of SGTs is questionable.

Our study group comprised of 3 cases of AdCC and we found that the nuclear staining was negative for PR. This is in accordance with the studies performed by-Nasser MS, Faquin WC, Yogeshwar P (2003)[6], Ito FA, Ito K, Coletta RD, Vargas PA, Lopes MA (2009)[16], Safoura S, Mohamad E, Nosrat AE, Fereshte E (2011)[20], Omar TA, Eldidi F, Nawar WM (2013)[21], They concluded that PR does not play a role in tumorigenesis and hormone therapy in AdCC. No association was found between PR and different grades of AdCC.[20]

Our results for AdCC were not in accordance with the studies performed by Ozono S, Onozuka M, Sato K, Ito Y (1992)[23], Shick PC, Riordan GP, Foss RD (1995)[24], Barrera JE, Shroyer KR, Said S, Hoernig G, Melrose R, Freedman PD et al (2007)[25] and Kolude B, Adisa A, Adeyemi B, Lawal A (2013).[22] These authors concluded that PR expression may be of possible prognostic and therapeutic value in some cases of AdCC. Also authors concluded that p53 aberrations may be involved in AdCC tumor progression and that ER and PR may play a role in AdCC development.[25]

Our study included 3 cases of CA ex PA. All cases were negative for nuclear PR staining. This is in accordance with the studies conducted by Tarakji B, Nassani MZ, Sloan P

(2010)19. They concluded that carcinoma arising in PA was not dependent on endocrine function.

Our results for Ca ex PA were not in accordance with the study conducted by Nasser MS, Faquin WC, Yogeshwar P (2003).[6] Their study did not relate the role of PR in the histogenesis of SGTs and hence, concluded that the clinical management of such tumors on the basis of hormonal therapy is doubtful.

In our study, 2 out of 3 cases of Ca ex PA showed faint cytoplasmic expression of PR. This is in accordance with the studies conducted by Tarakji B, Nassani MZ, Sloan P (2010).[19] The authors also observed progesterone positivity in the cytoplasm in 5 out of 27 cases. They concluded that cytoplasmic positivity was non-specific and that carcinoma arising in PA was not dependent on endocrine function.

We included 1 case of PLGA in our study which showed negative results with respect to PR protein expression. This is in accordance with the study conducted by Kolude B, Adisa A, Adeyemi B, Lawal A (2013).[22] They concluded that due to the small number of cases taken and rarity of some histological variants of SGTs, a definitive pattern of PR expression could not be assessed.

Many previous studies performed on the expression of ER and PR in SGTs have reported conflicting results. The difference in the results may be due to various factors which include gender, age, specific tumoretiology, IHC technique, fixation of tissue, antigen retrieval and/or type of antibody used and the pathological interpretation of the staining due to lack of strict evaluation criteria.[12,16,19] It is possible that tumor cells lose this marker as they become tumoral. Based on these findings, we suggest that PR does not play a role in prognosis, tumor progression or staging of the salivary gland tumors.[10-16]

In our study, the limitations include a smaller sample size, hence, a better PR expression and association between PR expression, hormonal therapy and prognosis of SGTs could not be confirmed. Also, we could not include all benign and malignant salivary gland tumors in our study which needs further window of exploration. The difference in the results in the present study may be due to various factors which include gender, age, specific tumor etiology, IHC technique, fixation of tissue, antigen retrieval and/or type of antibody used and the pathological interpretation of the staining due to lack of strict evaluation criteria.

