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Research Article

FINE NEEDLE ASPIRATION CYTOLOGY OF SALIVARY GLAND LESIONS WITH CATEGORIZATION BASED ON MILAN SYSTEM – A 5-YEAR RETROSPECTIVE STUDY

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ABSTRACT

Objectives: Fine needle aspiration cytology (FNAC) is a well-established technique for initial assessment of salivary gland lesions. The Milan system for reporting salivary gland cytopathology (MSRSGC) was introduced to provide a guide for diagnosis and management of salivary gland lesions according to risk of malignancy (ROM) in different categories.

Methods: A 5-year retrospective study was conducted to reclassify the salivary gland lesions from previous diagnosis. Clinical data, FNAC, and histopathology report was retrieved and cases were reclassified according to the Milan system of classification. Risk of malignancy was calculated for each category. The positive predictive value, negative predictive value, and diagnostic accuracy of FNAC was calculated.

Results: A total of 314 cases were evaluated cytologically. Histopathology was available in 81 cases. The distribution of cases in different categories according to the Milan system was 1.27% (Cat I), 48.4% (Cat II), 1.91% (Cat III), 38.21%, (Cat IV A), 2.22% (Cat IV B), 3.18% (Cat V), and 4.77% (Cat VI). Overall risk of malignancy reported was 0%, 0%, 50%, 14.7% (Cat IV A), 66.66% (Cat IV B), 83.3%, and 100%, respectively. Overall sensitivity, specificity, positive predictive value, and negative predictive value was 70.58%, 93.75%, 75%, and 92.30%, respectively. Diagnostic accuracy was 88.89%.

Conclusion: MSRSGC is a useful system for conveying risk of malignancy (ROM) and deciding further treatment protocol and, hence, improves overall patient care and management.

Keywords: Fine needle aspiration cytology, Salivary gland lesions, The Milan system, Risk of malignancy.

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INTRODUCTION

Salivary gland lesions constitute 3–6% of all head-and-neck pathologies [1], so accurate diagnosis of these lesions is mandatory for adequate management. Due to diverse and overlapping morphological spectrum of these lesions, there is a need for uniform classification system [1,2-4]. Milan system for reporting salivary gland cytopathology (MSRSGC) was thereby conceptualized in 2015 by American Society of Cytopathology and International Academy of Cytology (IAC) [5]. The Milan system is a six tier classification providing risk stratification, that is, risk of malignancy (ROM) for each ascending risk category rather than a binary benign or malignant assessment for each individual case. This is an essential step toward improving overall effectiveness of salivary gland FNA and to foster better communication between clinicians and institutions to improve overall patient care [5].

This system is still at a preliminary stage. Therefore, an attempt was made to reclassify the salivary gland lesions retrospectively into six categories as proposed by this system to determine the cytological concordance, disconcordance, and assessment of risk stratification by calculating the ROM for individual categories.

METHODS

A 5-year retrospective study was done for salivary gland lesion cases where FNAC reports and clinical data were available covering the period July 2016 to June 2021 in the Department of Pathology, GMC, Patiala. Data was retrieved and reclassified by two independent pathologists using Milan system (MSRSGC) of classification as follows: Category I: Non-diagnostic, Category II: Non-neoplastic, Category III: Atypia of undetermined significance (AUS), Category IV A: Neoplasm benign, Category IV B: Salivary gland neoplasm of uncertain malignant potential (SUMP), Category V: Suspicious for malignancy, and Category VI: Malignant [6].

The histopathological reports wherever available, were compared, statistical analysis was done and risk of malignancy (ROM) was calculated for each category. The cytosmears of all disconcordant cases were re-examined to ascertain the possible cause of errors. The specificity, sensitivity, positive predictive value, negative predictive value, and diagnostic accuracy of FNAC were calculated.

ROM was calculated using number of cases which turned out to be malignant on histopathology in each category versus the total number of cases in which histopathology was available.

RESULTS

A total of 314 cases of FNAC done in a period of 5 years were re-evaluated, out of which 60.82% (n=191) were males and 39.17% (n=123) were females with male to female ratio of 1.55:1. The parotid gland was most commonly involved followed by submandibular gland and minor salivary glands.

