Original Article

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Pathology Section

Significance of Nuclear Morphometry in Salivary Gland Neoplasms: A Cross-sectional Study

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ABSTRACT

Introduction: Fine Needle Aspiration Cytology (FNAC) is one of the commonest and most efficient investigation done for salivary gland swellings, which is of much help in diagnosing inflammatory, benign and malignant lesions. The morphology of nucleus determines the behaviour of the cell/tissue as benign or malignant. Nuclear morphometry analysis is done by using various software.

Aim: To determine the nuclear morphometry parameters in salivary gland neoplasm of FNAC smears by Image J software and to determine its association with the cytological diagnosis.

Materials and Methods: This was a retrospective laboratory observational cross-sectional study done for four years (2018-2021) at a tertiary healthcare centre, attached to Sri DevarajUrs Medical College, Kolar, Karnataka, India. All the FNAC smears of salivary gland neoplasms were considered and classified by cytomorphology as benign and malignant tumours. All smears were analysed using Image J software for nuclear morphometric parameters such as area of the nucleus, perimeter of the nucleus, feret diameter, minimum ferret and skewness. The nuclear morphometric findings of benign and malignant tumours were compared and statistical analysis was doneby using Student's t-test, p-value of <0.05 was considered statistically significant.

Results: A total of 52 cases were studied. The average age of presentation of benign and malignant salivary gland neoplasm was 38.7 and 49.4 years, respectively. Among, 30 (57.6%) benign neoplasms there were 23 (76.7%), pleomorphic adenoma was the commonest and among 22 (42.4%) malignant squamous cell carcinoma 16 (72.7%) deposits was the commonest. The nuclear morphometry analysis showed that the mean values of area (p-value ≤0.001), perimeter (p-value≤0.001), ferret diameter (p-value≤0.001) and minimum ferret (p-value=0.001) of malignant lesions were comparatively higher than benign and was statistically significant.

Conclusion: Nuclear morphometry along with routine cytopathological evaluation will improve the accuracy of diagnosis of neoplastic lesions of salivary gland. This information can be used to plan better treatment and predict prognosis of the disease. Digital morphological analysis helps in obtaining quantitative values from qualitative data which can be utilised further for automation.

Keywords: Benign neoplasm, Fine needle aspiration cytology, Malignant neoplasm, Salivary gland neoplasm

INTRODUCTION

The FNAC is one of the most important, frequently used and routine diagnostic tool in cytology. Salivary gland neoplasms are initially and commonly investigated using FNAC because of easy approach and better post procedure recovery when compared to biopsy [1,2]. Even though it's done commonly, it is not an easy task as all these neoplasms show diversity in their cytomorphology extensively and there is also a chance of overlapping of cytomorphological features between neoplasms [1,3]. These salivary gland tumours are often seen showing heterogenicity with variable components such as cystic, solid and necrotic lesions. There is always a subjective variability among the opinions given by various pathologists when they miss to observe the subtle architectural, morphological and cytological patterns of the cells [1,3,4].

The innovative method which is cytomorphometric analysis of the nucleus helps in reducing the subjective variability among various pathologists by converting the nuclear cytomorphological features into quantitative data which ultimately helps in minimising the bias and errors in diagnosis and to differentiate between benign and malignant neoplasms [3,5,6]. This helps particularly in cases where there is difficulty in categorising the tumour.

Many studies are done and good amount of literature is available on morphometry analysis of neoplasms of breast and oral cavity. Limited data is available regarding the salivary gland neoplasms and its diagnostic role. The morphometry study is especially useful in overlapping nuclear characteristics of neoplasms of salivary gland [1,2]. Hence, this study was taken up to study various parameters of nuclear morphometry in both benign and malignant neoplasms of salivary gland. The objective was to determine nuclear morphometry parameters in salivary gland neoplasm fine needle aspiration smears by Image J software and to associate with the cytological diagnosis

MATERIALS AND METHODS

The present study was a retrospective laboratory observational cross-sectional study conducted in Department of Pathology, at a tertiary healthcare centre, attached to Sri Devaraj Urs Medical College, Kolar, Karnataka, India, on the FNAC smears of suspected cases of neoplasms of salivary gland for the period of four years (January 2018-December 2021). The cases were analysed in the year 2022. Ethical clearance was obtained from Institute Ethics Committee (IEC) (DMC/KLR/IEC/135/2022-23, dated 08.09.2022) before the start of the study. The smears were screened and further categorised as benign and malignant based on cytomorphological features as per the 5th edition World Health Organisation (WHO) classification [7]. Histopathological diagnosis of biopsy was considered as gold standard.

Inclusion criteria: FNAC smears of salivary gland neoplasms with smears showing clear nuclear and cytoplasmic features were included in the study.