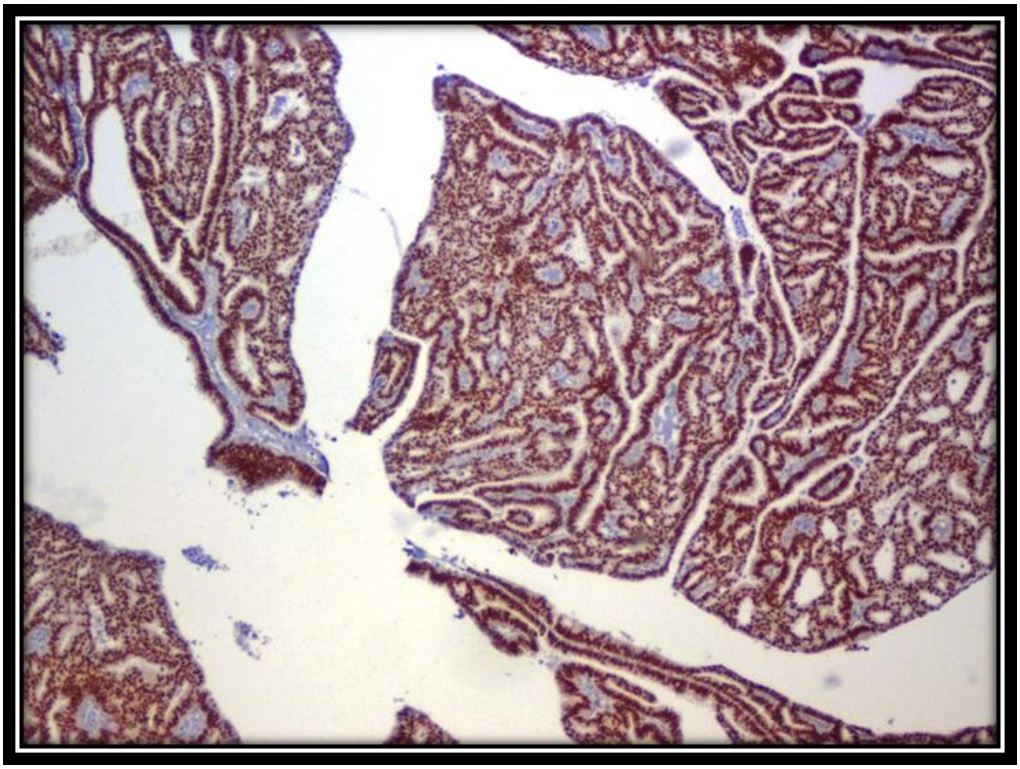
SUMMARY AND CONCLUSION

Present study was taken up to study the expression of progesterone receptors in benign and malignant salivary gland tumors and to compare the expression of progesterone receptors in benign and malignant salivary gland tumors using immunohistochemical technique.

