Expression of galectin-3 in adenoid cystic carcinoma & mucoepidermoid carcinoma of salivary glands- a study from Pakistan

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Abstract

Background: Adenoid cystic carcinoma (AdCC) and mucoepidermoid carcinoma (MEC) are the commonest salivary gland malignancies in Pakistan constituting almost 75% of all malignant salivary gland tumours.
Objective: The objective of this study was to determine the expression of galectin-3 (Gal-3) in AdCC and MEC of salivary glands and to see its relationship with histological differentiation in these tumours.
Method: This descriptive study was conducted at the Department of Morbid Anatomy and Histopathology/ Oral Pathology, University of Health Sciences Lahore, Pakistan. Biopsies and detailed clinical data of 20 cases each of adenoid cystic carcinoma and mucoepidermoid carcinoma reported at local tertiary care hospitals from Jan. 2014 to Sep. 2015 were retrieved. The histologic diagnosis was made on Hematoxylin and Eosin staining. The tumours were graded into grades I, II & III according to the most recent grading criteria. AdCC was studied with respect to its morphological patterns (tubular, cribriform and solid) while MEC was studied with special concern to the cell types seen in it (mucous, intermediate, squamous and clear cells). Immunohistochemical expression of galectin-3 was determined in these tumours with respect to histological grades, patterns and cell types seen.
Results: Moderate positivity (55%) for anti-galectin-3 antibody was the most frequently observed score for galectin-3 in both MEC and AdCC. Moderate positive (55%) staining reaction was followed by weak positive (30%) staining reaction in AdCC. Total score for antigalectin-3 antibody, positive stromal reaction (intensity) and location of cellular signals for anti-galectin-3 antibody were all found to be significantly associated with grades of AdCC. Also, histological pattern of AdCC (tubular, cribriform and solid) were significantly associated with type of anti-galectin-3 staining pattern of cells (p<0.001). In MEC, moderate positive (55%) staining for anti-galectin-3 antibody was followed by strong positive reaction (30%). The total score for anti-galectin-3 antibody was significantly associated with grades of the tumour and lymph node status. Also, the type of staining reaction in cells was significantly associated with cell type (mucous, squamous and intermediate cells).
Conclusion: It can be concluded from the current study that expression of Gal-3 decreases with decreasing differentiation in parenchyma of malignant tumours while its expression in tumour extracellular environment increases with increasing grade of the tumour. Also, it can be concluded that nuclear expression of Gal-3 is associated tumour differentiation and cytoplasmic expression with tumour cell proliferation.

Keywords:*Adenoid Cystic Carcinoma; Galectin-3; Mucoepidermoid Carcinoma*

1. Introduction

Adenoid cystic carcinoma (AdCC) and mucoepidermoid carcinoma (MEC) are the commonest salivary gland neoplasms. Regarding the prevalence of these tumours, some studies have reported AdCC to be commonest while others have named MEC to be the commonest salivary gland malignancy (Vuhahula 2004 p. 15-23, Long-jiang et al. 2008 p. 187-92, Ettl et al. 2012 p. 267-83, Kızıl et al. 2013 p. 112-20).
Adenoid cystic carcinoma is a slow growing but aggressive tumour of the salivary glands with a prolonged clinical course and late distant metastasis (Gondivkar 2011 p. 231-36). Twenty-five percent AdCC arise in the parotid gland and 60% from the minor salivary glands(Bradley 2004 p.127-132) with palate as its most frequent site (Kolay et al. 2014 p. 195-98). Adenoid cystic carcinoma occurs across a wide age range of 3rd to 9th decades of life, with a peak incidence in 4th to 7th decades of life (Wang et al. 2012 p. 879-886). This tumour exhibits a varied clinical course with one group showing persistent fulminating course, early metastasis and death in 3 years and the other group has been described as "patient and the tumour existing in symbiosis" resulting

in wide infiltration of structures by the tumour (Singla et al. 2015 p. 182-86).

Mucoepidermoid carcinoma is seen in both children and adults and has been named as commonest malignancy of salivary glands in children (Ozawa et al. 2008 p. 414-18). It occurs in major and minor salivary glands with a frequency of 53% and 47% respectively. Parotid gland and palate are most common sites for the major and minor salivary glands respectively (Eveson et al. 2005 p. 219-20). Worldwide reported incidence of MEC is 0.44 per 100,000 (Adesina et al. 2012 p.210-13). The age range for MEC is third to fifth decade with mean age of 45 years (Luna 2006 p. 293-307) and a female predilection with male to female ratio of 2:3 (Eveson et al. 2005 p.219-20). Clinically, it appears mostly as an asymptomatic swelling (Luna 2006 p. 293-307). However, the clinical features vary according to the grade of the tumour as most low and intermediate grade MECs are slow growing and painless masses, while high grade MECs are fast growing, painful fixed to the underlying skin or tissue, show facial nerve paralysis, ulceration and trismus (Ranganath et al. 2011 p. 66-69).

Galectin-3 is a beta-galactoside-binding, multifunctional chimera protein member of the galectin family. It is composed of an N-terminal domain (ND), a repetitive collagen-like sequence rich in glycine, proline and tyrosine, and a C-terminal domain (CD) (Costa et al. 2014 p. 1-9). Gal-3 is expressed on variety of cells including epithelial cells, myeloid cells, ductal cells fibroblasts, chondroblasts, osteoblasts etc. (Dumic et al. 2006 p. 616-35). The cytoplasm, nucleus, cell surface and extracellular environment are important sites of expression (Costa et al. 2014 p.1-9).

Gal-3 has several important roles in cancer development including progression, adhesion, invasion, angiogenesis, immunosuppression and metastasis (Costa et al. 2014 p. 1-9, Newlaczyl &Yu 2011 p. 123-28). Nuclear Gal-3 expression shows anti-tumour effect whereas cytoplasmic Gal-3 expression shows tumour promotion (Califice et al. 2014 p. 7527-36).

