ORIGINAL RESEARCH

Cytomorphological Study of Salivary Gland Lesions using the Milan System of Reporting

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Abstract
Background: Fine-needle aspiration cytology (FNAC) of salivary gland is used worldwide for the diagnosis and management of salivary gland tumours. It provides a minimally invasive, quick, safe, cost-effective, and accurate outpatient technique that is extremely useful in identifying a substantial subset of salivary gland lesions and thus reduces unnecessary invasive surgical procedure in patients.
Objective
1. To study the pattern of the lesions of salivary glands by "the Milan system of reporting for Salivary Gland Cytopathology".
2. To categorize the cytopathology of salivary gland lesions by the Milan system.
3. To create a uniform reporting system for salivary gland cytopathology.
4. To assist in the effective communication of the results to the clinicians.
5. To compare the cytopathology diagnosis with histopathology (wherever available).
Materials and Methods: The present study was carried out in the Department of Pathology, Pt. J.N.M. Medical College, Raipur (CG) and associated Dr. Bhim Rao Ambedkar Memorial Hospital Raipur (CG), prospectively for two years. Approval from the Institutional Ethics And Scientific Committees was obtained.
Result: Out of the total 86 cases, 54 were males and 32 were females. The Male: Female ratio was 1.6:1.The mean age of the subjects was 45.5 years. Under the Non-Neoplastic category, Sialadenitis was the most frequent diagnosis comprising 14 cases (51.9%). A total of 21 cases out of 86 cases were reported in the Benign neoplasm category. Pleomorphic adenoma was the most common diagnosis comprising 17 of 21 benign cases (80.9 %). Mucoepidermoid Carcinoma was the most frequent malignancy, comprising 8 cases out of 25 (32%) cases under malignant category. ROM (risk of malignancy) for each category was calculated. ROM for non-diagnostic, non-neoplastic, AUS, benign neoplasm, SUMP, SFM, and malignant categories were 0%, 14.2%, 0.0%, 7.6%, 0%, 66.6%, and 100% respectively.

Conclusion: The FNAC reporting with the MILAN system, when undertaken meticulously, is an effective way of communication with the clinicians, avoiding a surgical procedure in some cases and thereby tremendously aiding the patient care in terms of reducing financial burden and most significantly, avoiding psychological trauma. Considering the high accuracy, sensitivity and specificity rates of FNAC diagnosis observed in the present study, it can be aptly concluded that FNAC when categorized by the MILAN system, further improves diagnosis, communication and risk stratification.

Key words: Salivary gland, MILAN, Cytology.

Introduction

Fine-needle aspiration cytology (FNAC) of salivary gland is used worldwide for the diagnosis and management of salivary gland tumours. It provides a minimally invasive, quick, safe, cost-effective, and accurate outpatient technique that is extremely useful in identifying a substantial subset of salivary gland lesions and thus reduces unnecessary invasive surgical procedure in patients. In addition, it guides the further management strategy.[1-5] A separate meta-analysis suggested that inclusion of salivary gland FNAC in clinical decision making can reduce the overall cost of treating salivary gland tumours.[6] Many studies have reported excellent sensitivity and specificity of FNAC to differentiate between neoplastic and non-neoplastic lesions. Among different studies, the sensitivity of FNAC ranges from 86% to 100%, and specificity ranges between 90%–100%.[7-11] When FNAC is used to sub classify the neoplasm, the accuracy ranges drop to 48% to 94%.[1,12-13]. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) is a standardized reporting system designed to address the above-mentioned limitations and facilitate communication between pathologists and clinicians, with the ultimate aim to help guide the clinical management of salivary gland lesions. The MSRSGC is a seven tiers diagnostic system of cytology findings for salivary gland lesions. Each category has a corresponding suggestion of risk of malignancy (ROM) and suitable management strategies. It would also allow exchange of data between various laboratories.[14-16] The present study aims to categorize salivary gland lesions as per MSRSGC that aids in communication between cytopathologists and clinicians, evaluate proposed Risk of Malignancy (ROM) and thus recommend for clinical intervention, enhancing patient care.

Aim and Objectives

Aim

To study the pattern of the lesions of salivary glands by "the Milan system of reporting for Salivary Gland Cytopathology".

Objectives

1. To categorize the cytopathology of salivary gland lesions by the Milan system.
2. To create a uniform reporting system for salivary gland cytopathology.
3. To assist in the effective communication of the results to the clinicians.
4. To compare the cytopathology diagnosis with histopathology (wherever available).

Material and Methods

The present study "Cytomorphological study of salivary gland lesions using The Milan System of Reporting" was carried out in the Department of Pathology, Pt. Jawahar Lal Nehru

Memorial (Pt. J.N.M.) Medical College, Raipur (Chhattisgarh) and associated Dr. Bhim Rao Ambedkar Memorial (Dr. B.R.A.M) Hospital Raipur (CG) prospectively for two years, from Dec. 2018 to Aug. 2020. Approval from the Institutional Ethics And Scientific Committee was obtained.

Design
Observational, hospital based.