On cytological examination, Category II was the largest category (48.4%) followed by Category IV A (38.21%). Pleomorphic adenoma was the most common benign tumor while mucoepidermoid carcinoma was the most common malignant tumor seen. Six cases were reclassified as AUS, seven cases reclassified as SUMP, and ten cases as suspicious of malignancy. Corresponding histopathological diagnosis was available for 81 cases of 314 cases (25.79%).

The cases having insufficient cellular material, background debris, and extensive air drying artifact on cytology were reclassified in Category I.

Histopathology was available in one out of four cases which proved to be of pleomorphic adenoma.

Thirty cases out of 152 cases of Category II had histopathological follow-up. Five cases proved to be of benign neoplasm (Three cases diagnosed on cytology as benign cellular aspirate proved to be pleomorphic adenoma while two cases diagnosed as cystic lesion on cytology proved to be Warthin tumor). No malignant case was found on histopathology. Overall ROM for Category I and II was 0%.

Category III consisted of smears having low cellularity with metaplastic changes and atypical cells inconclusive of malignancy. Histopathological follow-up of four cases was available. Two cases were diagnosed as pleomorphic adenoma, one case as mucoepidermoid carcinoma (Fig. 1), and one case as low-grade lymphoma. Overall ROM for this category was 50%.

Category IV A had histopathological follow-up of 34 cases out of 120 cases. Out of ten cases of basal cell adenoma diagnosed on cytology, histopathology was available in three cases (two cases proved to be of pleomorphic adenoma while one as basal cell adenoma). Nineteen cases of pleomorphic adenoma showed concordance on histopathological follow-up (Fig. 2), while four proved to be malignant (three cases as adenoid cystic carcinoma and one case as low grade mucoepidermoid carcinoma). One case of Warthin tumor diagnosed on cytology proved to be of mucoepidermoid on follow-up. One case of oncocytoma proved to be of Warthin tumor on histopathology (Fig. 3). Overall ROM reported in this category was 14.7%.

Seven cases were reclassified in Category IV B, where cytomorphological features were suggestive of a neoplasm but distinction between benign and malignant neoplasm was not possible. Histopathology was available in three cases; one case was diagnosed as pleomorphic adenoma (Fig. 4) and two cases as adenoid cystic carcinoma. Overall ROM calculated as 66.6%.

Category V had follow-up in six out of ten cases. Five cases proved to be malignant while one case revealed disconcordance (one case reclassified as suspicious for adenoid cystic carcinoma proved to be cellular pleomorphic adenoma on follow-up (Fig. 5), with ROM of 83.3%.

In Category VI, follow-up was available in three cases out of 15, all proved to be malignant with risk of malignancy 100% (Fig. 6).

Overall concordance was noted in 66 cases (Tables 1 and 2). There were 12 true positive, 60 true negative, four false positive, and five false

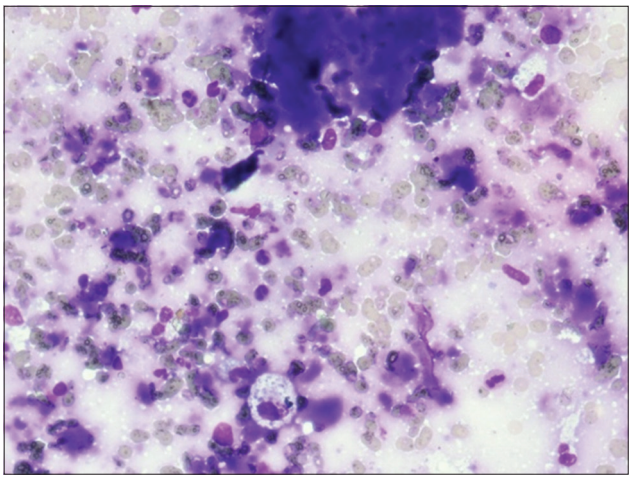

Fig. 1: AUS – This aspirate shows abundant background mucin, few atypical cells, debris, and few vacuolated epithelial cells which were not sufficient for a specific diagnosis. Follow-up proved to be mucoepidermoid carcinoma (MGG ×400)