The molecular mechanism (Fig.1) underlying the process of Gal-3 mediated neoplastic transformation is partly ascribed to interaction of Gal-3 and activated oncogenic K-Ras (Ras-GTP) which results in strong activation of Raf-1 and PI3-K. Also, it inhibits the hydrolysis of activated K-Ras (Elad-Sfadia et al. 2014 p. 34922-30). Other mechanisms include regulation of cell cycle by its binding to β-catenin and interaction with transcription factors like cyclin-D and c-Myc ultimately resulting in cell proliferation and tumour growth (Yang et al. 2008 p. 1-24).
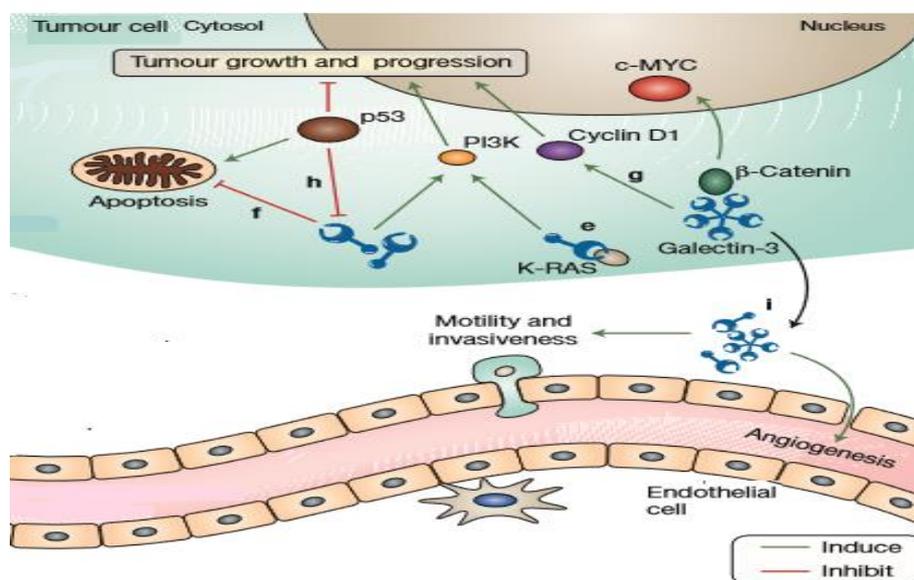
Fig. 1: Molecular Mechanism of Gal-3 Mediated Neoplastic Transformation (Yang Et Al. 2008 P.17).

Up-regulation of Gal-3 is associated with pancreatic cancer, hepatocellular carcinoma, diffuse large B-cell lymphoma, nasopharyngeal carcinoma patients, colorectal cancer, clear cell renal carcinomaand melanoma and acts as a tumour suppressor in breast and endometrial cancers (Song et al. 2014 p. 185-91).

Normal salivary glands have shown cytoplasmic positivity in secretory duct cells. Galectin-3 positivity is shown to be related with metastasis in AdCC (Teymoortash et al. 2014 p. 51-56).

Keeping in view the variety of roles played by Gal-3 in neoplastic transformation, tumour progression, metastasis, apoptosis and angiogenesis in various tumours, variations in reports that studied its role in salivary gland cancers and paucity of reports in literature that studied relationship of Gal-3 in salivary gland tumours in Pakistan, this study was designed to determine the immunohistochemical expression Gal-3 in adenoid cystic carcinoma& mucoepidermoid carcinoma of salivary glands and to determine its association with histological grades and subtypes in these tumours.

2. Subjects and methods

This study was conducted at the Department of Morbid Anatomy and Histopathology/Oral Pathology, University of Health Sciences, Lahore. A total of 40 biopsies (20 each) of AdCC & MEC of salivary glands reported at Histopathology Departments of University of Health Sciences, King Edward Medical College/Mayo hospital, Sheikh Zaid hospital andFatima Jinnah Medical College /Ganga Ram Hospital, Lahore from January, 2015 to September, 2015 were included in the study. Detailed clinical data was retrieved from the respective departmental records.

2.1. Hematoxylin &eosin staining

Paraffin embedded tissue sections were made from biopsy specimens. Tissue sections of 4μm were cut using rotary microtome and were stained with hematoxylin and eosin stain. Diagnosis of adenoid cystic carcinoma & mucoepidermoid carcinoma was confirmed and their histological grades were determined according to the grading criteria provided by Spiro & Auclair respectively (Spiro et al. 1974 p.512-20 & Auclair et al. 1992 p. 2021-30) (Table 1 & 2 respectively).

Table 1: Histological Grading of Adenoid Cystic Carcinoma (SpiroEt Al. 1974 P. 512-20)

Grade	Grading parameter
I	Mostly tubular or cribriform (no stipulations on minor solid components)
II	50% solid
III	Mostly solid

Table 2: Histological Grading of Mucoepidermoid Carcinoma (Auclair Et Al. 1992 P. 2021-30)

Histopathological feature	Point value
Cystic component < 20%	2
Neural invasion	2
Necrosis	3
4 or more mitosis/10 hpf	3
Anaplasia	4
Tumour grade	Point score
Low	0-4
Intermediate	5-6
High	7-14

2.2. Immunohistochemistry

About 4 μm thick tissue sections were cut with the help of rotary microtome and taken on Poly-L-lysine coated slides for immuno-histochemical staining with anti-Gal-3 antibody.

Two sections were taken from each block, dried at 60ºC for 50 minutes followed by dewaxing in xylene and rehydration in alcohol. Next, the slides were placed Coplin jars containing citrate buffer (pH 6.0) solution and then in hot water bath (95^{0}C) for 40 minutes in order to retrieve antigens (Heat Induced Epitope Retrieval). After removing the slides from hot water bath, they were allowed to cool at room temperature and hydrogen peroxide was added to block endogenous peroxidase activity followed by thorough washing with PBS (phosphate buffered saline).Sections were then incubated with 1-2 drops of protein blocker for 10 minutes to block endogenous enzymatic activity and then again washed with PBS. This was followed by incubation with primary antibody (anti-Gal-3 antibody, catalog no. PB9081 (Boster Biological Technology Co., Ltd.) diluted to concentration of 1/20 μg/ml (suggested dilution by the manufacturer) for 1 hour. Then, sections were incubated successively with Biotinylated Secondary Antibody for 10 minutes and Streptavidin Peroxidase Reagent for 10 minutes before application of DAB (di-amino-benzidine) (2 minutes) to avoid false positive staining. All incubation steps were separated by thorough washing with PBS. Counter staining with hematoxylin was done followed by dehydration and mounting of sections with coverslips using DPX.

