

Centromere Protein F (CENPF): A novel marker for salivary gland pathology

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Abstract

Background: Salivary gland tumours are relatively uncommon, and there exists considerable diagnostic difficulty. This is due to individual lesions having diverse histopathological features, presence of number of types and variants, and overlapping histological features in different tumour entities.

Aim: The current study aimed at assessing the expression of centromere protein F (CENPF) in benign and malignant salivary gland tumours and to evaluate the efficacy of CENPF as a proliferative marker to aid in the diagnosis of malignancy so that it will help in surgical pathology practice.

Materials and Methods: The study group involved 20 cases of benign salivary gland tumours, 20 cases of malignant salivary gland tumours, and 10 normal salivary gland tissues. All the cases were subjected to immunohistochemical analysis for CENPF expression and were assessed by two independent observers and further taken up for evaluation.

Statistical Analysis: The results were analysed statistically among different groups using analysis of variance (ANOVA) or Kruskal–Wallis test with Chi-squared test using IBM's Statistical Package for the Social Sciences (SPSS) version 17.0.

Results: CENPF expression in normal salivary gland was negative with gradual increase in expression from benign salivary gland tumours to malignant salivary gland tumours. CENPF expression was high in malignant salivary gland tumours.

Conclusion: Findings of the study suggest that CENPF can be regarded as a new cell proliferation marker for malignant salivary gland tumours.

Keywords: Biomarker, CENPF, immunohistochemistry, salivary gland tumours

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INTRODUCTION

Salivary gland tumours make up about 3% of all head and neck neoplasms, and they are relatively uncommon. Most commonly affected sites are parotid gland, submandibular

gland, and minor salivary glands, where the sublingual gland is very rarely affected.^[1] There exists considerable diagnostic difficulty due to individual lesions having diverse histopathological features, presence of number of

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types and variants, and overlapping histological features in different tumour entities.

Even though haematoxylin and eosin (H&E) staining is considered as the gold standard method used for the diagnosis, immunohistochemistry can enhance the accuracy and can be a helpful tool in cases that cannot be assessed by histological examination, such as the cell nature and differentiation status, cell proliferation, and tumour protein expression. Immunohistochemistry plays an important role in the diagnosis of salivary gland tumours and is always helpful in supporting the histological assessment. Unfortunately, there is not enough data available regarding immunohistochemical analysis of salivary gland tumours that can be applied to surgical pathology practice.^[2]

Various studies have been done in studying the incidence of salivary gland tumours across USA, Brazil, Jordan, Japan, Africa, and Nigeria. But only a few studies have been done so far to see the pattern of distribution of salivary gland lesions in Asian countries especially in India, thus indicating the scarcity of epidemiological data of these lesions.^[3]

Centromere protein F (CENPF) is a 350 KD transient kinetochore protein that is also known as mitosin in humans, Lek 1 in mouse, and CMF-1 in chicken. It is located at the outer kinetochore plate and is expressed in a cell-cycle dependent manner. It has a complex and dynamic expression and localization pattern. CENPF level is low in G1 phase but increases sharply in S phase as a nuclear protein. At the G2/M transition, CENPF re-localises to the nuclear envelope where it contributes via NudE/EL to dynein/dynactin recruitment to the nuclear envelope. Following the progression of M phase, mitosin is hyperphosphorylated and exhibits localisation at the kinetochores, spindle poles, and midbody. It is subjected to rapid degradation at the end of mitosis.^[4-6]

Various studies show overamplification of CENPF in various malignancies, but to our knowledge literature review shows only one study done on CENPF expression in salivary gland tumours.^[7] The current study aimed at assessing the expression of CENPF in benign and malignant salivary gland tumours and to evaluate the efficacy of CENPF as a proliferative marker to aid in the diagnosis of malignancy so that it will help in surgical pathology practice.

MATERIALS AND METHODS

The current study involved 10% formalin-fixed paraffin-embedded (FFPE) tissues of histopathologically

diagnosed cases of 20 benign salivary gland tumours, 20 malignant salivary gland tumours, and 10 normal salivary gland tissues from the archives. The study proposal was approved by the Institutional Ethics Committee, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai. The clinical and histological parameters included in the study were obtained from the records of the patients documented in the department.