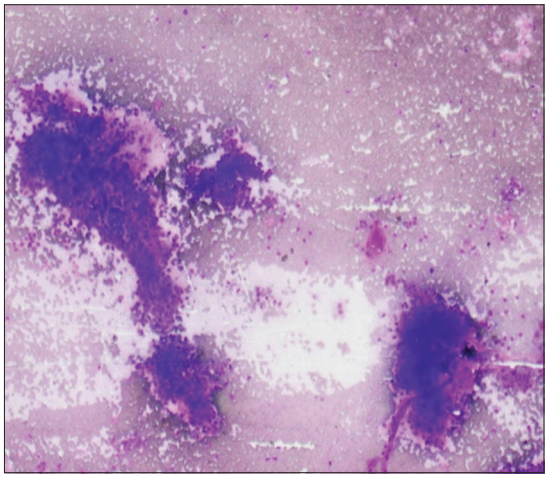

Fig. 2: Benign neoplasm – Smears show classical features of pleomorphic adenoma with magenta staining fibrillary matrix and myoepithelial cells (MGG ×100)

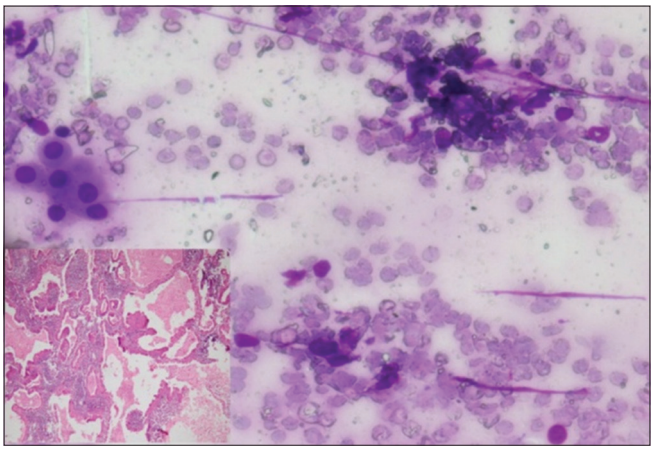

Fig. 3: Benign neoplasm – Smears show few sheets of on cocytes with debris in the background diagnosed as oncocytoma. Follow-up proved to be Warthin tumor (MGG ×400). Inset shows Warthin tumor on HPE (H&E ×100)

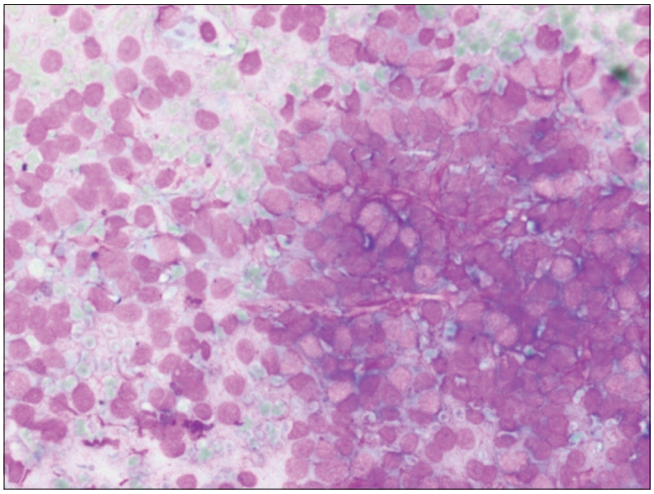

Fig. 4: SUMP – Smears show high cellularity of epithelial cells with focal nuclear atypia, loss of cohesion, and scant matrix. Follow-up proved to be cellular pleomorphic adenoma (MGG ×400)

Table 1: Cytological diagnosis and corresponding histopathological diagnosis

MSRSGC categories		No of cases	Histopathology available	Final histopathological diagnosis
I	Non diagnostic	4	1	Pleomorphic adenoma (1)
II	Benign cellular aspirate	18	5	Pleomorphic adenoma (3) Chronic sialadenitis (2)
	Acute sialadenitis	28	1	Acute sialadenitis (1)
	Chronic sialadenitis	51	11	Chronic sialadenitis (11)
	Sialadenosis	17	-	
	Granulomatous sialadenitis	6	-	
	Reactive lymphadenopathy	1	-	
	Cystic lesions	31	13	Retention cyst (11) Warthin tumour (2)
III	AUS	6	4	Pleomorphic adenoma (2) Lymphoma (1) MEC (1)
IV A	Basal cell adenoma	10	3	Pleomorphic adenoma (2) Basal cell adenoma (1)
	Pleomorphic adenoma	98	23	Pleomorphic adenoma (19) Adenoid cystic ca (3) MEC (1)
	Warthin tumor	9	7	Warthin tumor (6) MEC (1)
	Oncocytoma	2	1	Warthin tumor (1)
	Benign spindle cell lesion	1	-	
IV B	SUMP	7	3	Adenoid cystic ca (2) Pleomorphic adenoma (1)
V	Suspicious for MEC	7	4	MEC (4)
	Suspicious for Adenoid cystic ca	2	2	Adenoid cystic ca (1) Cellular pleomorphic adenoma (1)
	Suspicious for Ca Ex PA	1	-	
VI	MEC	6	3	MEC (3)
	Metastases	9	-	