Human colon cancer tissue was used as positive control for Gal-3 while omitting the primary antibody step in peroxidase-labelled streptavidin-biotin technique provided negative control for Gal-3.

Gal-3 expression was evaluated on basis of extent and intensity of immunolabeling in tumour cell cytoplasm/nucleus. Quantification for anti-galectin-3 antibody staining was done using the criteria utilized by Remmelink (Remmelink et al. 2011 p. 543-56).

The **intensity** of staining was evaluated in a score ranging from 0-3(IS) (qualitative variable):

0: negative,1: weak,2: moderate,3: strong.

The **proportion** of positive tumour cells was evaluated in a score ranging from 0-4 (PS) (quantitative   variable).

0: 0% +ve cell s, 1: 1-25% +ve cells, 2: >25-<75% +ve cells, 3: >75%-100% +ve cells

A **final score**, labelled as a total score (TS), was given by multiplying the IS and the PS.

0 = Negative, 1-3 = weak positive (+1), 4-6 = moderate positive (+2), 7-9 = strong positive (+3).

The histological and immunohistochemical data was analyzed statistically using SPSS 20.0. Chi-square and Fischer Exact tests were applied and p-value <0.05 was considered to be statistically significant.

3. Results

The mean age of patients with AdCC was 41.50±12.224 years, an age range of 22-70 years and a female predilection of 1.5:1. Minor salivary glands were the commonest site involved (80%) of which palate was most frequently affected (37.5%) followed by maxilla (25%). For MEC mean age of patients was 32.35±13.674 years, an age range of 9-60years and male predilection (1:1.5). Parotid

gland (70%) was the commonest site involved followed by maxilla (10%).

The histological grades noted in adenoid cystic carcinoma and mucoepidermoid carcinoma are summarized in Table 3.

Table 3: Histopathological Grades of Mucoepidermoid Carcinoma and Adenoid Cystic Carcinoma

Tumour morphology	Grade I		Grade II		Grade III	
	F	%	F	%	F	%
Adenoid cystic carcinoma	16	80	01	05	03	15
Mucoepidermoid carcinoma	07	35	05	25	08	40

3.1. Adenoid cystic carcinoma

The tumour cells in adenoid cystic carcinoma were basaloid with small hyperchromatic nuclei and scant eosinophilic cytoplasm. These cells were arranged in tubular, cribriform and solid patterns, varying in proportions in one neoplasm (Table 3).

The total score (TS) for anti-Gal-3 antibody staining in adenoid cystic carcinoma is summarized in Table 4.

Table 4: Total Score (TS) and Staining Pattern of Antigal-3 Antibody in Different Histological Grades of Adenoid Cystic Carcinoma

Anti-Gal-3 positive score	I		II		III		Total		p-value
	F	%	F	%	F	%	F	%	
Total Score (TS)									
Weak positive(1-3= +1)	03	15	0	0	03	15	06	30	
Moderate positive (4-6= +2)	10	50	01	05	0	0	11	55	0.03
Strong positive(7-9= +#)	03	15	0	0	0	0	03	15	
Stroma (intensity)									
Negative0	03	15	0	0	01	05	04	20	
Weak positive+1	08	40	0	0	0	0	8	40	
Moderate Positive +2	05	25	01	05	0	0	06	30	0.007
Strong positive+3	0	0	0	0	02	10	02	10	
Anti-Gal-3 antibody staining pattern									
Cytoplasmic	13	65	01	05	0	0	14	70	
Nucleocytoplasmic	02	10	0	0	0	0	02	10	0.012
Weak cytoplasmic	01	05	0	0	03	20	04	20	

Moderate positivity (n=11 i.e. 55%) was the commonest staining reaction seen with anti-Gal-3 antibody in cases of adenoid cystic carcinoma (Table 4).

The staining pattern of cells also varied among the grades of tumour with grade I tumours (Fig. 2-3) showing predominantly cytoplasmic and nucleocytoplasmic staining and grade III tumour cells showing weak cytoplasmic staining (p=0.012) (Fig. 5). In the grade II AdCC, the staining pattern was strong in tubular areas and weak in solid areas (Fig. 4).

(A)
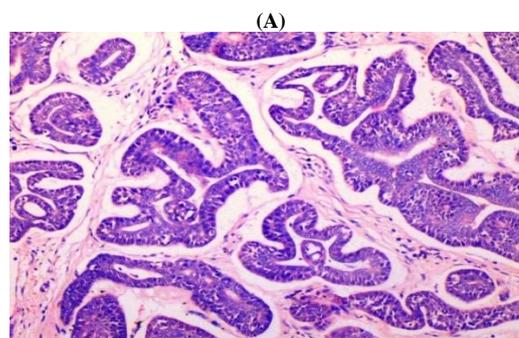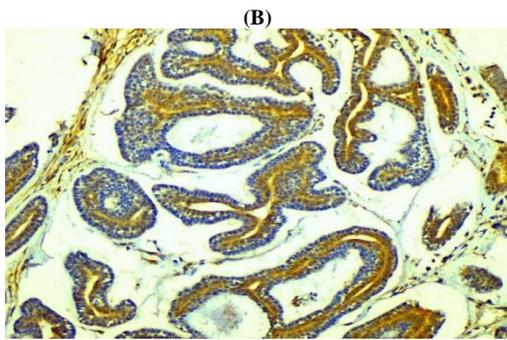
Fig. 2: Photomicrographs (A) Showing Tubular Pattern (Grade I) of Adenoid Cystic Carcinoma (H&E; 10X) & (B) Showing Strong Nucleocytoplasmic Positivity (Galectin-3; 10X).