Data Collection
Cross sectional.

Study Setting
Department of Pathology Pt. J.N.M Medical College Raipur (C.G) and associated Dr. B.R.A.M. Hospital, Raipur (CG).

Study Period
Two years.

Inclusion criteria
The present study included a total number of 86 cases presenting to the outpatient department with salivary gland lesions referred for cytology. Patients willing to participate in the study were requested to fill a consent proforma.

Exclusion criteria
Excluded were those subjects with lesions contraindicated for FNAC, patients not willing to participate in the study, or refusing consent for the FNAC procedure.

Result
The study included 86 patients with salivary gland swellings referred to cytology section. The patients FNAC diagnoses were categorized as per MSRSGC. Histopathological diagnoses were available in 44 cases.
Out of total 86 cases, 54 were males & 32 were females, as in Table no. 6 and Chart No. 1. The M: F ratio was 1.6:1
The present study included total number of 86 patients. The youngest patient was of age 11 years, and the oldest patient was of age 85 years. A maximum number of cases were in the age group of 61-70 years. The mean age was 45.5 years

Chart No. 1: Gender Wise Distribution of Cases

Chart 2: Age Wise Distribution Of The Cases
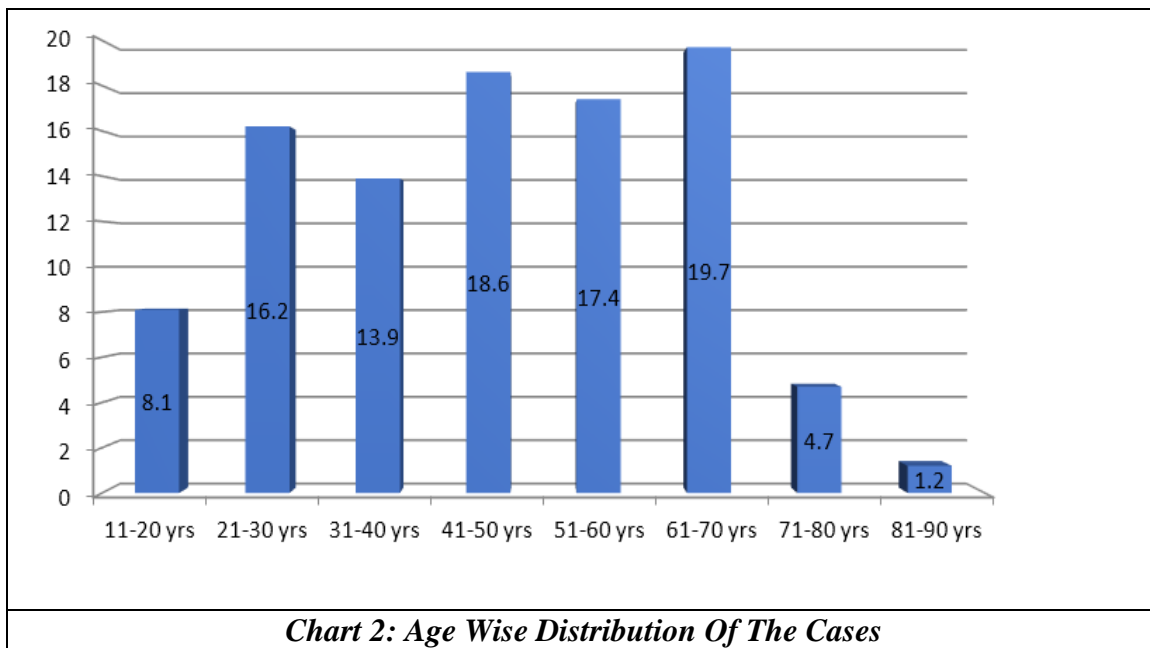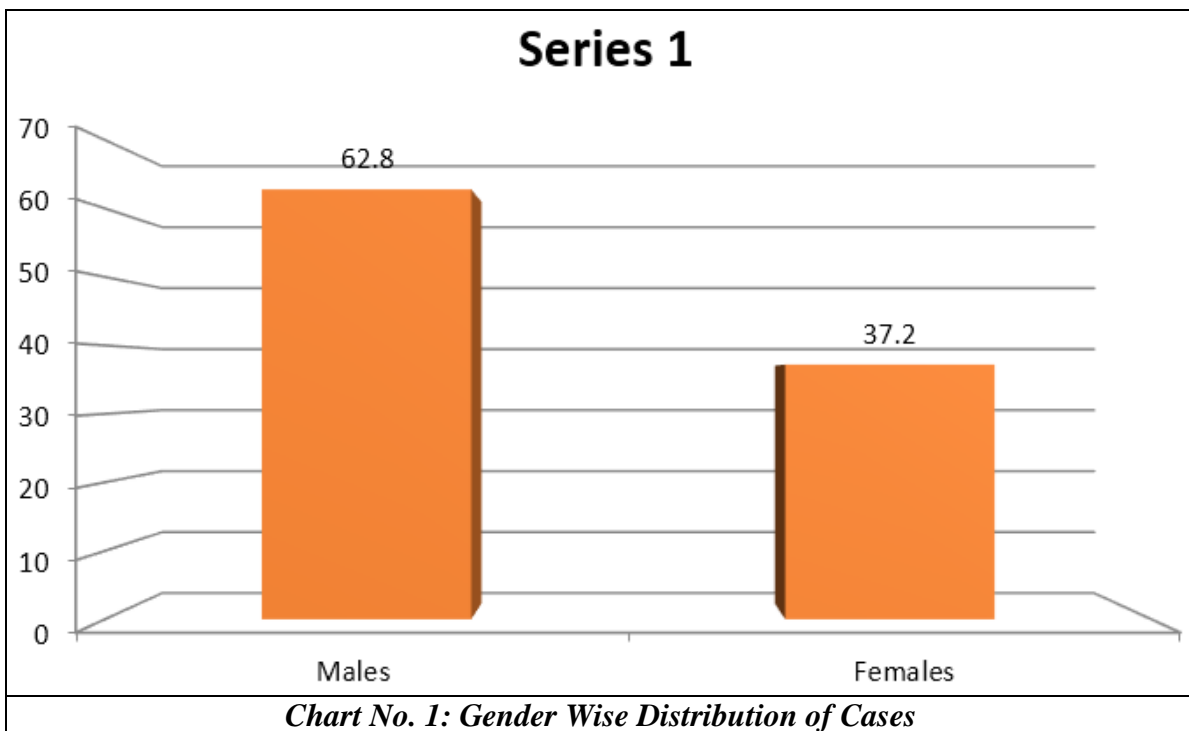
The study included patients attending the OPD for salivary gland swellings, being referred subsequently to the cytology section of Department of Pathology.