The study samples were divided into three groups

- **Group I:** Normal Salivary Gland ($n = 10$)
- **Group II:** Benign Salivary Gland Tumours ($n = 20$)
- **Group III:** Malignant Salivary Gland Tumours ($n = 20$)

Group I comprised of 10 normal salivary gland tissues; group II comprised of 12 pleomorphic adenomas, 3 basal cell adenomas, 1 cystadenoma, 2 myoepitheliomas, and 2 Warthin tumours.; and group III comprised of 6 mucoepidermoid carcinomas, 9 adenoid cystic carcinomas, 1 adenoid cystic carcinoma ex pleomorphic adenoma, 2 acinic cell carcinomas, and 2 carcinoma ex pleomorphic adenomas.

All the study samples were stained with routine H and E stain and were also subjected to immunohistochemical analysis for CENPF expression.

Immunohistochemistry

All the cases were subjected to CENPF immunostaining using CENPF antibody (polyclonal NB 100-88157, Novus Biologicals- Rabbit, dilution 1:200). Ewing's sarcoma was selected as the control group, as given in the protocol of the kit.

The immunostained slides were evaluated semi-quantitatively. The intensity of staining and the percentage of cells were evaluated and scored. The degree of immunostaining was assessed and scored independently by two investigators blinded from the clinical parameters, according to both intensity and extent of staining. Only cells stained in the nucleus were taken into account. The entire tissue sections were observed to assign scores.

Based on the previous studies done,^[8,9] the sum of the intensity scores and extent scores were used as final staining scores (0–7). For the purpose of statistical evaluation, tumours having a final staining score of less than 3 were grouped as low CENPF expression and greater than or equal to 3 were grouped as high CENPF expression.

Statistical analysis

The results were analysed statistically among different groups using analysis of variance (ANOVA) or Kruskal–

Wallis test with Chi-squared test using IBM's Statistical Package for the Social Sciences (SPSS) version 17.0. *P*-values less than 0.05 were considered as statistically significant [Tables 1 and 2].

RESULTS

We studied the expression of CENPF in 20 benign salivary gland tumours, 20 malignant salivary gland tumours, and 10 normal salivary gland tissues. Only cells stained in the nucleus were taken into account. The age group of the study sample ranged from 5 years to 83 years. The mean age of the patients in group I, group II, and group III were 31.5 years, 43.8 years, and 48.4 years, respectively. The mean age of patients in the positive group was 49.14 years and the mean age of patients in the negative group was 35.64 years. The distribution of sex in the study was found to be 32 males (64%) and 18 females (36%). Among 32 males, 19 cases (59.4%) expressed CENPF and among 18 females, 9 cases (50%) expressed CENPF.

Site distribution in group II and group III included 6 different sites. Among the 40 cases, 17 (42.5%) were from the parotid gland, 17 (42.5%) were from the palate, 3 (7.5%) were from the submandibular gland, 1 (2.5%) was seen in the sublingual, 1 (2.5%) was seen in the base of the tongue, and 1 case (2.5%) was seen in the retromolar trigone.

Positive expression of CENPF was seen in 14 out of 17 cases (82.4%) of the palate, 11 out of 17 cases (64.7%) of the parotid, 1 out of 3 cases (33.3%) of the submandibular gland, and 1 each (100%) of the sublingual and retromolar trigone. The base of the tongue showed no CENPF expression. Pearson's Chi-squared test was performed, and the result was statistically significant ($P < 0.05$).

Three out of 20 cases (15%) of benign salivary gland tumours had reactive lymphadenitis. One case (5%) had lymph node involvement in malignant salivary gland tumours. Five out of 20 cases (25%) of malignant salivary gland tumours showed recurrence.

All the cases in group I stained negative for CENPF. Out of 20 cases in benign salivary gland tumours, 40% of cases showed positive CENPF expression. One hundred percent of cases from malignant salivary gland tumours showed positive CENPF expression [Figure 1].