Fig. 3: Photomicrograph A-C Showing (A) Cribriform Pattern (Grade I) of Adcc (H&E; 4x), (B) Showing Strong Nucleocytoplasmic Positivity (Galectin-3; 4x) & (C) Showing Moderate Cytoplasmic Positivity (Galectin-3; 20x).

The tubular pattern showed cytoplasmic and nucleocytoplasmic staining (Fig. 2), solid pattern showed weak cytoplasmic positivity only (Fig. 5) but cribriform pattern showed all the three staining reactions in cells (Fig. 3).

Fig.4: Photomicrograph A-B Showing (A) Grade II Adenoid Cystic Carcinoma with Both Solid (S) and Tubular (T) Components (H&E; 10X) & (B) Showing Weak Positive Staining In Solid (S) and Moderate To Strong in Tubular (T) Components (Galectin-3; 10X).
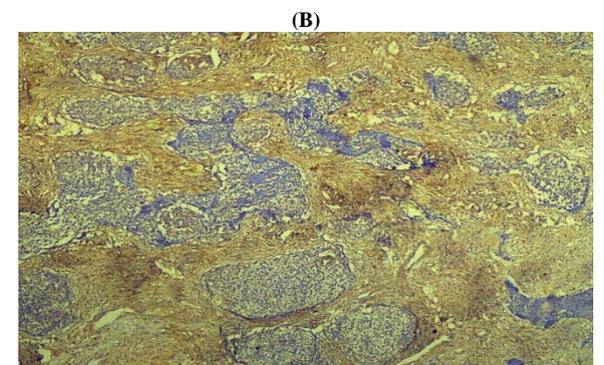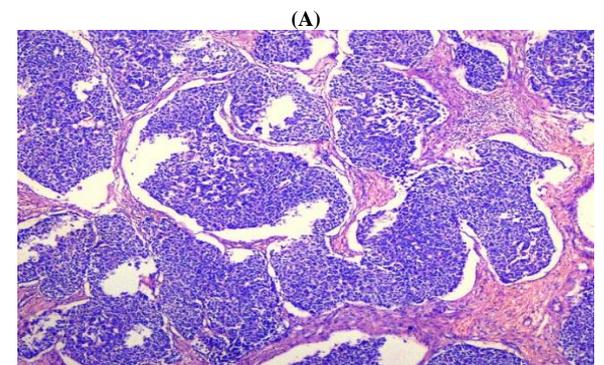
Fig. 5: Photomicrographs A &B Showing (A) H &E Stained Sections of Solid Variant (Grade III) of Adenoid Cystic Carcinoma (H&E; 10x) & (B) Weak Cytoplasmic Staining (Galectin-3; 4X). Note the Contrast between Tumour Parenchyma (Arrows) and Tumour Stroma (Arrowheads).

In the stroma, weak positivity was found to be most common pattern in grade I tumours while strong positivity was seen only in

cases of grade III AdCC (Fig. 5 B). Thus, stromal positivity was associated significantly with grade of tumour (p= 0.007).

Histological variants of AdCC and differential staining pattern of tumour cells for Gal-3 were significantly associated (p value <0.001) (Fig. 6).
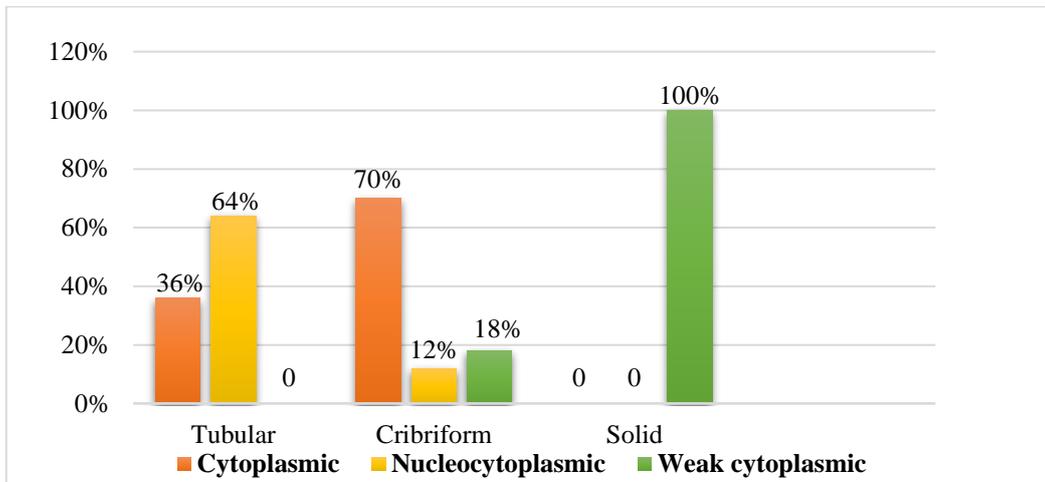
Fig. 6: Staining Pattern of Anti-Gal-3 Antibody in Different Histological Subtypes of Adcc (P<0.001).

3.2. Mucoepidermoid carcinoma

Diagnosis of mucoepidermoid carcinoma rests on identification of three cell types' i.e. mucous cells, intermediate cells and squamous cells within a variety of morphological patterns (Fig. 7).
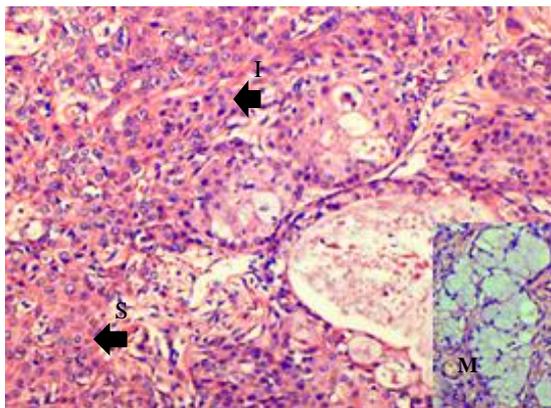
Fig. 7: Photomicrographs Showing Intermediate (I), Squamous(S) and Mucous (M) Cells (Inset) (H&E; 20X).