Out of a total 86 patients, maximum number of 54 patients had swellings in the parotid region, which contributed 62.8 % of all cases.

Chart No. 3: Site Wise Distribution Of The Cases
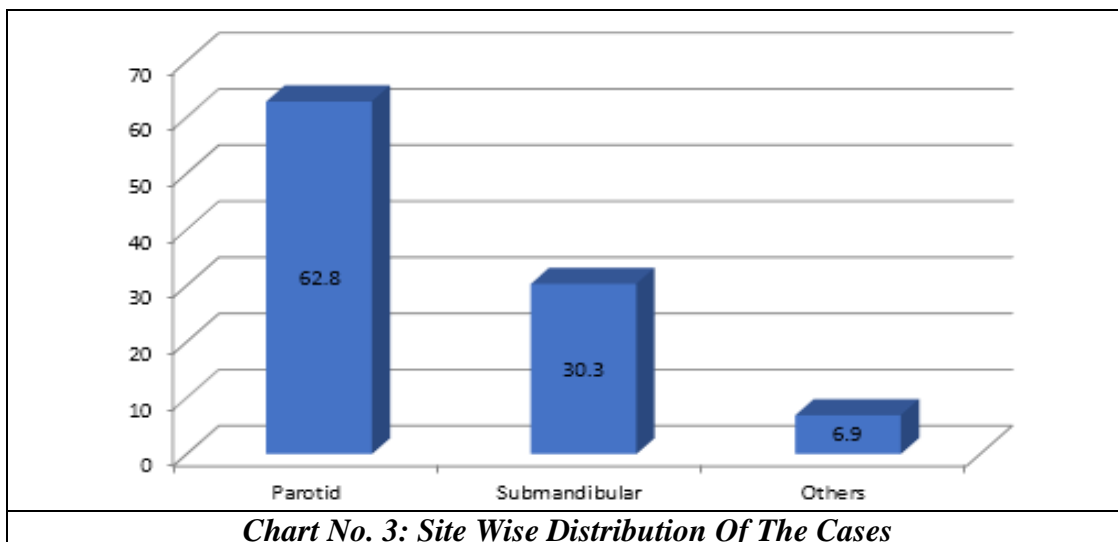
All cases were categorized by cytology as per the MSRSGC within I - VI groups as depicted in table 1

| Categories	No. of cases	Percentage (%)
Category I :Non-diagnostic	7	8.1
Category II. Non-Neoplastic	27	31.4
Category III. Atypia of undetermined significance (AUS)	2	2.3
Category IV. Neoplasm A. Neoplasm: Benign	21	24.4
B. Neoplasm: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)	0	0.0
Category V. Suspicious for malignancy	4	4.7
Category VI. Malignant	25	29.1
Table No. 1: Categorization of Cases Based on MSRSGC		

A total of 27 cases fell in the Non- Neoplastic category of MSRSGC of which Sialadenitis was the most frequent non-neoplastic diagnosis comprising 14 cases.

