The importance of team work of cytologist and surgeon in preoperative diagnosis of intraoral minor salivary gland tumours

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Case report

The Importance of Team Work of Cytologist and Surgeon in Preoperative Diagnosis of Intraoral Minor Salivary Gland Tumours

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A B S T R A C T

Tumours arising from oral minor salivary glands may exhibit an overlap of clinical and morphological features that may produce diagnostic and therapeutic dilemmas. The aim of this study is to asses the value of fine needle aspiration cytology (FNAC) in differentiation of benign and malignant tumours and to render a specific diagnosis. We evaluated the team work of surgeon and cytologist to improve diagnostic accuracy. Two steps are important for accuracy: sampling aspirate that should be done together by surgeon and cytologist and cytological microscopic analysis of the smears that should be performed by an experienced cytologist. The study included 132 patients with intraoral minor salivary gland tumours between 2002 and 2011. Adequate material was obtained from 121 (91.7%) patients. FNAC was usually performed by cytologist in a team with maxillofacial surgeon at cytology department that is more convenient for preparing the samples and especially for ROSE procedure (rapid-on site evaluation) of smears. In such a way the cytologist checked the adequacy of samples and decided whether some ancillary techniques should be used and therefore repeat FNAC. A total of 82 patients underwent surgery, 40 with malignant and 42 with benign tumours. Preoperative cytological diagnoses were compared with histopathological ones using histopathology as a gold standard. The most common benign tumour was pleomorphic adenoma and among malignant tumours adenoid cystic carcinoma. The most commonly affected site was the palate. The team work of surgeon and cytologist achieved specificity of 95.1%, sensitivity of 97.6% and diagnostic accuracy of 96.3%. We can conclude that although subclassification of some tumour types of salivary glands remains poor, FNAC is invaluable in patient triage and therefore should be considered in the first line investigations of these lesions by the cytologist and surgeon.

***Key words**: FNA, cytology, ROSE, team work, minor salivary gland, tumours, diagnostic accuracy*

Introduction

Salivary gland tumours comprise less than 3% of all tumours of head and neck: the parotid gland is the most frequently involved (64–80%) while minor salivary glands are less commonly affected (9–23%)[1,2].

The minor salivary glands are numerous – comprise 600 to 1000 small glands widely distributed throughout the upper aerodigestive tract. Although they seem to be relatively simple organs, they are composed of various epithelial cells like duct, basal, acinar and myoepithalial cells and non-epithelial cells like fibrous tissue, muscular or fat tissue cells. They are all capable of rapidly entering the cell cycle and are therefore possible targets for neoplastic transformation producing more than 60 types of different tumours that are divided into 5 main groups according to the World Health Organisation histological classification in 2005: malignant epithelial, benign epi-

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thelial, soft tissue, haematolymphoid and secondary tumours[2]. Perhaps no tissue in the body is more capable of producing such a diverse histopathological and cytological expression than salivary tissue.

Intraoral minor salivary gland tumours are unique in their morphological complexity, variability and pattern overlapping and therefore commonly result in diagnostic problems. They are less common than tumours of major salivary glands (generally account for 15% of all salivary gland neoplasms) and cause limited experience of cytologists as well as pathologists[3]. Another problem for cytological evaluation is sampling inadequacy because of the characteristics of the tumour itself like desmoplasia, cystic change or increased vascularity and because of the difficulties in reaching and aspirating the lesion[4].

Fine needle aspiration cytology (FNAC) has proved to be very useful in preoperative diagnosis of different salivary gland lesions preventing unnecessary operations in 70% of patients with a non-neoplastic lesion and in 79% of patients with a metastasis[5]. Heller et al. recommend the performance of fine needle aspiration in almost all patients with salivary gland lesions because cytological evaluation resulted in a change in the clinical approach to 35% of the patients[6]. Although definitive subclassification of some lesion types of salivary glands remains poor, FNAC is invaluable in patient triage[7]. Despite that, FNAC studies of the intraoral minor salivary gland masses are few and limited[8–11]. Most of them are case reports[12–19] or studies of minor and major salivary glands together[20–24] or studies of all lesions of oral and maxillofacial region[25,26].

The purpose of this study is to asses the value and accuracy of FNAC in the differentiation between cysts, benign and malignant tumours and, if possible, to render a specific diagnosis using histopathology as a gold standard. The surgical treatment can be very different, depending on the position, size and malignant or benign nature of the tumours. Therefore we want to point out the importance of team work of cytologist and surgeon and the presence of a cytologist on site for avoiding sampling error and improving the diagnostic accuracy knowing the clinical data about the patients and minor salivary gland lesions.