High grade MEC (8; 40%) was the predominant grade in the current study closely followed by grade I (7; 35%) and II (5; 25%). Intra-cystic component (<20%), 4 or more mitosis/10 high power field and anaplasia were significantly associated with grade of MEC having p-value= 0.005, 0.007 and <0.001 respectively, Regarding metastasis, 9(45%) cases of MEC showed nodal metastasis. Also, cartilage invasion (grade I) and metastasis of bone by tumour cells (grade III) was noted in 1(5%) case each.

Table 6 summarizes the anti-Gal-3 antibody staining scored in the three grades of MEC and the staining pattern in cells.

Table 6: Total Score (TS) and Staining Pattern of Anti-Gal-3 Antibodyin Different Histological Grades of Mucoepidermoid Carcinoma

Anti-Gal-3 positive score	I F	%	II F	%	III F	%	Total F	%	p-value
Total Score (TS)									
Weak positive(1-3= +1)	0	0	01	05	02	10	03	15	
Moderate positive(4-6= +2)	02	10	04	20	05	25	11	55	0.049
Strong positive(7-9= +#)	05	25	0	0	01	05	06	30	
Stroma									
Weak positive	04	20	01	05	05	25	10	50	
Moderate Positive	03	15	01	05	02	10	06	30	0.113
Strong positive	0	0	03	15	01	05	04	20	
Anti-Gal-3 antibody staining pattern									
Cytoplasmic	07	35	04	20	05	25	16	80	
Nucleocytoplasmic	0	0	01	05	03	15	04	20	0.194

As shown in table 6, moderate positivity (n=11 i.e. 55%) was the commonest staining reaction seen with Gal-3 antibody in cases of mucoepidermoid carcinoma.

The total score (TS) for Gal-3 antibody immunostaining was significantly associated with the grade of MEC (p= 0.049). Ten (50%) cases showed weak stromal positivity, 06 (30%) showed moderate positivity and 04(20%) showed strong positivity. The staining reaction of anti-galectin-3 antibody in various grades of MEC are shown in Figures 8-10.

(A)

(B)

(C)
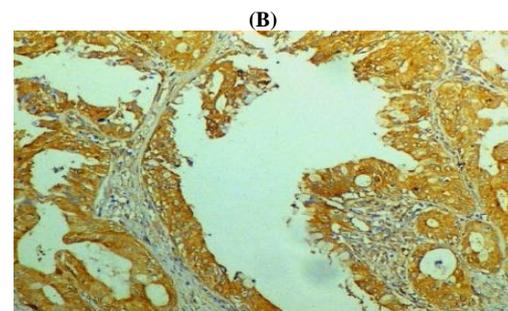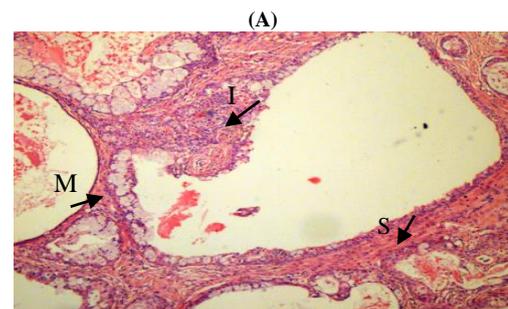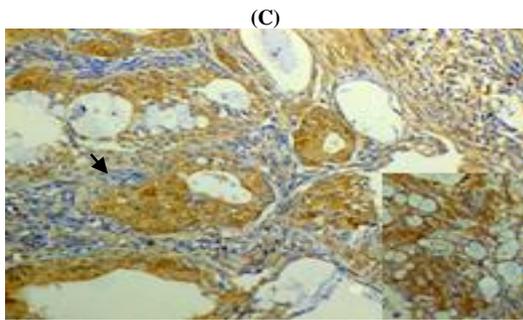
Fig. 8: Photomicrographs A-C Showing (A) Cystic Spaces (Arrow) Lined By Mucous (M), Intermediate (I) & Squamous (S) Cells (H&E; 4x),(B) Strong Cytoplasmic Positivity (Galectin; 10x) & (C) Shows Strong Cytoplasmic Positivity in Mucous Cells (Inset), Nucleocytoplasmic in Squamous Cells (Arrow) &Weak Cytoplasmic in Intermediate Cells (Arrowhead) (Galectin-3; 20X).

(A)

(B)

(C)
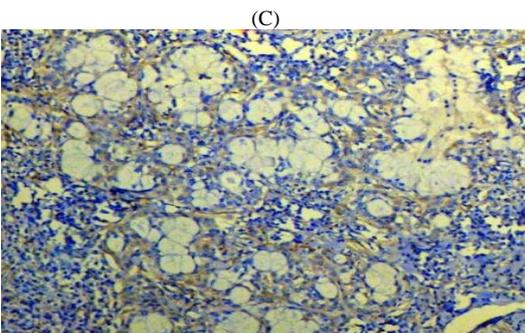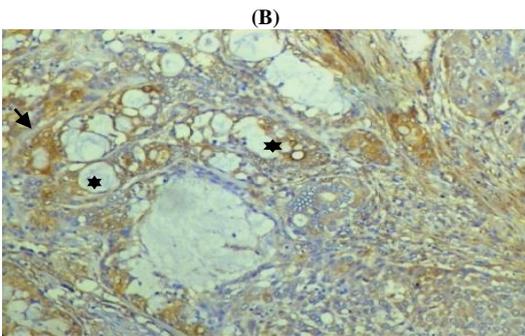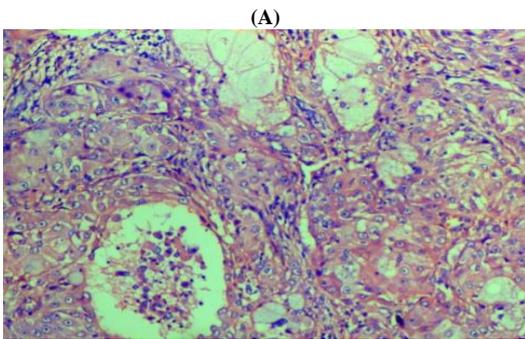
Fig. 9: Photomicrographs A-C Showing (A) H&E Stained Sections of Grade II MEC (10X), (B) Moderate Positivity and Some Squamous (Arrow) and Mucous Cells ( ) Showing Strong Cytoplasmic Positivity (Galectin-3; 10X) & (C) Weak Positivity (Galectin-3; 10X).