Chart No. 4: Distribution of the Frequency of Non-Neoplastic Category
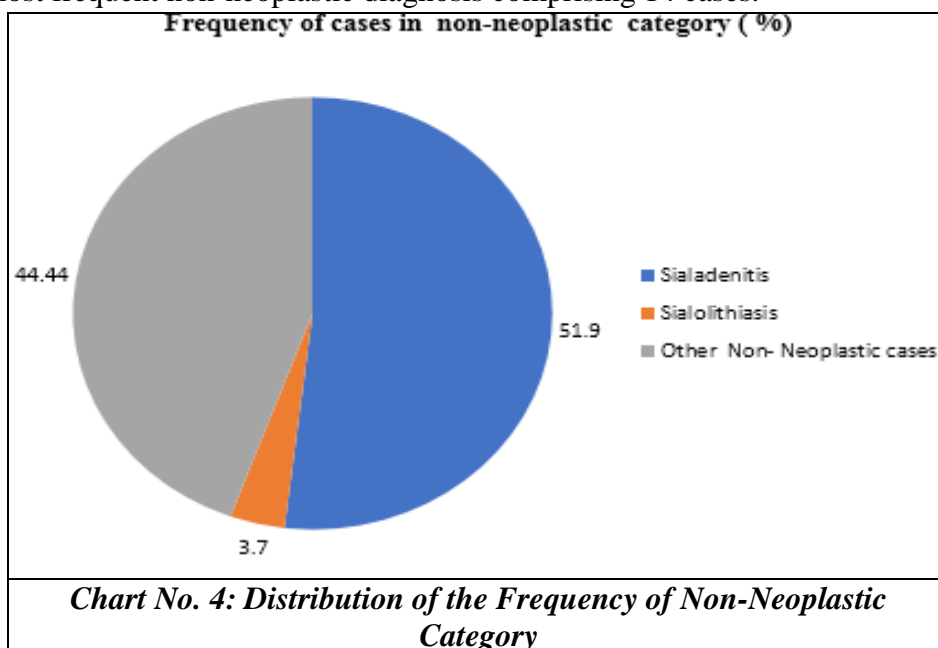
A total of 21 cases out of 86 cases fell in the Benign neoplasm category of MSRSGC. Pleomorphic adenoma comprised 17 of 21 cases.

Chart No. 5: Distribution of the Frequency Of benign Neoplasm Category
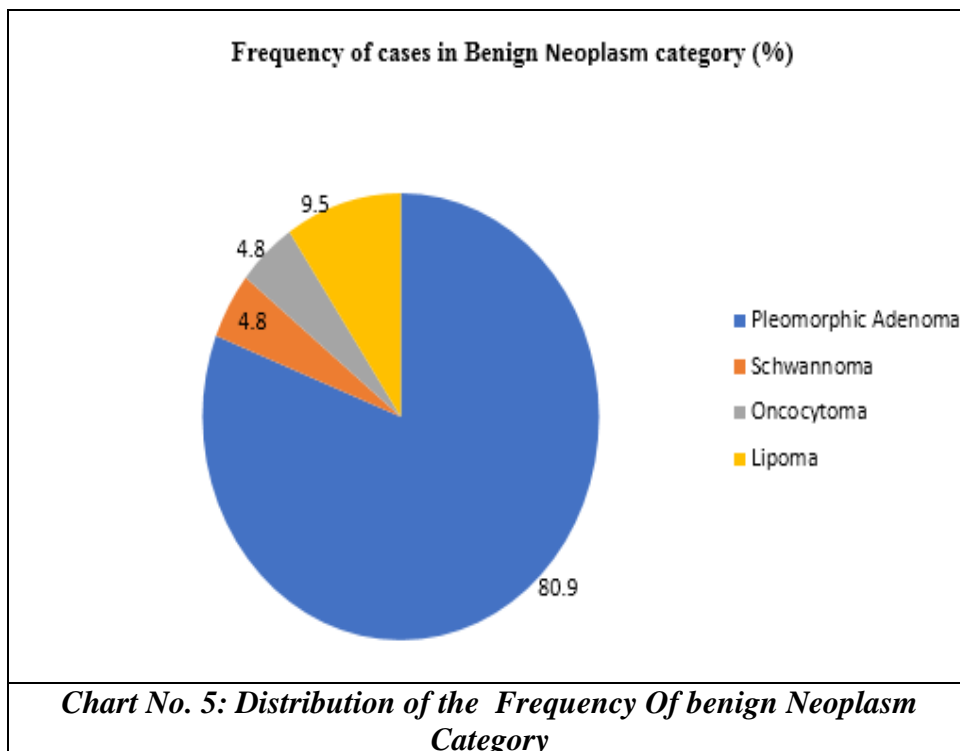
A total of 25 out of 86 cases, fell in the Malignant neoplasm category of MSRSGC of which Mucoepidermoid Carcinoma was the most frequent malignancy comprising 8 cases (32%).