Patients and Methods

This is a retrospective study which includes a total of 132 patients with intraoral minor salivary gland lesions and to whom FNAC was performed at the Department of clinical cytology and cytometry at Dubrava University Hospital in Zagreb, Croatia.

At our hospital FNAC of minor salivary gland lesions have been performed by cytologist usually in a team with maxillofacial or oral surgeon because of the position of the lesion inside oral cavity that is sometimes difficult to reach (Figure 1). It can be done either at surgery clinic or at department of cytology that is more convenient for preparing the samples (smears for cytological examination and saline solutions for flow-cytometry) and especially for ROSE procedure (rapid-on site evaluation) of

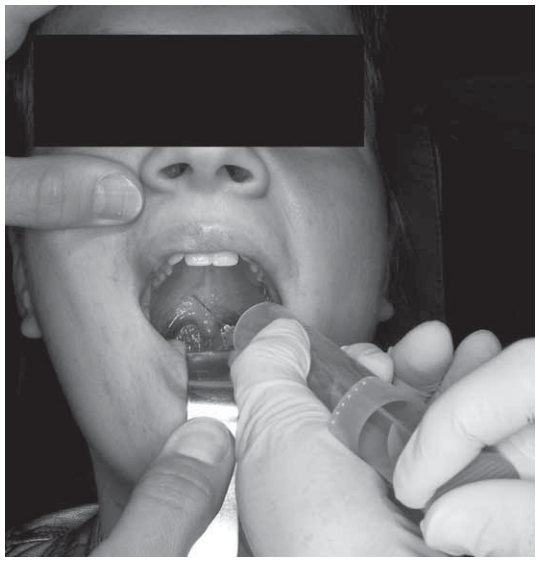

Fig. 1. Cytologist and maxillofacial surgeon together perform fine needle aspiration of intraoral lesion on palate.

smears. Usually one aspiration per lesion was performed in all patients. Prior to the aspiration, clinical history was taken (data about other diseases that patients suffer of and about therapy that has influence on cells' morphology like chemotherapy, radiation or hormones therapy) and physical examination was done after discussing the clinical details with the surgeon. FNAC was performed with or without local anaesthetic using 10 or 20 ml syringe and 23-gauge needle with or without syringe holder. The air dried smears were stained with Pappenheim and Hemacolor rapid staining and one slide per lesion was fixed in 96% ethyl alcohol and later stained with Papanicolaou technique. ROSE (rapid-on site evaluation) procedure includes rapid staining of chosen cytological slide that takes 30 seconds and on-site microscopic examination of aspirated material allowing cytologist to check the adequacy of samples and to decide whether some ancillary techniques are needed to establish a precise diagnosis and to subtype the aspirated tumours. FNAC was repeated if the slides were not adequate or if we needed more material to add cytochemistry, immunocytochemistry or flow-cytometry for immunophenotyping or DNA analysis to the standard cytological microscopic analyses. The cytological diagnoses were compared with histopathological ones using histopathology as a gold standard.

Results

Within 9,5 years, from January 2002 to July 2011, 132 FNAC of intraoral minor salivary gland lesions have been performed to 64 female and 68 male, ages from 9 to 101 years old (median 55 years) and processed at the Department of clinical cytology and cytometry in Dubrava University Hospital, Zagreb. We acquired adequate material from 121 (91.7%) patients and 11 aspirates were inadequate due to blood, few cells, matrix even when the FNAC was repeated. Out of 121 patients 82 (67.8%) un-

derwent surgery: 42 (51.2%) with benign and 40 (48.8%) with malignant tumours according to the histopathological diagnosis (Figure 2). The rest of 39 (32.2%) patients have not been operated on but have been followed up because the cytological diagnoses were inflammation or reparatory changes (Figure 2). The most commonly affected site was the palate in 49 (59.8%) patients followed by the buccal mucosa in 16 (19.5%) patients and the floor of the mouth in 8 (9.7%) patients (Table 1). The tongue, upper and lower lip were affected in only 9 (11%) patients (Table 1). Tumours arising in buccal mucosa were predominantly benign whereas those located in the palate and the floor of the mouth were mostly malignant (Table 1). The most common benign tumour was pleomorphic adenoma diagnosed in 25 patients followed by cyst diagnosed in 11 patients (Table 2). The rest of 6 be-