Exclusion criteria: The smears with inadequate staining, showing overlapping of nuclei, with abundant necrotic and degenerative material, abundant inflammatory cell infiltrate, mucous and blood, unlabelled smears and broken slides were excluded from the study.

Study procedure: The aspirate smears were stained with haematoxylin and eosin stain. The nucleus of suspected cells from all the smears was focussed at 400X magnification with the help of objective lens on Zeiss Primostar microscope with attached digital camera. Among the cells, five nuclei which were clearly visible were captured and were subjected to morphometrical analysis using image J software. This software identifies the selected areas of cells and quantifies the parameters. The nuclear morphometric parameters analysed were such as area of the nucleus, perimeter of the nucleus, feret diameter, minimum ferret and skewness [8].

1. Area: Area of the nuclear membrane defined with outline.
2. Perimeter: Length of the defined area's boundary.
3. Feret diameter: Longest distance measured between any two selected points within the selected boundary.
4. Minimum feret: Minimum calliper diameter.
5. Skewness: Third order moment about the mean.

STATISTICAL ANALYSIS

All the values were entered in Microsoft excel sheet and analysed by Statistical Package for the Social Sciences (SPSS) software version 20.0. The data were analysed using independent sample test. Mean and Standard Deviation (SD) values were calculated for all the parameters in both benign and malignant categories. The p-value was obtained using Student's t-test and p-value of <0.05 was considered as statistically significant.

RESULTS

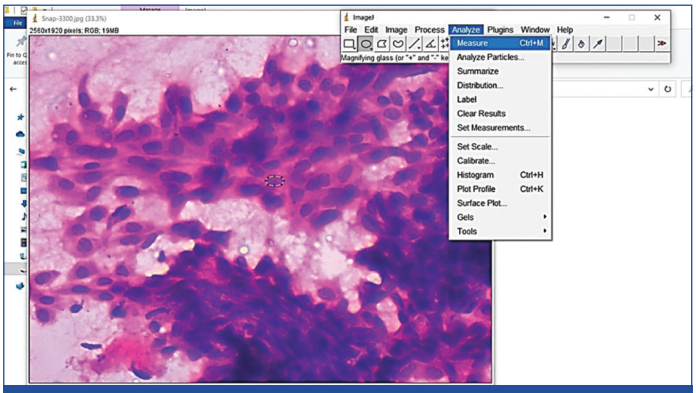
A total of 52 cases were studied, of which 30 (57.6%) cases were benign and 22 (42.4%) malignant by cytomorphology [Table/Fig-1] and confirmed by histopathology. The age range of patients for benign and malignant salivary neoplasms was 15 to 85 years and 16 to 75 years, respectively. The average age of presentation in benign and malignant salivary gland neoplasm was 38.7 and 49.4 years, respectively. Among 30 benign salivary gland neoplasms there were 17 males and 13 females with ratio of 1.3:1. Among 22 malignant salivary gland neoplasms there were 12 males and 10 females with ratio of 1.2:1.

Benign neoplasms		N=30, n (%)
1	Pleomorphic adenoma	23 (76.7)
2	Monomorphic adenoma	2 (6.7)
3	Warthin's tumour	5 (16.6)
Malignant neoplasms		**N=22, n (%)**
1	Squamous cell carcinoma deposit	16 (72.7)
2	Mucoepidermoid carcinoma	4 (18.1)
3	Acinic cell carcinoma	1 (4.6)
4	Small round cell carcinoma	1 (4.6)

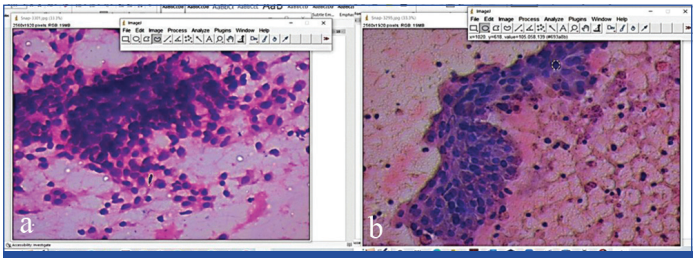
[Table/Fig-1]: Distribution of benign and malignant neoplasms in the present study.

Cytology of pleomorphic adenoma showed benign ductal and myoepthelial cells arranged in cohesive clusters, ductal pattern and in singles along with fragments of chondromyxoid stroma. Background was haemorrhagic. Warthin's tumour showed oncocytic cells arranged in cohesive clusters along with polymorphous population of lymphocytes in a dirty amorphous eosinophilic background. Monomorphic adenoma aspirates showed plasmacytoid epithelial cells, some having vacuolated cytoplasm arranged in clusters and in glandular pattern. Background showed erythrocytes.