Chart No. 6: Distribution of the Frequency for Malignant Neoplasm Category
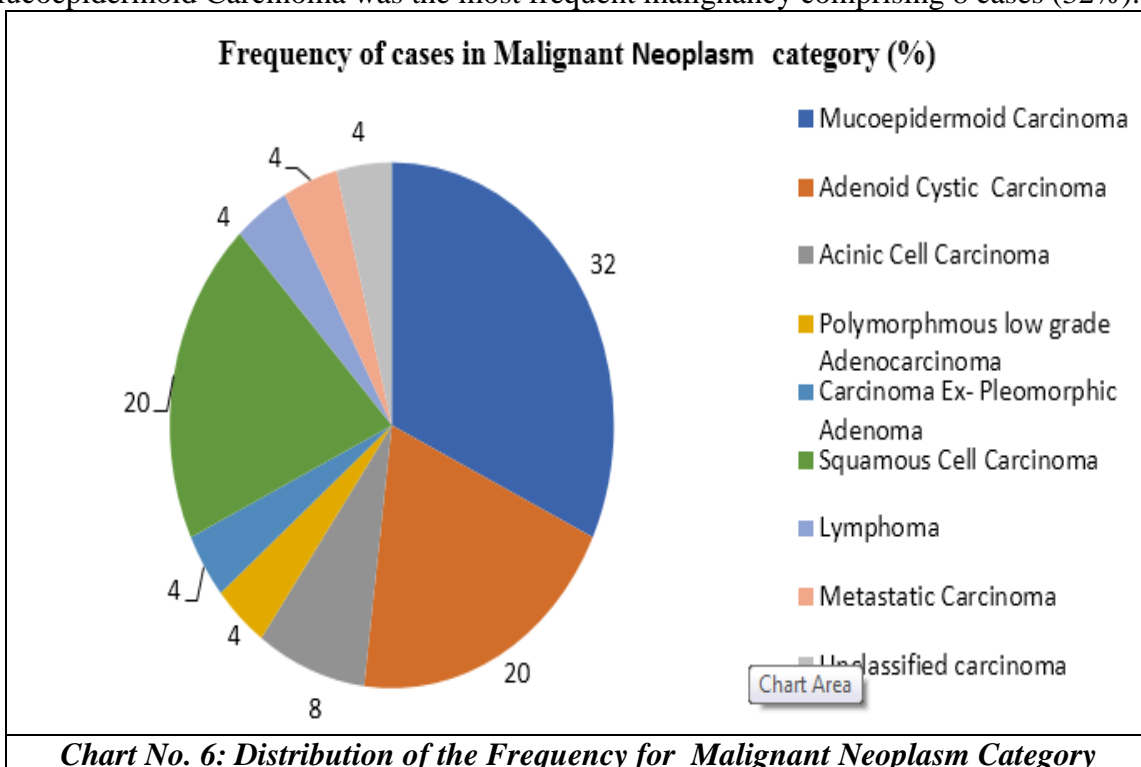
The cytological reports of MSRSGC were compared with the histopathological diagnosis. Out of the 44 available cases for histopathological comparison, there were 41 concordant and 03 discordant cases. The concordant ratio was 93.2 % and discordant ratio was 6.8%. Out of 86 cases 44 specimens were available for histopathological examination. There was a single false positive case (2.2%) and 2 false negatives cases (4.5%). In the present study, out of the cases in the non- neoplastic category, one case was diagnosed as mucoepidermoid

carcinoma on histopathology. ROM in this category was calculated as 14.2%. Among the cases in benign neoplasm category, one case was diagnosed as Adenoid Cystic Carcinoma on histopathology. A ROM of 7.6% was calculated for this category. In the suspicious for malignancy, and malignant categories, a ROM of 66.6% and 100% respectively was calculated. The kappa statistic for the degree of agreement between FNAC and histological examination results was 0.86. The chi- square statistic with Yates correction was 29.5155. The p-value was <0.00001. (Significant at p<0.05). The observations of the study were found to be statistically significant.

Discussion

A total of 86 cases were included in the study, of which 54 (62.8%) were males and 32 (37.2%) were females, with a Males: Females ratio of 1.6:1. Similar study of **Chen et al (2019)**[17] in which M:F =1.5:1 was observed, where 338 patients were included, with 203 males and 135 females. **Pujani et al (2018)**[18] found similar results with M:F= 1.4 :1 with 88 males and 62 females. **Hafez et al (2019)**[19] also studied 118 cases where M:F= 1.3:1 where 66 patients were male and 52 patients were females. However **Erkka Tommola et al (2019)**[20] observed 138 cases with M:F = 0.86:1 where females (74) outnumbered males (64). **Griffth et al (2018)**[21] also studied 281 cases where 137 males and 144 females, with M:F= 0.95:1. In the present study, age of the patients ranged from 11 to 85 years. Out of 86 cases, most cases were in the 6th or 7th decades of life with a maximum in the age group 61-70 years (17 out of 86). The Mean age observed was 45.5 years. **Das et al (2004)**[22] observed 712 patients aged between 6 months to 91 years with peak incidence in the 3rd to 5th decade with mean age 37 years. **Geir Tryggvason et al (2013)**[23] studied 543 cases and found 144 cases were 66 yrs or older (26.5%) and mean age was 54.1 years. **Rossi et al (2017)**[24] observed 515 cases with age ranging from 1-43 years and mean age of 16.4. **Karuna et al (2019)**[25] studied 105 cases, highest incidence (23.8%) was found between 31 and 40 years. **Kala et al (2019)**[26] also observed 293 cases according to age and found largest number of cases were seen in age group 21 to 40 years (46.1%) followed by 41–60 year age group (27%). **Katta et al (2019)**[27] observed salivary gland pathologies most commonly in the fifth decade of life confirming 37.68% of cases.