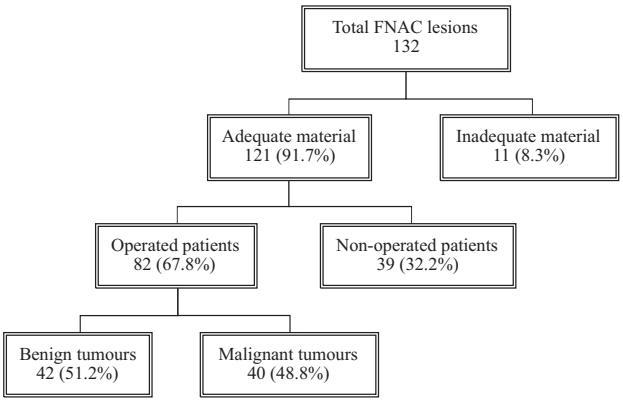

Fig. 2. Data of FNAC of small salivary gland lesions (n=132).

TABLE 1
ANATOMIC SITE DISTRIBUTION OF INTRAORAL MINOR SALIVARY GLAND TUMOURS (N=82)

Tumours	Anatomic site						
	Palate	Buccal mucosa	Tongue	Upper lip	Lower lip	Floor of the mouth	Total
Benign	23	10	2	1	3	3	42
Maligant	26	6	1		2	5	40
Total	49	16	3	1	5	8	82

TABLE 2
COMPARISON OF CYTOLOGICAL AND HISTOLOGICAL DIAGNOSIS OF INTRAORAL MINOR SALIVARY GLAND TUMOURS (N=82)

Cytological diagnosis		Histological diagnosis														
		MALIGNANT									BENIGN					
		adcc	mec	scc	cexpa	plga	acc	lec	NHL	expl	pa	ba	schw	cys	cana	myep
MALIGNANT	adcc	16				1[c]					1[a]					
	mec		9								1[a]					
	scc			2												
	cexpa				2											
	plga					4										
	acc						2									
	lec							1								
	NHL								1							
	expl									1						
BENIGN	pa	1[b]									23					
	ba											2				
	schw												2			
	cys													11		
	cana														1	
	myep															1
	Total	17	9	2	2	5	2	1	1	1	25	2	2	11	1	1

adcc=adenoid cystic ca; mec=mucoepidermoid ca; scc=squamous cell ca; cexpa=ca ex pleomorphic adenoma; plga=polymorphous low grade adenoca; acc=acinic cell ca; lec=lymphoepithelial ca; NHL=B-NHL (maltoma); expl=extranodal plasmocytoma; pa=pleomorphic adenoma; ba=basal cell adenoma; schw=schwanoma; cys=cyst; ca=canalicular adenoma; myep=myoepithelioma;
[a] – false positive; [b] – false negative; [c] – false subtype or tumor

nign tumours were basal cell adenoma and schwanoma detected in 2 patients each and canalicular adenoma and myoepithelioma in one patient each (Table 2). The most common malignant tumour was adenoid cystic carcinoma diagnosed in 17 patients, mucoepidermoid carcinoma in 9 patients and polymorphous low-grade adenocarcinoma in 5 patients (Table 2). The remaining 11 malignant tumours were squamous cell carcinoma (2 patients), carcinoma ex pleomorphic adenoma (2 patients), acinic cell carcinoma (2 patients) and lymphoepithelial carcinoma, B-non-Hodgkin lymphoma (MALT) and extramedullary plasmacytoma each in one patient (Table 2). Comparison between cytological and histopathological diagnosis showed that there were 79 accurate and 3 false cytological diagnoses (Table 2). There were 2 false positive cytological diagnoses: one pleomorphic adenoma was diagnosed as adenoid cystic carcinoma and another one as a low grade mucoepidermoid carcinoma (Table 2). In Figure 3a the cause of erroneous cytological diagnosis of mucoepidermoid carcinoma is presented: a cluster of atypical metaplastic squamous cells with hyperchromatic nuclei and anisonucleosis and lack of mucus. Histopathology of this tumour shows tissue of mucoid and myxoid appearance intermingled with finger-like processes of epithelial and myoepithelial cells typical of pleomorphic adenoma (Figure 3b). There was one false negative cytological diagnosis: adenoid cystic carcinoma was diagnosed as cylindromatous pleomorphic adenoma because of stromal cores resembling hyaline globules surrounded by numerous myopeithelial and epithelial cells in pseudopapillary formations (Figure 4a, Table 3). Histopathology showed cribriform pattern with nests of cells with cylin-