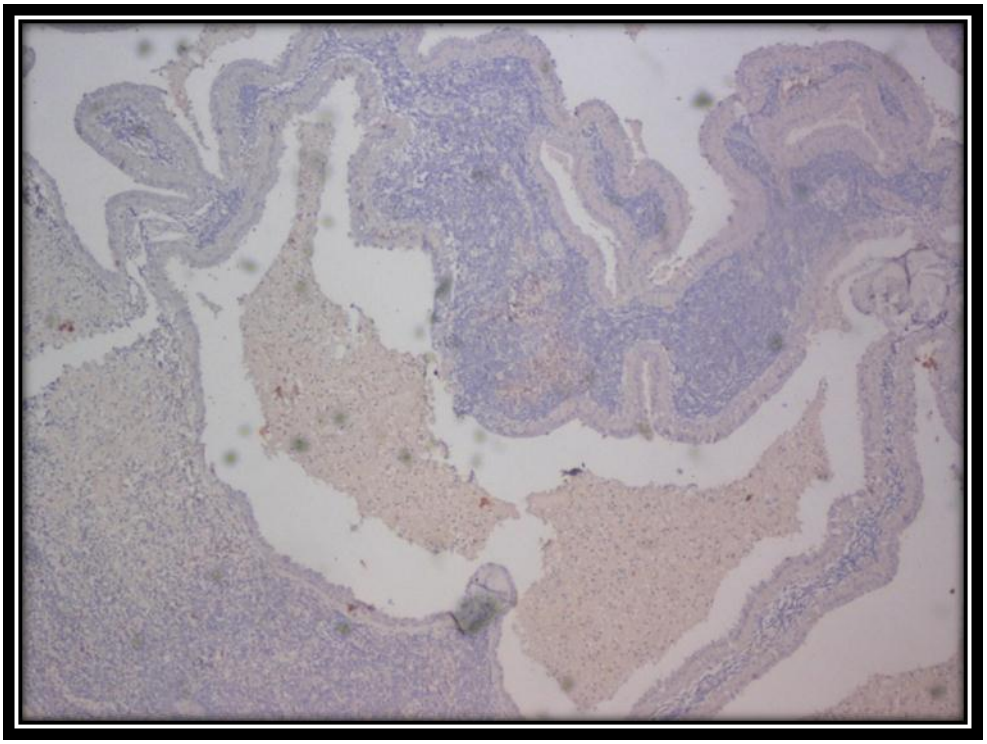
From the study, we concluded that-

1. Expression of PR was not observed in all the 30 cases of benign and malignant salivary gland tumors (Group-I and Group-II).
2. Secondly, faint cytoplasmic staining was observed in 4 benign and 2 malignant SGTs (Table-1,2,3 and 4; Graph-V and VI)

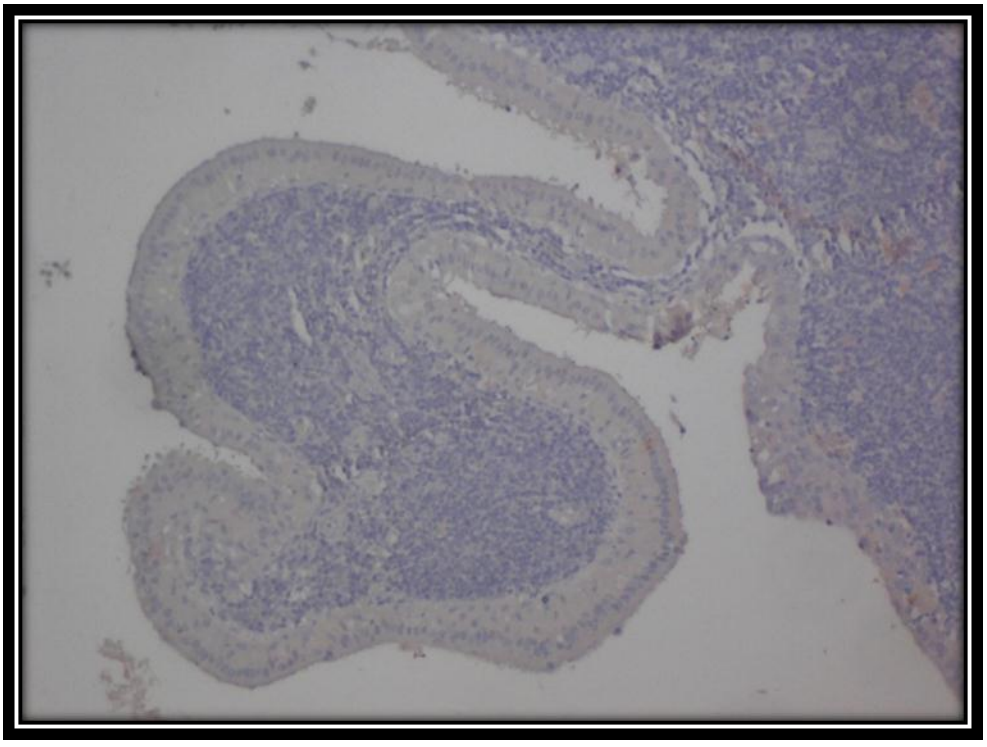
Hence, we conclude that the role of PR in tumorigenesis of PA, WT, MEC, AdCC, Ca ex PA and PLGA is doubtful and therefore, targeted hormonal therapy for such lesions demand further studies.