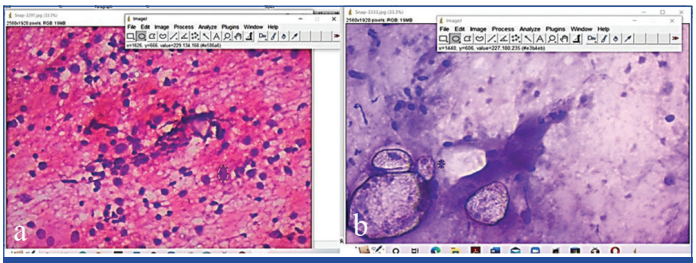
The aspirates of squamous cell carcinoma deposits showed polyhedral cells having pleomorphic, hyperchromatic nucleus with moderate amount of eosinophilic cytoplasm arranged in discohesive clusters and in singles. Background showed haemorrhage. The smears of mucoepidermoid carcinoma showed polyhedral cells and ductal epithelial cells having hyperchromatic and pleomorphic nucleus, cytoplasm pale eosinophilic/vacuolated, arranged in glandular pattern and in singles. Background showed necrosis and erythrocytes. The cytology of acinic cell carcinoma showed cells having eccentric nucleus, vesicular nucleus with prominent nucleoli, pale basophilic/vacuolated cytoplasm arranged in acinar pattern with haemorrhage in the background. Small round cell carcinoma showed round epithelial cells having hyperchromatic nucleus arranged in singles in a haemorrhagic background Morphometric quantification of following parameters was done by using image J software [Table/Fig-2-4].



[Table/Fig-2]: Image J software showing nuclear morphometry analysis in Fine Needle Aspiration Cytology (FNAC) smear in a case of pleomorphic adenoma (H&E X400).



[Table/Fig-3]: Image analysis on benign tumours: (a) Pleomorphic adenoma; (b) Warthin's tumour (H&E X400).



[Table/Fig-4]: Image analysis on malignant neoplasms: (a) Squamous cell carcinoma; (b) Mucoepidermoid carcinoma (H&E X400).

The nuclear morphometry analysis showed that the mean values of area, perimeter, ferret diameter, minimum ferret and skewness among benign tumours was 35.49±19.25, 22.94±6.62, 8.66±2.40, 5.42±1.83, 0.55±0.13, respectively and for malignant tumours was 78.69±42.72, 34.63±9.79, 12.71±3.54, 8.31±2.63, 0.11±0.08, respectively. The value of each parameter except skewness was comparatively higher in malignant tumours than in benign and was statistically significant [Table/Fig-5].

DISCUSSION

The incidence of salivary gland tumours in the world is approximately 0.4-13.5/100,000 population. Among the tumours arising in the head and neck region, salivary gland tumours constituted <3%. The incidence among Indian population is 1.7%. The prevalence reported in a study by Kalyani R et al., showed that the salivary gland cancers constituted 0.84% of all cancers, in males it constituted 1.17% of

S. No.	Parameter	Benign Mean±SD	Malignant Mean±SD	p-value (Student's t-test)
1	Area	35.49±19.25	78.69±42.72	≤0.001
2	Perimeter	22.94±6.62	34.63±9.79	≤0.001
3	Feret diameter	8.66±2.40	12.71±3.54	≤0.001
4	Minimum feret	5.42±1.83	8.31±2.63	≤0.001
5	Skewness	0.55±0.13	0.11±0.08	0.039

[Table/Fig-5]: Comparison of mean values of nuclear morphometry in benign and malignant neoplasms.

higher in benign tumours [Tables/Fig-5]. This indicates that various parameters used in nuclear morphometry play a very important role in differentiating benign and malignant neoplasms of salivary gland. A study by Chaurasia J et al., have shown that the analysis of digital images for nuclear morphometry helped in differentiating benign and malignant salivary gland tumours [Table/Fig-6] [1].

There are a few benign tumours in salivary gland such as pleomorphic adenoma and basal cell adenoma which may mimic some of the malignant lesions such as adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma due to heterogenicity of

S. No.	Study	Area Mean±SD		Perimeter Mean±SD		Feret diameter Mean±SD		Minimum ferret Mean±SD		Skewness Mean±SD	
		Benign	Malignant	Benign	Malignant	Benign	Malignant	Benign	Malignant	Benign	Malignant
1	Chaurasia J et al., 2020, Bhopal [1]	42.64±15.00	66.17±35.84	25.47±5.19	34.21±12.56	7.68±1.48	9.75±2.70	-	-	-	-
2	Present study,2023, Kolar	35.49±19.25	78.69±42.72	22.94±6.62	34.63±9.79	8.66±2.40	12.71±3.54	5.42±1.83	8.31±2.63	0.55±0.13	0.11±0.08

[Table/Fig-6]: Mean values of nuclear morphometry in benign and malignant neoplasms in various studies compared to the present study [1].

all male cancers and in females 0.58% of all female cancers [9]. In present study, both males and females were almost equally affected in benign tumours but slight male preponderance was noted in malignant neoplasms. Average age of presentation in males for benign lesions was 42.7 years and in females it was 31.6 years. Average age of presentation in males for malignant lesions was 52.5 years and in females it was 44.6 years. Study by Chaurasia J et al., regarding salivary gland neoplasms showed that the minimum age of presentation was 17 years and maximum being 75 years [1]. The average age of presentation for benign tumours was 38.7 and for malignant tumours it was 49.4 in the present study. The salivary gland neoplasms commonly involve major salivary glands compared to minor salivary glands [10].