MEC: Mucoepidermoid carcinoma, Ca Ex PA: Carcinoma ex pleomorphic adenoma

negative cases. Overall sensitivity, specificity, positive predictive value, and negative predictive value were 70.58%, 93.75%, 75%, and 92.3%, respectively. Overall diagnostic accuracy was 88.89%.

DISCUSSION

FNAC is a safe and minimally invasive first line diagnostic tool for the evaluation of salivary gland lesions and it provides useful information for clinical management of patients [7-9]. MSRSGC is a newer system

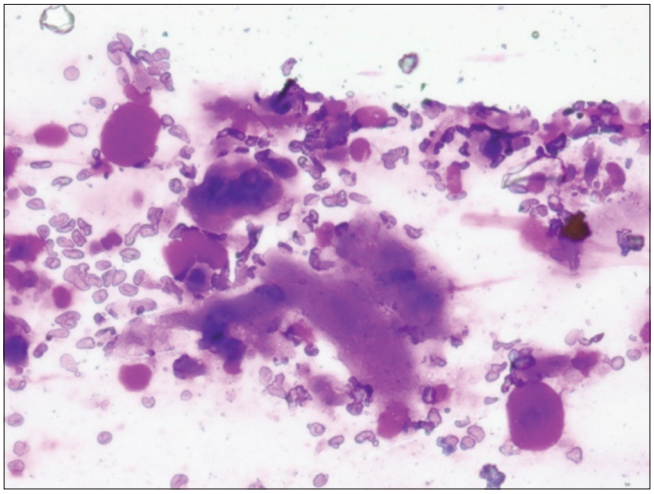

Fig. 5: Suspicious for adenoid cystic carcinoma – Smears show few hyaline globules with groups of cells having scanty cytoplasm and high N/C ratio. Typical spherical globules with adherent tumor cells absent. Follow-up proved to be cellular pleomorphic adenoma (MGG ×400)

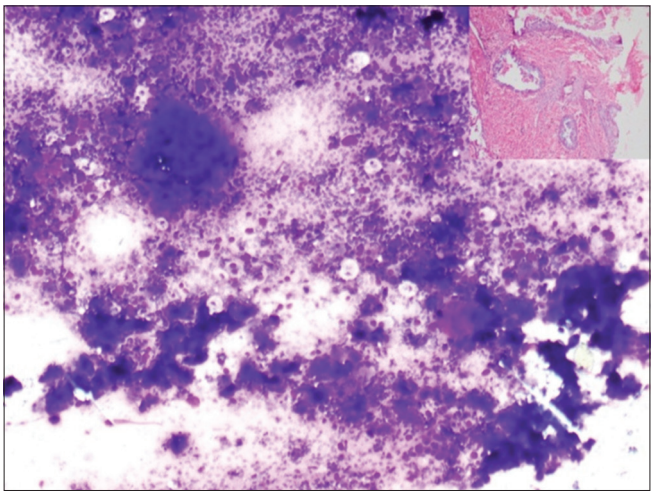

Fig. 6: Malignant – Smears show abundant background mucin and debris along with loose sheets of epithelial cells and mucinous cells. Follow-up proved it to be mucoepidermoid carcinoma (MGG ×100). Inset shows mucoepidermoid carcinoma on HPE (H&E ×400)

which classifies cytology of salivary gland lesions into six categories with ROM of 25%, 10%, 20%, 5% (IV A), 35% (IV B), 60%, and 90% for each category [5,6,10]. In our study, ROM for six categories is 0%, 0%, 50%, 14.7% (IV A), 66.66% (IV B), 83.3%, and 100%, respectively, and results are comparable to that provided in MSRSGC and other studies [5,11-13].

The ROM may represent an overestimation because it is based on cases that have undergone surgical excision and may have been impacted by patient demographics and institutional referral patterns [5].