DISCUSSION

Tumours arising from the salivary glands are relatively uncommon, and they comprise approximately 1% of all neoplasms in the whole body.^[2] The aetiology of salivary

gland carcinomas remains unclear. Most of the head and neck carcinomas are strongly associated with smoking and alcohol—they do not play a role in the salivary glands.^[10] Biomarkers are grouped into several classes like genomic markers, proliferation markers, and differentiation markers.^[11] Centromere protein F or CENPF is a 350 kD nuclear phosphoprotein that is involved in cell division.^[6] Autoantibodies to CENPF have been observed in various malignant tumours like breast carcinoma and lung carcinoma. The detection of anti-CENPF antibodies indicates increased cell division and dysfunctional cell cycle regulation during carcinogenesis.^[7,12] Therefore, it acts as a suitable marker for evaluating proliferation of cells by immunohistochemistry.^[13] CENPF is upregulated in various tumours like lung cancer, non-Hodgkin's lymphoma, mantle cell lymphoma, and oesophageal carcinoma.^[14,15]

Till date, only one study by Shigeishi *et al.*^[7] has been carried out to evaluate the expression of CENPF in salivary gland tumours. In this study, we used polyclonal antibody CENPF for studying its expression in salivary gland tumours using immunohistochemistry. Ewing's sarcoma was used as positive control, and it showed strong nuclear staining with CENPF antibody [Figure 2].

There was negative expression of CENPF in all 10 cases of normal salivary gland [Figure 3]. This is in accordance with the study done by Shigeishi *et al.*^[7] that showed that

Table 1: Significance in expression of CENPF in different study groups

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-squared test	30.519	2	0.000 (Significant)

$P < 0.05$ was considered significant, df: degrees of freedom

Table 2: Table showing final scores of CENPF being analysed statistically among different groups using ANOVA and found to be highly significant

	Sum of Squares	df	Mean Square	F	P
Final score					
Between groups	234.150	2	117.075	52.731	0.000
Within groups	104.350	47	2.220		
Total	338.500	49			

$P < 0.05$ was considered significant, df: degrees of freedom

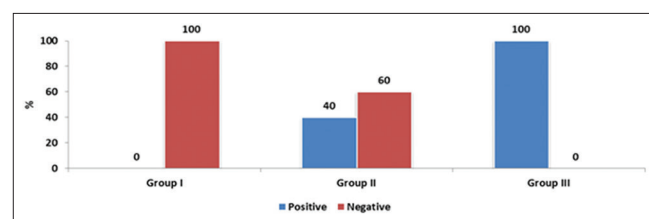


Figure 1: Bar diagram showing expression of CENPF in different study groups

normal submandibular salivary gland tumours showed no immunoreactivity to CENPF in immunohistochemistry. This may be due to the fact that only intercalated duct reserve cell can undergo proliferation when compared to salivary gland acini.

Out of 20 cases of benign salivary gland tumours, 8 (40%) showed positive CENPF expression and 12 (60%) showed negative CENPF expression [Figures 4 and 5]. In a similar study conducted by Shigeishi *et al.*^[7] on CENPF gene expression in human salivary gland tumours, benign salivary gland tumours showed weak CENPF staining.

Out of 20 cases of malignant salivary gland tumours, all cases showed positive CENPF expression [Figures 6–8]. In a similar study conducted by Shigeishi *et al.*^[7] on CENPF gene expression in human salivary gland tumours, three of eight mucoepidermoid carcinomas, two of four acinic cell carcinomas, two of four adenoid cystic carcinomas,

and one of one malignant myoepithelioma were positive for CENPF.

In our study, when considering the final score, only group II and group III were taken into account since all cases from group I showed negative (0%) CENPF expression. A final score of less than 3 was considered as low CENPF expression, and a final score of greater than or equal to 3 was considered as high CENPF expression.

Out of 8 cases that showed positive expression of CENPF in benign salivary gland tumours (group II), 4 (50%) showed high CENPF expression and 4 (50%) showed low CENPF expression. Among the 4 cases from benign salivary gland tumours which showed high CENPF expression, 3 cases were pleomorphic adenomas and one case was myoepithelioma. The reason for high CENPF expression in four of the benign salivary gland

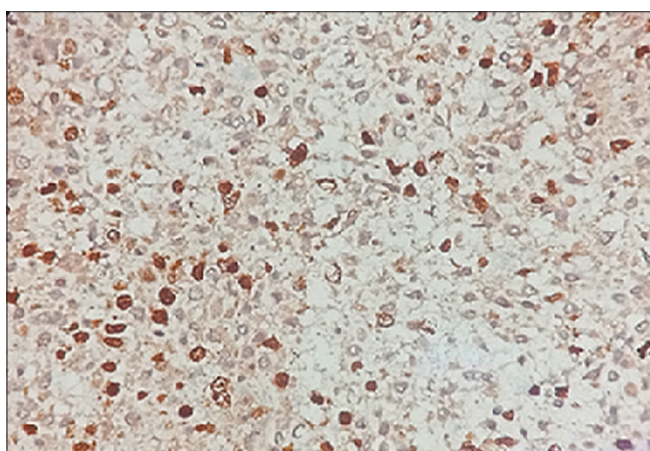


Figure 2: Photomicrograph showing nuclear staining with CENPF (×400) in positive control (Ewing's sarcoma)