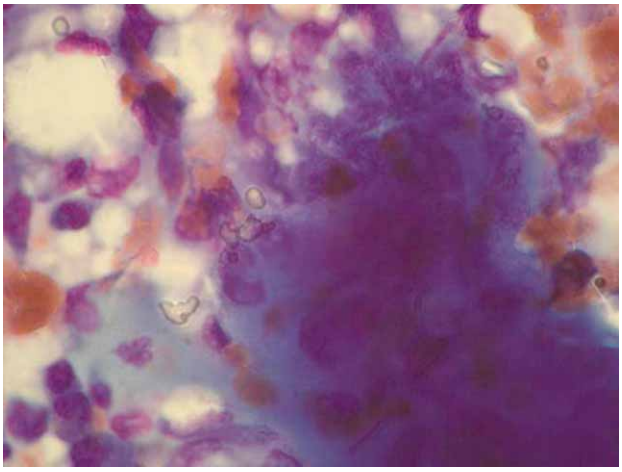

Fig. 3a. Cluster of atypical squamous metaplasia with hyperchromatic nuclei and anisonucleosis and lack of mucus – the cause of incorrect cytological diagnosis of low grade mucoepidermoid carcinoma instead of pleomorphic adenoma (Papenheim, 400x).

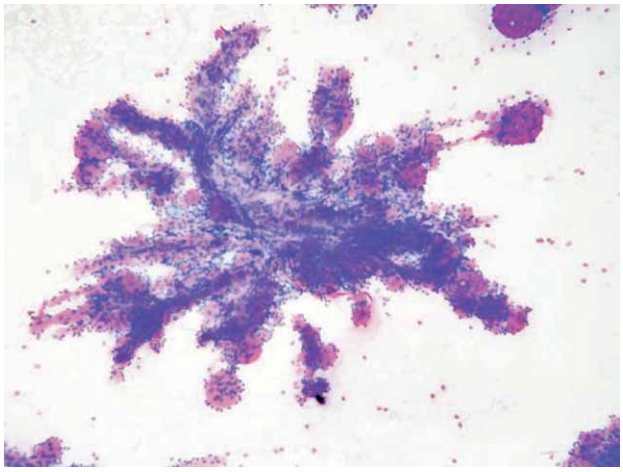

Fig. 4a. Stromal cores resembling hyaline globules surrounded by numerous myopeithelial and epithelia cells in pseudopapillary formations – the cause of incorrect cytological diagnosis of adenoid cystic carcinoma instead of pleomorphic adenoma (Papenheim, 400x).

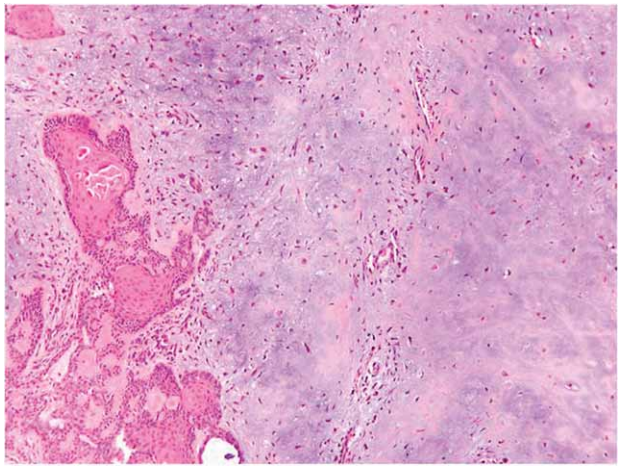

Fig. 3b. Tissue section shows tissue of mucoid and myxoid appearance intermingled with finger-like processes of epithelial and myoepithelial cells that is tipical for pleomorphic adenoma (Hematoxylin and eosin stain, 400x).

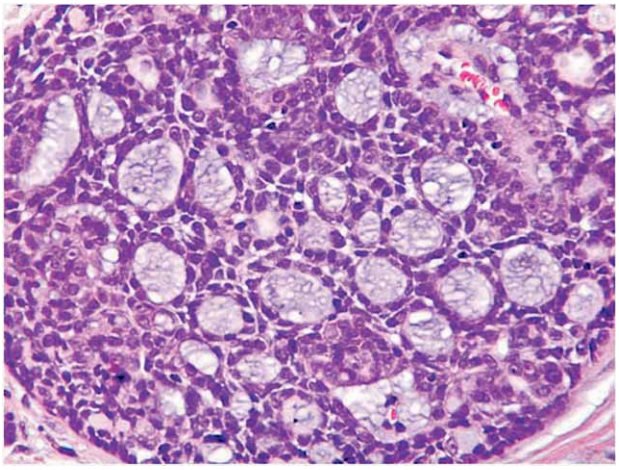

Fig. 4b. Adenoid cystic carcinoma: cribriform pattern with nests of cells with cylindromatous microcystic spaces filled with hyaline or basophilic mucoid material (Hematoxylin and eosin stain, 400x).