The present study has maximum cases in non-neoplastic category (48.4%) followed by benign neoplastic category (38.21%) which is similar to study done by Kala *et al*. [11].

Cytohistological disconcordance (Table 3) constitutes pitfalls in diagnosing salivary gland lesions on cytology. Disconcordance in our study was 17.2%. Our findings are similar to those of previous studies

Table 2: Cytological diagnosis, histopathological follow-up, concordance, disconcordance, and ROM

	Cat I	Cat II	Cat III	Cat IV A	Cat IV B	Cat V	Cat VI
Number of cases N (%)	4 (1.27)	152 (48.4)	6 (1.91)	120 (38.21)	7 (2.22)	10 (3.18)	15 (4.77)
H/P follow up (n)	1	30	4	34	3	6	3
Benign non neoplastic, n (%)	0	25 (83.3)	0	0	0	0	0
Benign neoplastic, n (%)	01	5 (16.6)	2 (50%)	29 (85.29)	1 (33.3)	1 (16.7)	0
Malignant, n (%)	0	0	2 (50%)	5 (14.7)	2 (66.6)	5 (83.3)	3 (100)
Concordance, n (%)	-	25 (83.3)	2 (50%)	29 (85.29)	2 (66.6)	5 (83.3)	3 (100)
Disconcordance, n (%)	-	5 (16.6)	2 (50%)	5 (14.7)	1 (33.3)	1 (16.7)	0
ROM (%)	0	0	33.3	14.7	66.6	83.3	100

H/P: Histopathology, ROM: Risk of malignancy

Table 3: Cytohistological correlation of disconcordant cases

MSRSGC	No. of cases	Cytological diagnosis	H/P Follow-up
I	-	-	
II	5	Benign cellular aspirate (3)	Pleomorphic adenoma (3)
		Cystic lesion (2)	Warthin tumor (2)
III	2	AUS (2)	pleomorphic adenoma (2)
IV A	5	Pleomorphic Adenoma (4)	Adenoid cystic ca (3)
			Low-grade mucoepidermoid ca (1)
		Warthin Tumor (1)	Mucoepidermoid ca (1)
IV B	1	SUMP	Pleomorphic adenoma (1)
V	1	Suspicious of adenoid cystic ca.	Cellular pleomorphic adenoma
VI	0		
Total	14/81		

conducted by Rohilla *et al.* [12] and Omhare *et al.* [14]. Disconcordance rates previously observed by various authors range between 6.9% and 21.8% [12,15-18].

Category I had histopathological follow-up in one out of four cases, which was diagnosed as pleomorphic adenoma. In two cases, fluid was aspirated with presence of occasional cystic macrophages and inflammatory cells. One had blood and few cells not meeting the criteria for adequacy. In such cases, authors suggest multiple passes from different planes and if still non-diagnostic and reaspiration under ultrasound guidance. ROM for this category was 0% which is low as proposed by Milan system [5]. Reason may be low sample size in this category.

Category II included 152 cases with histopathological follow-up available in 30 cases. Thirty-one cases presented as cystic lesion on cytology. Cystic lesions formed an important area of diagnostic pitfall as these can include a wide variety of lesions, namely, benign cysts comprising of simple retention cyst, mucocoele, lymphoepithelial cyst, along with benign tumors such as Warthin tumor, cystic pleomorphic adenoma, and malignant cystic lesions such as mucoepidermoid tumor and acinic cell carcinoma [12,19]. Three cases of benign cellular aspirate proved to be of pleomorphic adenoma. Review of these cases showed absence of typical stroma and scant cellularity. Two cases of cystic lesions proved to be of Warthin's tumor on histopathology. This is because cystic degeneration is commonly seen in Warthin's tumor. Review of this case showed smears to be of low cellularity with an occasional cluster of oncocytic cells which was missed. Aspirate from multiple sites in different planes along with aspiration of any residual mass under ultrasound guidance can help to achieve a more specific cytodiagnosis and to avoid false negative report in such cases. [19]. No malignant case was reported on follow-up, so ROM was 0%. This is in agreement with the proposed Milan system where the ROM ranges between 0–20% with an average of 10% [5]. Similarly, various studies have shown a risk of malignancy in concordance with our study [12,19,20].