In the present study, out of a total 86 patients, the most common site involved was parotid, with 54 patients which contributed 62.8 % of all cases, followed by submandibular comprising of 26 cases (30.3%) and 6 patients (6.9 %) had swelling at other sites (cheek, sublingual). Similar findings were observed in the studies of **Pujani et al (2018).**[18] wherein 94 out of 150 cases (62.6%) presented with parotid swellings and 51 cases (34%) involved submandibular gland swellings and other sites' involvement in 5 cases (3.3%). **Karuna et al (2019)**[25] studied 150 cases where 59 % had parotid gland involvement followed by submandibular gland (31.43%) and other sites 9.5%. **Hafez et al (2019)**[19] found that the most frequently involved salivary gland was the parotid gland (109 cases, 92.4%) followed by the submandibular gland (9 cases, 7.6%) with no other minor salivary gland involved. **Maleki et al (2019)** [28] observed that up to 80% of salivary gland neoplasms originate in the parotid gland, whereas only 7% to 11% occur in the submandibular glands.

Categorization Of Cases Based On MSRSGC
Distribution of the frequency of cases in Non-Diagnostic Category
In the present study, 7 cases out of 86 with 8.1% were Non-Diagnostic(ND) on FNAC. The MSRSGC rate of ND reporting is 10%, however, Rossi et al. in a survey of over 500 cytopathologists reports that approximately 50% or participants felt that 10 − 15 % is an acceptable upper limit for "nondiagnostic/unsatisfactory" cases[29]. Usually, those cases warrant a repeat FNA. Similar studies by **Savant et al (2018),**[30] **Viswanantan et al (2018)**[31] **and Kala et al (2019)**[26] have ND cases 9.2%, 12% and 6.1% respectively. The diagnostic terms "nondiagnostic", "inadequate", "insufficient", "unsatisfactory", "sampling error" were used in 67 studies. In 49 studies, these terms were used to describe the samples containing only blood, necrotic material, acellular cyst content, benign salivary gland tissue in the presence of a mass, or when there was insufficient cellularity or analyzable material to render a diagnosis based on the FNA specimen[32]

Distribution of the frequency of cases in Non-Neoplastic Category
There is a recognized risk of sampling error in this subset of salivary gland lesions and repeat sampling is highly recommended.[33] In the present study, 27 cases out of 86 were diagnosed under non –neoplastic category. Fourteen cases were Sialadenitis, 1 case was of sialolithiasis and other non neoplastic cases (Benign cystic lesion, inflammatory lesions, Suppurative lesions) constituted 12 cases. For comparison, the rate of nonneoplastic diagnoses is highly variable in the literature and ranges from 2% to 42%. Similar studies by **Pujani et al (2018)**[18] found Non-neoplastic cases accounted for 42% (63/150) with the most frequent diagnosis being sialadenitis (35/63) followed by sialadenosis. **Savant et al (2018)**[30] studied 199 cases and diagnosed 4 cases (2%) as non neoplastic which comprised cyst, florid non- necrotizing granuloma, lymphoepithelial cyst and sialodenitis and sialolithiasis. **Vishwananthan et al (2018)**[31] studied 627 cases and 179 cases were diagnosed as non neoplastic in which most common diagnosis was intraparotid Lymph node (36 cases). **Montezuma et al (2018)**[34] studied & compared old classification and Milan (new) classification system, found one case that was considered benign in the old classification and was not included in the NN or BN categories of the Milan classification corresponded to a cystic lesion with some atypical cells that was assigned as atypical by the new system.

Distribution of the frequency of cases in Atypia of undetermined significance (AUS)
The AUS category cases are those, where a neoplastic lesion cannot be completely ruled out. [18] In the present study 2 cases out of 86 (2.3%) were categorized as AUS. Both cases on histological follow up were found to be cases of sialadenitis and acute suppurative lesion. It was similar to the study of **Pujani et al (2018)**[18], where they found 2% cases as AUS. This is well within the goal of proposed MSRSGC system to keep the FNACs cases labelled as atypical to <10% [27]. **Kala et al(2019)**[26] observed 2.7% as AUS and found that presence of occasional basaloid cell with atypia might be the reason of categorization into AUS on FNAC. **Karuna et al (2019)**[25] observed that among 2 out of 76 cases of AUS, 1 case turned out to be pleomorphic adenoma and one case as low‑grade MEC (malignancy risk = 50.00%) on histological follow up.