FNAC is a minimally invasive method, used for diagnosis of salivary gland neoplasms. Many times the diagnosis based on cytology will be a challenging to the pathologists because of heterogenicity of the lesions and overlapping nuclear characteristics [1,10-12]. Morphometry using digital images plays a major role in such circumstances. By using this method one can elevate the accuracy of all the cytology reports. This is one of the most inexpensive tool that help us to reach the correct diagnosis [1,13,14].

Nuclear morphometry is one of the innovative, newest and advanced methods of application which has made remarkable changes in reaching precise diagnosis of salivary gland neoplasms. Study reports are available regarding morphometrical analysis of lesions in various organs. But not many studies are done and even the literature is limited regarding the nuclear morphometrical analysis of salivary gland neoplasms [1,2,15,16].

Chaurasia J et al., in their study analysed digital images of benign and malignant salivary gland neoplasms of uncertain malignant potential, and stated that cytomorphology along with nuclear morphometry will help in reaching accurate diagnosis and delivering appropriate treatment for the patient [1]. In another study by Obad-Kovačević D et al., who has done similar study using image analysis digitally on tumours of parotid gland concluded that morphometrical analysis helps much in cases where there is ambiguity in cytological diagnosis [2]. Zivkovic ND et al., did a study on metric analysis of nuclei on histopathology permanent sections of salivary gland neoplasms as polymorphous low-grade adenocarcinoma, pleomorphic adenoma, etc., and concluded that individual characteristic morphometrical analysis of nuclei are required for more accurate diagnosis [3].

In the present study, authors had assessed the morphometry of nuclear parameters between benign and malignant neoplasms. In all the parameters, mean values were higher in all malignant neoplasms when compared with benign neoplasms and was statistically significant except for the skewness which was seen

the cytomorphology of individual cells and its nuclei. In such cases, nuclear morphometry plays a major role in diagnosing the lesions because the parameters, especially area of nucleus and perimeter of the nucleus are higher in malignant neoplasms compared to benign ones [1,2].

In lesions, where tumours share similar or identical cytomorphological features, as basal cell adenoma and basal cell carcinoma, the smear shows basaloid cells in both cases. The distinction between two entities is difficult. In such cases, morphometrical analysis will be of immense help to the pathologist to sign out confidently even when there is scanty cellularity in the smear. Similar scenario can arise when the neoplastic cells are admixed with abundant amounts of extracellular matrix where digital morphological analysis will help in arriving at correct diagnosis [1,2].

The results which are obtained by the present study indicate that, if one consider this procedure in everyday practice, it will help in reaching precise diagnosis especially in cases with ambiguous cytomorphological diagnosis. Nuclear morphometry data can be used for automation, which is one of the emerging technologies, where artificial intelligence can be used to train the computer to diagnose the lesions which ultimately saves a lot of time for the pathologist as it can be used as an initial screening tool. The morphometry procedure has to be validated with large sample size and conducting muticentric studies before using the same in routine practice.

Limitation(s)

The limitations of the present study was, nuclear morphometry analysis findings was not correlated with immunohistochemistry or cytogenetics findings. The study had limited number of cases and was conducted in one tertiary healthcare centre. But the results which are obtained by the present study indicates that, if we consider this procedure in everyday practice, it will help in reaching precise diagnosis especially in cases with ambiguous cytomorphological diagnosis. Nuclear morphometry data can be used for automation, which is one of the emerging technologies, where artificial intelligence can be used to train the computer to diagnose the lesions which ultimately saves lot of time for the pathologist as it can be used as an initial screening tool. The morphometry procedure has to be validated with large sample size and conducting muticentric studies before using the same in routine practice.

CONCLUSION(S)

Nuclear morphometry study along with cytopathological evaluation will improve the accuracy of diagnosis in neoplastic lesions of salivary gland especially where there is overlap of nuclear features in aspirate cytology. The morphometry parameters as area, perimeter,

feret diameter and minimum feret were increased in malignant tumours than benign lesions. As morphometrical parameters are quantitative parameters, digital morphological analysis helps in getting quantitative values from qualitative data. This information can be utilised for artificial intelligence and telemedicine in differentiating benign lesion from malignant tumours of salivary gland especially in primary healthcare centre where there is non availability of cytopathologist.

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