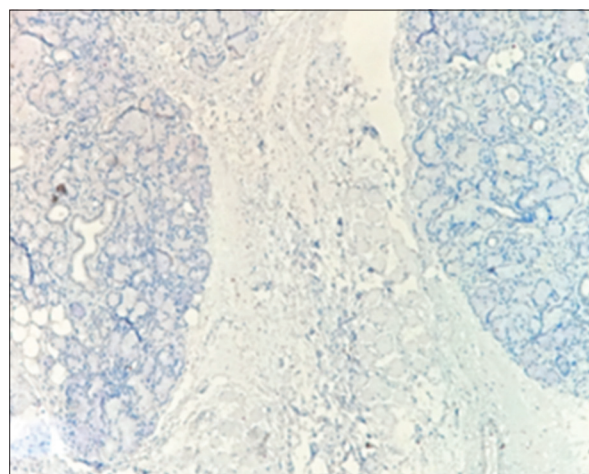


Figure 3: Photomicrograph showing negative CENPF expression (×100) in normal salivary gland

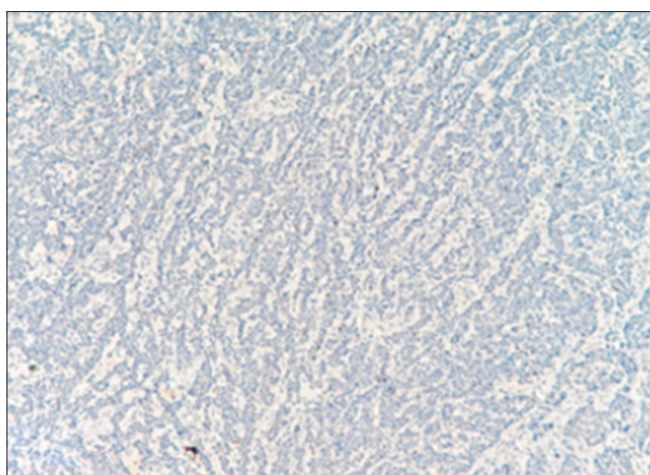


Figure 4: Photomicrograph showing negative CENPF expression (×100) in pleomorphic adenoma

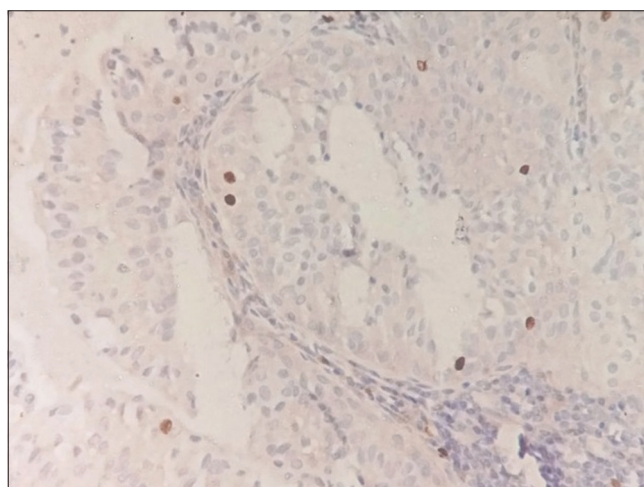


Figure 5: Photomicrograph showing low CENPF expression (×400) in Warthin tumour

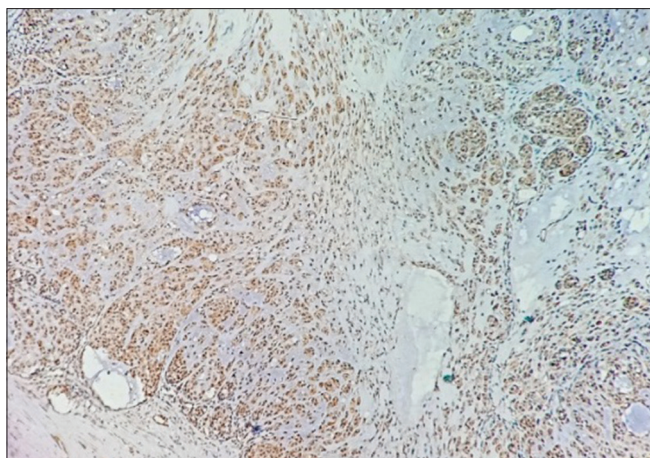


Figure 6: Photomicrograph showing >75% of staining with CENPF (×100) severe intensity in adenoid cystic carcinoma ex pleomorphic adenoma