Distribution of the frequency of cases in Benign Neoplasm category:

In the present study, 21 cases out of 86 (24.4%) were categorized as Benign Neoplasm (BN) where maximum cases were of Pleomorphic adenoma - 17 out of 21 (80.9%). Other 2 cases were Lipoma, 1 case Oncocytoma and 1 case Schwannoma. **Savant et al (2018)**[30] reviewed 199 cases and found 118 (59.2%) under Neoplasm Benign category, where Plemorphic adenoma was the most common diagnosis with 89 cases followed by other neoplasms such as Warthins Tumour, Monomorphic Adenoma and Oncocytoma. The diagnostic accuracy of this group was high due to the presence of the diagnostic features for these entities on cytology smears. Similarly, **Vishwananthan et al (2018)**[31] observed 627 cases and concluded that the most common benign neoplasm in the study cohort was pleomorphic adenoma (PA), comprising 16.9% of all cases. **Katta et al (2019)**[27] studied 69 cases and found 62.3% under Category IV A. **Kala et al (2019)**[26] studied 293 cases where BN comprised 98 cases 33.4%, and histological follow-up was available in 90 cases. PA constituted the highest percentage of all salivary gland neoplasm followed by Basal Cell Adenoma.

Distribution of the frequency of Cases in Suspicious for malignancy (SFM) category:

In the present study 4 cases out of 86 (4.7%) were categorized as Suspicious for malignancy. For 3 cases, histopathology tissue was available, wherein 2 cases were found to be malignant and 1 turned out be Warthins tumour. **Savant et al (2018)**[30] studied 199 cases and categorized 1. 5% cases in this category. They found that the presence of mucin, oncocytic cells and the absence of frank mitoses, or necrosis led to inclusion of cases in this category that were low grade mucoepidermoid carcinoma on resection instead of a higher category such as "Suspicious for Malignancy". **Farahani et al (2018)**[32] reviewed and meta-analyzed published literatures and found a total of 122 cases extracted from 17 studies were considered as "suspicious for malignancy" on FNA. On histologic follow-up, 4 cases were diagnosed as nonneoplastic, 43 as benign and 75 as malignant neoplasms. The present study findings are similar to study of **Karuna et al (2019),**[25] which studied 105 cases, 4.7% cases were categorized as SFM where in 76 cases histopathology was available, true malignancy was present in all 3/76 cases. **Chen et al (2019)**[17] observed 1020 cases and found 20 (2%) cases as SFM and after histopathological follow up of 7 cases which was available, two specimens (2/7, 28.6%) had benign histologic follow-up: one Warthin's tumor and one Chondroblastoma in the temporomandibular joint, with parotid gland involvement. Tyagi et al. reported that factors, such as poor stroma, hyaline globules, nuclear atypia and spindle shaped myoepithelial cells pose diagnostic challenges in this entity.[10]

Distribution of the frequency of cases in   Malignancy category:

The present study recorded 25 out 86 cases (29.1%) as malignant category, where most common malignancy was Mucoepidermoid carcinoma (8 cases out of 25) followed by adenoid cystic carcinoma (5 cases) and squamous cell carcinoma (5 cases), acinic cell carcinoma (2 cases), Polymorphous low grade adenocarcinoma , carcinoma ex pleomorphic adenoma, Lymphoma, metastatic carcinoma and other carcinoma each with 1 case. **Song et al (2018)**[35] reviewed 893 cases wherein 13.8% were categorized as malignant with most common malignancy as Squamous cell carcinoma (20 cases) followed by poorly

differentiated carcinoma (14 cases). Similar to our study of **Karuna et al (2019)[25]** wherein malignant cases were 17/105 (16.20%) with most frequent malignancy was Mucoepidermoid carcinoma (9 cases out of 105) followed by adenoid cystic carcinoma. **Tommola et al (2019)[20]** studied 138 patients with correlation to histopathology in 90 cases with surgical follow-up, there were 9 out of 90 (10%) cases that belonged to the category defined as malignant (Suspicious for Malignancy and Malignant Neoplasm) and all of them turned out to be true-positive cytological diagnoses. **Kala et al (2019)[26]** studied 293 cases where 172 cases had histological follow up, 44 cases (15%) were categorized as malignant, histopathological follow‑up of 40 cases was available, and 1 case was reclassified as PA, which was wrongly diagnosed as malignant: MEC on FNAC where presence of scattered atypical cells showing high nuclear cytoplasmic ratio lying in a myxoid background might had led to false diagnosis. **Maleki et al (2019)[28]** observed in multi- institutional study that, 'malignant' diagnosis was rendered for 13.9% of the cases (102 of 734); 61.8% (63 of 102) had surgical follow up. After the exclusion of lymphoma cases (21%), most malignant submandibular gland tumors in the study were adenoid cystic carcinoma (ACC; 20%), squamous cell carcinoma (20%), carcinoma ex pleomorphic adenoma (8.5%), or mucoepidermoid carcinoma (MEC; 7.5%).