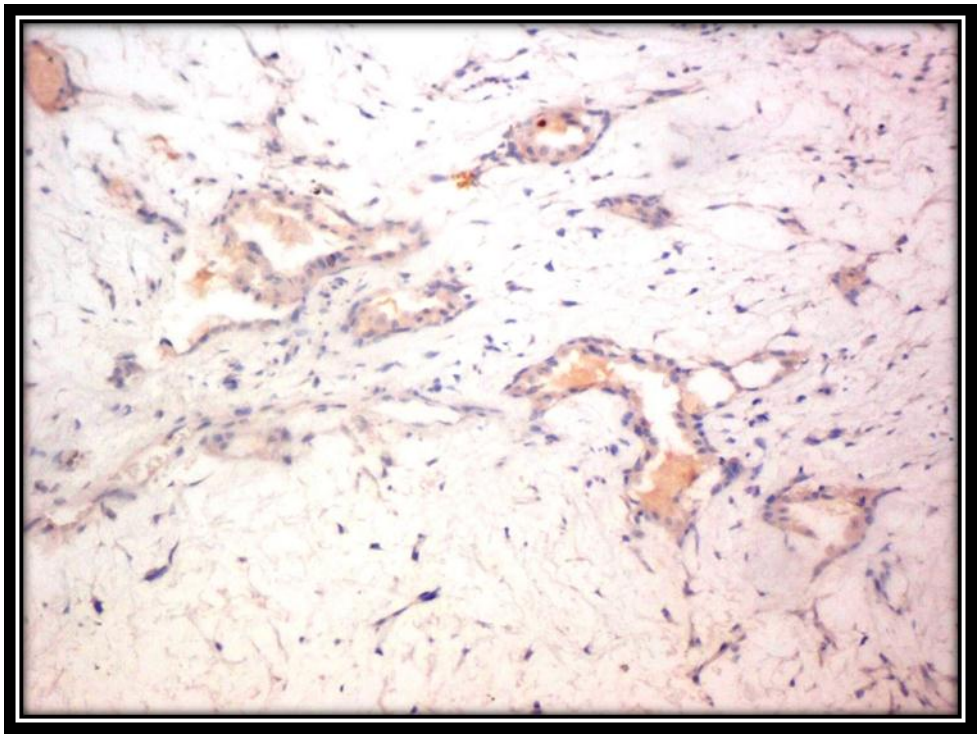
PMG-1: Nuclear positivity of PR protein in Ductal Carcinomaof the Breast (Control)(Magnification 20X)



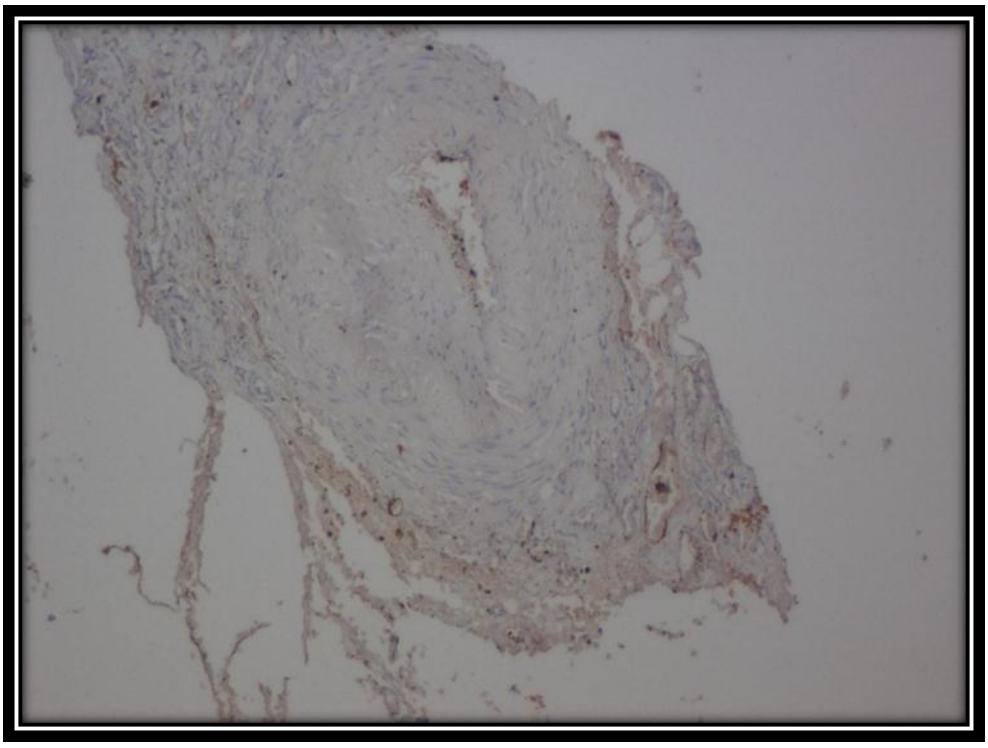
PMG: 2**-Cytoplasmic PR expression in cells of the oncocytic epithelium in WT (Group-I)(Magnification 20X).**