In Category III, histopathology was available in four out of six cases. Two cases proved to be of pleomorphic adenoma. These cases had focal areas of high cellularity and few cells showing metaplastic changes, so were put in Category III on cytology. However, characteristic chondromyxoid stroma seen typically in pleomorphic adenoma was not appreciated in smears. One case showed few cells having atypia which turned out to be mucoepidermoid carcinoma. Review of this case revealed mucin in the background. Another case revealed prominent lymphoid component with limited atypia. Histopathology revealed low-grade lymphoma. ROM was 50%. This suggests that lesions were not adequately sampled on FNAC. It is suggested that repeat FNAC under ultrasound guidance/surgery as recommended by Milan system are appropriate management strategies for this category. In aspirates with atypical lymphoid proliferation, flow cytometry/immunocytochemistry should be considered to rule out a lymphoproliferative disorder [5].

Category (IV A) has 120 cases with histopathological follow-up available in 34 cases. This category revealed five malignant cases on histopathology. Out of four cases diagnosed as pleomorphic adenoma on cytology, three proved to be of adenoid cystic carcinoma, and one case as low-grade mucoepidermoid carcinoma on histopathology. Review of the three cases of adenoid cystic carcinoma showed that the aspirates were highly cellular with scant matrix. Such cases should be interpreted with caution as hyaline globules of adenoid cystic carcinoma can mimic matrix in globules of pleomorphic adenoma [5]. Review of the fourth case which proved to be low-grade mucoepidermoid carcinoma showed that the matrix had mucoid appearance rather than fibrillary which was missed. One case of Warthin tumor diagnosed on cytology proved to be low-grade mucoepidermoid carcinoma on follow-up. Cystic fluid was aspirated in this case. Smears revealed low cellularity with minimal nuclear atypia and presence of oncocytic cells. Review of smears revealed mucin and which was missed and led to this diagnostic pitfall. Low-grade mucoepidermoid carcinoma is a common cause of false negative salivary gland FNAC. If cystic fluid is obtained, the lesion should be reaspirated from solid areas and any background mucin in such cases should be interpreted with caution. Risk of malignancy in this category was found to be 14.7% which is in concordance with study done by Jha *et al*. [21], Kala *et al*. [11], and Rohilla *et al*. [12]. Conservative surgery is the best management for this category.

Category IV B had follow-up in three cases out of seven. One case was histopathologically diagnosed as cellular pleomorphic adenoma. This case had high cellularity of ductal epithelial cells with scanty matrix

and squamous metaplasia on cytology. Other two cases had occasional hyaline globules with few clusters of small basaloid tumor cells. They proved to be cases of adenoid cystic carcinoma. Surgical excision is generally indicated for such cases or repeat aspiration combined with immunocytochemistry may yield a more specific diagnosis. In our study, risk of malignancy of this category was found to be 66.6% which was in correlation with various studies [11,12,20,21].

Category V had follow-up in six cases out of ten. Seven cases were reclassified as suspicious for mucoepidermoid carcinoma, of these; four had follow-up and proved to be mucoepidermoid carcinomas. One case reclassified as suspicious for adenoid cystic carcinoma on cytology proved to be cellular pleomorphic adenoma with squamous metaplasia on follow-up. The possible explanation for misdiagnosis on cytology could be due to metaplastic cells simulating atypical cells of a malignant tumor. ROM for this category our study was 83.3%, which is in line with the results of the previous studies [11,12,20].

Category VI included 15 cases with histological follow-up available in three cases all of which were malignant, thus giving ROM of 100%, which is in concordance with the previous studies [20-22]. All lesions in this category must be managed by surgery.

By incorporating MSRSGC for diagnosing salivary gland lesions, a sensitivity, specificity, and accuracy of 70.58%, 93.75%, and 88.89%, respectively, were obtained. Similar results were obtained by Rajwanshi *et al.* [7] and Singh *et al.* [19].

CONCLUSION

MSRSGC is a useful system for risk assessment and deciding further treatment protocol. The smaller number of cases reclassified on cytology and even a lesser number of cases that we received for histopathological correlation along with the retrospective study design are the limitation of this study. Further studies including larger sample size utilizing proposed Milan plan are required for prospective application of the study.

AUTHOR CONTRIBUTION

Dr. Poonam Singal: Writing of manuscript and interpretation of data. Dr. Monika Garg: Writing of manuscript and analysis of data. Dr. Amrinder Kaur: Proof reading of manuscript. Sukriti Bansal: Collection of data and statistical analysis.

CONFLICT OF INTEREST

None.

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