(A)

(B)
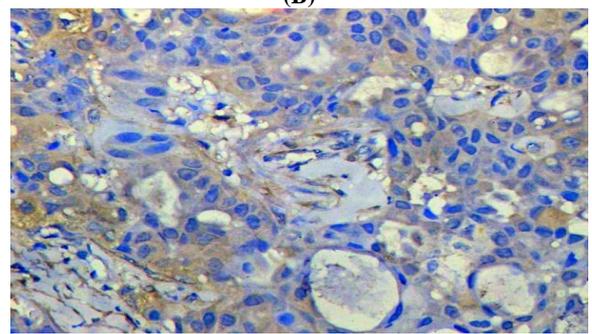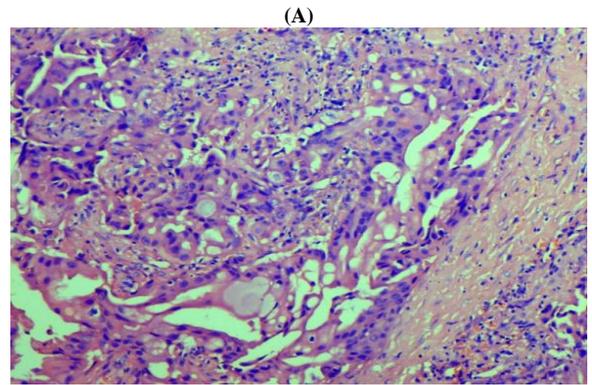
Fig. 10: Photomicrographs A &BShowing (A) H &E Stained Sections of Grade III MEC (10X) & (B) Weak Positive Staining (Galectin-3; 20X).

Moderate to strong staining reaction was noted for anti-galectin-3 score in metastatic sites (Fig. 11).

(A)

(B)

(C)
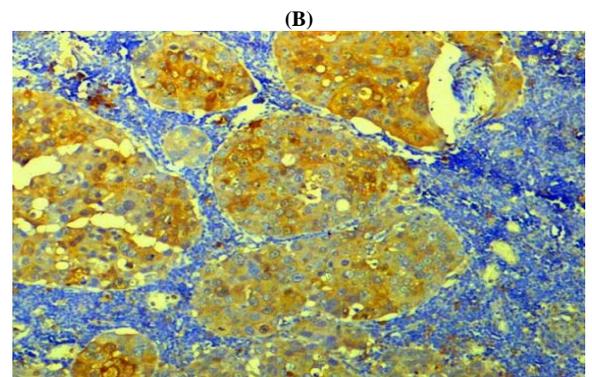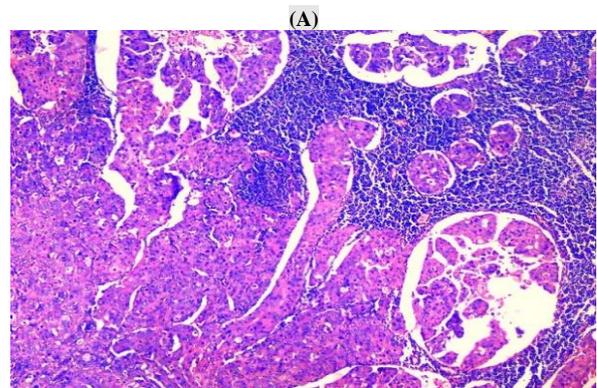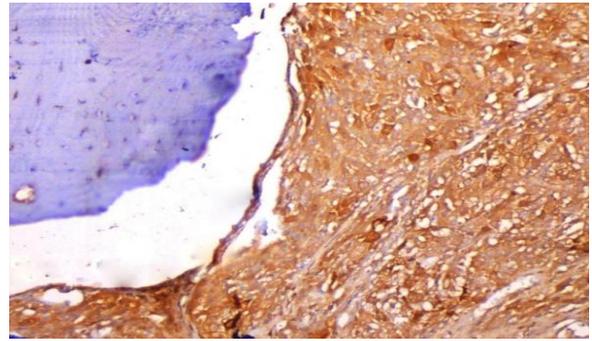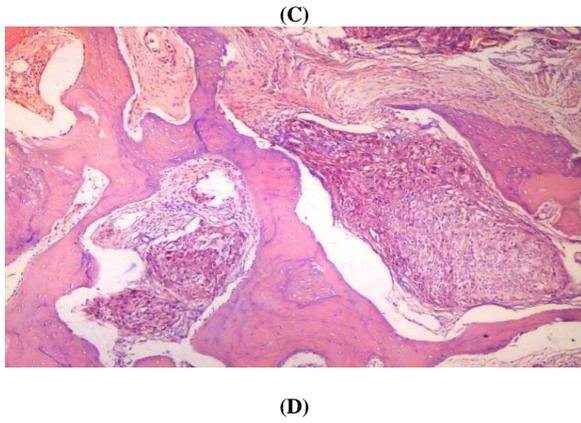
Figure 11: Photomicrographs A-D Showing (A) Metastatic Deposits in Lymph Node (H&E; 10X), (B) Moderate Staining in the Metastaic Deposits (Galectin-3; 10X.), C Showing Metastatic Deposits in the Bone (H&E; 10X) and (D) Strong Positive Reaction in Metastatic Deposit (Galectin-3; 20X).