Range of ROM from different studies when calculated category wise were as follows
Non- Diagnostic- 0%-33%, Non-Neoplastic-0 to 100%, AUS-0% -100%, Neoplasm: Benign-Neoplasm: Benign-0%-7.6%, SUMP-0%-50%, SFM- 0%-100%, Malignant- 93.3%-100%.
MSRSGC is based on risk stratification[19]. In the present study, category wise ROM was rendered and results were; Non‑diagnostic (ND)- 0.0%, which is similar with results of **Rohilla et al (2017) [36], Savant et al (2018) [30] , Karuna et al, (2019)[25],Tommola et al (2019)[20]** while **Hafez et al (2019)[19]** found 33.3% ROM as 3 cases which were interpreted as ND or non-diagnostic on cytology proved to be Warthin's tumor, pleomorphic adenoma and metastatic papillary thyroid carcinoma on histopathological follow-up.
In the Category II: Non‑neoplastic (NN)- ROM calculated was14.2%. Similar results were observed by **Hafez et al[19]** (2019) with 11% as ROM. Tommola et al [20] observed 100.0% ROM. The author found 1 case under non neoplastic category which was diagnosed as Lymphoma on histopathology. Thus ROM was biased by one falsely-negative case.
In the Category III: Atypia of undetermined significance (AUS), ROM was 0% contrary to study by **Rohilla et al (2017)[36]** with ROM 100%, where 2 cases were in the atypical category. These were the cases with which they had difficulty in distinguishing the aspirates as neoplastic or nonneoplastic. The smears in these 2 cases showed epithelial cells exhibiting mild atypia present against a mucinous background. In one case, the cytology was reported with a differential diagnosis of a retention cyst versus a cystic neoplasm, and in the other, the differential diagnosis was a retention cyst versus a low-grade MEC. Both cases turned out to be low-grade MECs on follow-up histopathology.
In the Category IVa: Neoplasm: benign (NB) ROM was 7.6%, similar to previous studies of **Rohilla et al (2017), Song et al (2019), Karuna et al (2019)** in which ROM was calculated as 7.3% , 2.2%, 2.4% in this category respectively.
In the Category IVb: Neoplasm: salivary gland neoplasm of uncertain malignant potential (SUMP) ROM was 0.0%, as none of the case was categorized as SUMP.

In the Category V: Suspicious of malignancy (SM) ROM was 66.6%, similar results were observed by previous study; **Hafez et al (2019)** with ROM 60%. While **Rohilla et al (2017)** calculated 0% ROM as there was no case in the suspicious category.

Similarly, 100% ROM was observed in Category 6: Malignant (M) which was also calculated by various other authors; **Savant et al (2018), Hafez et al (2019) and Tommola et al (2019).**

In the present study, after statistical analysis Diagnostic accuracy, Sensitivity, specificity, Positive predictive value and negative predictive value were 93.1 %, 91.3%, 95.2 %, 95.4 % and 90.9 % respectively. Sensitivity specificity ranged from 61.5% -94.6% and 84 %-100% respectively, as found in previous studies. After comparison of statistics of various studies, Chen et al (2019) [97] had Diagnostic Accuracy of 80.1% and highest Diagnostic Accuracy, 99% was observed by Thiryayi et al (2018) [113] In the present study, FNAC of salivary gland lesions were categorized as per MSRSGC and correlated with gold standard histopathology. The chi- square statistic with Yates correction was 29.5155. The p-value was <0.00001. (Significant at p<0.05). The observations of the study were found to be statistically significant. The kappa statistic for the degree of agreement between FNAC and histological results was 0.86.

Conclusions

The categorization of Salivary gland lesions using MILAN system is a reliable method when it is used for risk stratification. However, to obtain the benefit by the system, the FNAC diagnoses must be examined and compared with the final definitive diagnosis. Regular evaluation and audits of FNAC diagnosis with final paraffin section diagnosis should be conducted by both surgeon and pathologist as part of quality assurance and sustenance to determine the most appropriate management. It is important for the pathologist to obtain more clinical information, observation and to appreciate the clinical situation. In clinically suspected malignancy, more than one or repeat samples must be examined in order to decrease the false-negative diagnosis on FNAC. The pathologist faces diagnostic dilemmas not only because of overlap in morphological features among different types and grades of tumors, but also due to aberrant differentiation within the same lesions. Technical skill and diagnostic expertise are essential for FNAC diagnosis and categorization of cases by the MILAN system, therefore every surgeon and pathologist must accept certain limitations and responsibilities, enabling the patient receiving maximum benefit. We believe that critical self examination, strict quality control and pathologists' training could improve the accuracy rate even further. It will also serve to improve communication, and the understanding of the capabilities and limitations of the MILAN system. We believe that similar continuous monitoring of salivary gland reporting by MILAN system should be performed to recognize the benefits and the reasons of misinterpretations and, if possible, to reduce them and pave way for further improvement.

Within the scope and limitations of the present study, the following recommendations could be drawn:

1. Utilize MILAN system for all FNAC reporting of salivary gland pathologies and follow-up of all of the surgically treated cases.
2. Use of FNAC in conjunction with Rapid On Site Evaluation (ROSE) with clinical and radiological views in all cases to effectively reduce diagnostic dilemmas.

3. Organize expert training sessions and conduct regular self audits of FNAC reporting by MILAN system, in order to improve the accuracy further.

Conflict of Interest: Nil

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