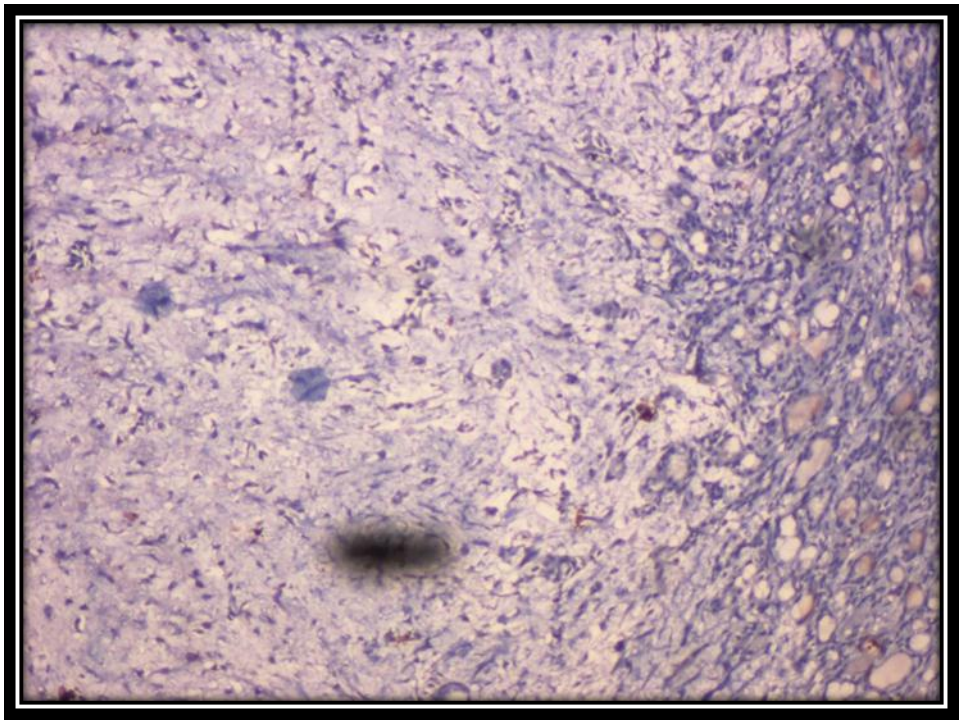
PMG-3: Cytoplasmic PR expression in cells of the oncocytic epithelium in WT (Group-I)(Magnification 40X).



PMG-4: Cytoplasmic PR expression in few ductal cells in Ca ex PA (Group-II)(Magnification 20X).



PMG-5: Cytoplasmic PR expression in fewmyoepithelial and stromal cells in PA (Group-I)(Magnification 20X).



PMG-6: Negative PRexpression in PA (Group-I)(Magnification 20X).

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