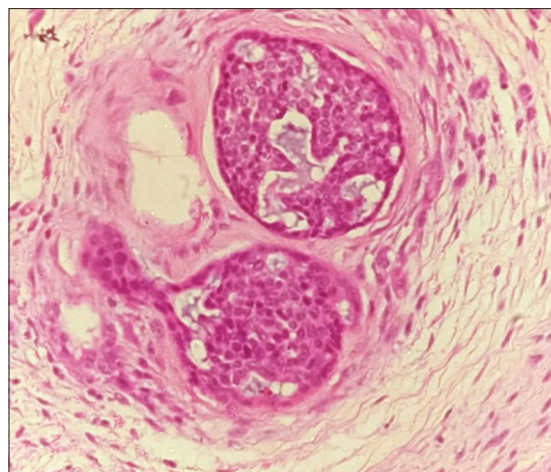


Figure 7: Photomicrograph showing neural invasion in adenoid cystic carcinoma (H&E, ×400)

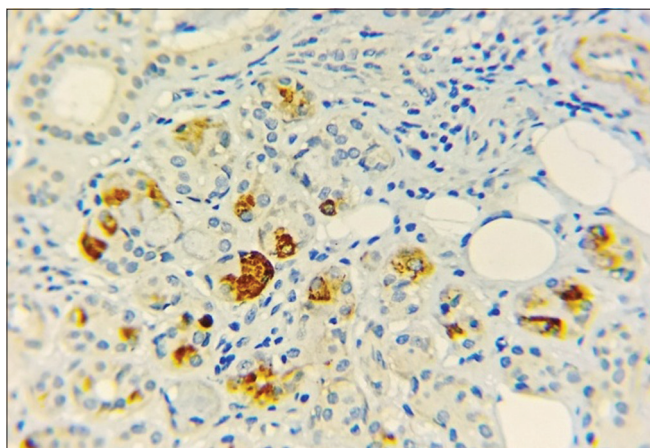


Figure 8: Photomicrograph of area of neural invasion in adenoid cystic carcinoma showing >75% of staining with CENPF (×400), severe intensity

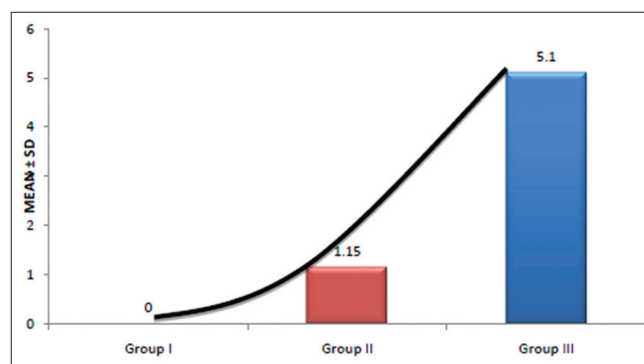


Figure 9: Image showing summary of study results

tumours may be because of the fact that cells may be rapidly proliferating and these lesions have more chances of undergoing malignant transformation.

Among 20 malignant salivary gland tumours, 19 (95%) showed high CENPF expression and 1 (5%) showed low CENPF expression. That one case was diagnosed as mucoepidermoid carcinoma. The reason for low CENPF expression in this case may be because of the fact that mucoepidermoid carcinoma was cystic type and was of low grade.

Malignant salivary gland tumour with lymph node metastasis and recurrence has high CENPF expression.

Thus, in the present study expression of CENPF in various study groups revealed that normal salivary gland tumours did not express CENPF. The gradual increase in the expression of CENPF was noted from benign

salivary gland tumours (40%) to malignant salivary gland tumours (100%) with statistical significance [Figure 9].

CONCLUSION

Together with our study results and knowledge about the role of CENPF as a proliferative marker for malignancy, CENPF may play a role in increased tumour aggressiveness. Since the number of cases included in our study was small, further analyses with increased number of cases are needed for further studies. So the present study suggests CENPF may be an additional diagnostic tool for salivary gland carcinomas.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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