(D)

The TS of Gal-3 and positive lymph node status were associated significantly with each other p =0.006 (Fig.12).
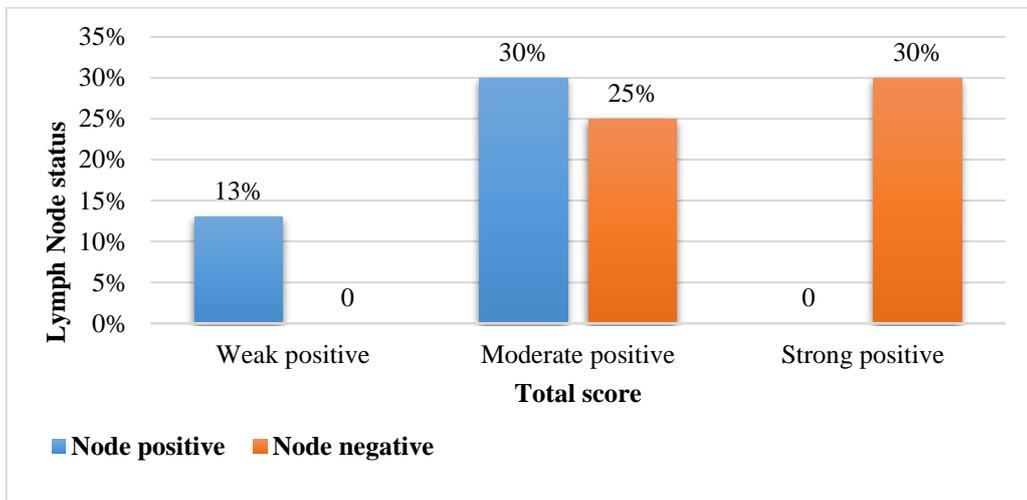
Fig. 12: Relation between Anti-Gal-3 Antibody Total Score (TS) and Lymph Node Status (P=0.006)

Regarding staining pattern of cells, mucous cells showed strong cytoplasmic positivity, intermediate cells showed weak cytoplasmic positivity and squamous cells showed both cytoplasmic and nuclear plus cytoplasmic signals i.e. nucleocytoplasmic (Fig. 13).
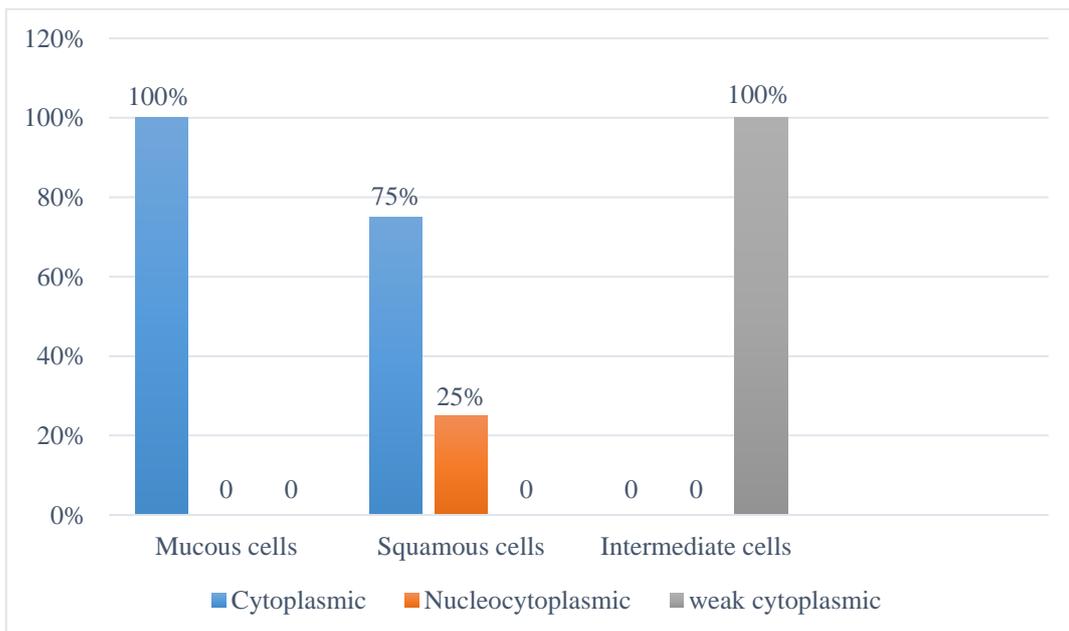
Fig. 13: Staining Pattern of Anti-Gal-3 Antibody in Variety of Cells inMEC (P<0.001).

Cells in MEC and predominant staining pattern in them was related significantly with a p-value <0.001 (Fig. 13).

4. Discussion

Gal-3 is a unique molecule, which has been extensively studied since it discovery in various pathologies. It regulates several steps in cancer development which include proliferation, progression, adhesion, invasion, angiogenesis, immunosuppression, metastasis and apoptosis depending upon its subcellular location (Newlaczyl & Yu 2011 p.123-28). Its upregulation in some cancers and downregulation in others is associated with cancer development and poor prognosis (Song et al. 2014 p. 185-91). Studies have reported that it is associated with tumour differentiation resulting in decreased expression with decreasing differentiation in colorectal carcinomas (Arfaoui-Toumi et al. 2010 p. 1-8) and stronger expression is noted in colon and gastric cancers that metastasized to other sites (Okada et al. 2006 p. 1369-76, Wu & Gan 2007 p. 1731-33, Huang & Liu 2008 p.1358-61).

In cancers of salivary glands, Gal-3 has been studied in adenoid cystic carcinoma and polymorphous low grade adenocarcinoma (Teymoortash et al. 2006 p. 51-56, Ferrazzo et al. 2007 p. 580-85, El-Nagdy et al. 2013 p. 131-39). In the current study, 40 cases were evaluated for expression of Gal-3, 20 cases each of AdCC and MEC. All the cases were positive for anti-Gal-3 antibody. Expression was mainly cytoplasmic but nucleocytoplasmic expression was also noted (not exclusively). No single case of exclusively nuclear staining was noted. However, the expression of galectin-3 was not significantly associated with the type of salivary gland tumour (p=0.337) in the current study.

In the present study, the expression of Gal-3 in peri-tumoral normal salivary glands, adjacent to the tumour tissue, was restricted to the ductal compartment. It was strongly expressed in cytoplasm of cells. Acini were negative for anti-Gal-3 antibody.These results are in harmony with the study of Teymoortash who reported strong cytoplasmic positivity for normal salivary gland ducts and weaker acinar expression (Teymoortash et al. 2006 p. 51-56). In contrast, Ferrazzo reported nuclear expression in cells of ducts (Ferrazzo et al. 2007 p.580-85).

In the current study, IHC score, intensity of stromal staining and pattern of staining were all significantly related with grades of AdCC (p= 0.03, 0.007 & 0.012 respectively). Most cases showed cytoplasmic staining in tumour cells of which 14(70%) cases showed moderate to strong cytoplasmic reaction while 4(20%) cases showed weak cytoplasmic reaction. Only 2(10%) cases showed nucleocytoplasmic staining. These results are in concordance with some studies (Teymoortash et al. 2006 p. 51-56,El-Nagdy et al. 2013 p. 131-39) while different from others (Ferrazzoet al. 2007 p.580-85) who reported nuclear expression to be predominant pattern of staining in AdCC (p<0.001). Most of grade I and all grade II AdCC showed moderate positive reaction for Gal-3 in contrast to grade III tumours, all of which showed weak positivity (Fig. 2-5). These results are in accordance with Xu who reported higher expression of Gal-3 in low grade tumours and weaker in high grade tumours (Xu et al. 2000 p. 271-76). In contrast, others have reported stronger staining reaction in solid subtype than in cribriform or tubular (Teymoortash et al. 2006 p. 51-56, El-Nagdy et al. 2013 p. 131-39).

The pattern of staining was also of significance when related to patterns of AdCC (Fig. 2-5). Nucleocytoplasmic staining was seen in 64% of tubular pattern (Fig. 2) while 70% of cribriform pattern showed moderate to strong cytoplasmic staining (Fig. 3) and 100% of solid pattern showed weak cytoplasmic staining in tumour parenchyma (Fig. 5).Tubular pattern is most differentiated while solid is least differentiated thus the staining pattern of cells for anti-Gal-3 in these patterns & this is in accordance with the conclusion made by Ferrazzothat nuclear expression is associated with tumour differentiation rather than progression in AdCC (Ferrazzo et al. 2007 p. 580-85).

In contrast to the recent study, El-Nagdy concluded that higher expression of Gal-3 has potential role in imparting aggressiveness to the solid ACC (El-Nagdy et al. 2013 p.131-39). In the recent study, staining pattern of cells in solid AdCC was weaker but the stroma was strongly positive (Fig. 5) presenting a sharp contrast to the lightly stained tumor cell islands of Gal-3 positive tumor cells. Extracellular expression of Gal-3 is reported to be associated with tumour invasion, metastasis and angiogenesis (Costa et al. 2014 p. 1-9).

Mucoepidermoid carcinoma revealed moderate to strong Gal-3 positivity mainly in the cytoplasm of squamous and mucous cells (Fig. 8C). Squamous cell also showed variable nucleocytoplasmic staining along with cytoplasmic staining. This is in accordance to the study conducted by Piantelli (Piantelli et al. 2002 P. 3850-56).They also showed that staining of squamous cells was more intense where the cells were associated with keratin pearl i.e. where they were more differentiated and decreased with decreasing differentiation. Mucous cells exhibited moderate to strong cytoplasmic positivity for Gal-3 while intermediate cells showed weak positivity in all cases (Figure 8C) (p<0.001). This in contrast to the findings reported by Remmelink who reported only intermediate cells to be positive for Gal-3 (Remmelink et al. 2011 p. 543-56).. In the current study, increasing grade of MEC was associated with decreasing Gal-3 expression (p=0.049). In one case of MEC, bony invasion by the tumour was encountered, it showed diffuse strong positivity in the metastatic deposits. Similarly, most tumours that metastasized to lymph nodes and bone showed strong Gal-3 positivity in metastatic site than in primary tumour site (Fig. 11). This higher positivity for anti-Gal-3 antibody in metastatic tumour sites and in stroma of intermediate and high grade MEC is in accordance with the role that Gal-3 mediates steps of tumour invasion and metastasis (Nangia-Makker et al. 2010 p. 2530-541). All cases of MEC that showed strong positivity for anti-Gal-3 antibody in tumour parenchyma were negative for nodal metastasis, while all those which were weak positive showed positive lymph nodes (p=0.006).

In view of above discussion, it seems that overexpression of Gal-3 is related to formation and progression of neoplasms in salivary glands. Next, it is observed that nucleocytoplasmic expression was seen in few squamous cell in MEC and tubular pattern of AdCC which are well differentiated structures. So, these findings are in accordance with the suggestion that nuclear expression of Gal-3 is associated with histologically evident tumour differentiation (Ferrazzo et al. 2007 p. 580-85). On the other hand, cytoplasmic expression is mainly seen in malignant tumours in the current study which has been related to cell proliferation, cell cycle progression and acquisition of anti-apoptotic profile by the tumour cells (Song et al. 2014 p. 185-91). Also, it is noted in this study that as the grade of malignancy increases from low to high, expression of Gal-3 decreases in the tumour cells and increases in the surrounding extracellular space where it mediates functions like cell-matrix adhesion, cell activation and angiogenesis (Argüeso & Panjwani2011 p. 2-3).

5. Conclusion

It can be concluded from the current study that expression of Gal-3 decreases with decreasing differentiation in parenchyma of malignant tumours while its expression in tumour extracellular environment increases with increasing grade of the tumour.Galectin-3 may play an important pathogenetic role in metastasis of malignant tumours owing to its homotypic adhesion to tumour cells and heterotypic adhesion of tumour cells with glycoconjugates in tumour's extracellular environment and with endothelial cells thus leading to invasion and migration of tumour cells and allowing them to reach metastatic sites. This is also evident from its strong expression in metastatic tumour sites in MEC. Also, it can be concluded that nuclear expression of Gal-3 is associated with histologically evident tumour differentiation and cytoplasmic expression with tumour progression.

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