TABLE 3
FINE NEEDLE ASPIRATION CYTOLOGY RELATIVE TO HISTOPATHOLOGY PER PATIENT BASIS (N=82)

	Positive predictive value (%)	Negative predictive value (%)	Specificity (%)	Sensitivity (%)	Diagnostic accuracy (%)
Patients	95.2	97.5	95.1	97.6	96.3

dromatous microcystic spaces filled with hyaline or basophilic material (Figure 4b). Thanks to our protocol the team-work of cytologist and surgeon achieved sensitivity of 97.6%, specificity of 95.1%, positive predictive value of 95.2%, negative predictive value of 97.5% and diagnostic accuracy of 96.3% (Table 3).

Discussion and Conclusion

Minor salivary gland tumours are a heterogeneous group of neoplasms with unknown aetiology and great histomorphological variation[27]. Tumours of intraoral minor salivary glands are generally stated to account for about 15% of all salivary gland neoplasms[28]. Although they are relatively uncommon, they generate significant interest because of the difficulties concerning cytological diagnosis. Despite that, there are few articles about cytology of minor salivary gland tumours, usually presented as a case report[12–19].

Most studies have shown that minor salivary gland tumours are more common in females than males with a ratio range up to 1.9:1, but in our study the tumours were more common in males with ratio range 1.1:1 similar to the study by Sahai and associates (1.4:1)[3,8]. The age range of the patients was similar to a large study by Waldron and associates from 8 to 100 years, but according to the literature data, we had the oldest patient with aspirated minor salivary gland tumour, the lady was 101 years old and suffered from benign basal cell adenoma[3].

The anatomic location of oral minor salivary gland tumours noted in the present study is consistent with that found in other major surveys: the palate is the most common location and accounts for between 41.7% and 66% of all cases and in our study 59.8% cases, predominantly malignant tumours[29,30].

In our series, the ratio of incidence of benign and malignant minor salivary gland tumours is consistent with other reports[3,20,27–30]. Pleomorphic adenoma is the most common benign tumour (25 of 42) and adenoid cystic carcinoma is the most common malignant tumour (17 of 40) what is consistent with histopathological reports of Eveson and Cawson, Yih and associates and Takahashi and associates as well as with the cytological report of Sahai and associates[8,28,31,32]. In other studies originating in the United States mucoepidermoid carcinoma is the most frequent malignant intraoral salivary gland tumour while in our study it is on the second place[3,29]. The third place among malignant minor salivary gland tumours in our report belongs to polymorphous low grade adenocarcinoma (PLGA) which is a distinctive salivary gland neoplasm with an almost exclusive propensity to arise from minor salivary glands and was named in 1984[9]. Four cases of PLGA were correctly diagnosed and one was misdiagnosed as adenoid cystic carcinoma in our study. Although specific histological criteria have improved the diagnosis of PLGA, cytological features are not yet completely defined and it is rather often misdiagnosed with adenoid cystic carcinoma although it is important to differentiate them because the adenoid cystic carcinoma is more aggressive[9,33]. Immunocytochemistry can be helpful because PLGA is usually positive for S-100, vimentin and AE1/AE3[34].

Besides this one false subtype of tumour we have 2 false positive and one false negative diagnosis in our study concerning three of the most frequent tumours: pleomorphic adenoma, adenoid cystic carcinoma and mucoepidermoid carcinoma. Distinguishing pleomorphic adenoma and adenoid cystic carcinoma is a major diagnostic problem for cytologist because of their cytological similarity in smears[8,24]. Both tumours may show pseudotrabecular and pseudocylindromatous architecture like in our misdiagnosed cases in both direction, one false positive and another one false negative. Sometimes cytochemistry, PAS and Alcian blue technique can be helpful as well as clinical data like pain or macroscopic appearance of aspirate. Cerulli and collaborators have investigated the accuracy of FNAC in 24 patients with pleomorphic adenoma and adenoid cystic carcinoma of minor salivary glands of the palate and got 91.6% accuracy and an error rate of 8.4%[10]. Another false positive case in this study was diagnosis of low-grade mucoepidermoid carcinoma instead of pleomorphic adenoma because of squamous metaplasia and lack of mucus that was similar described in the case report about pleomorphic adenoma mimicking mucoepidermoid carcinoma in parotid gland by Siddiqui and Wu[35].

Diagnostic difficulties in cytological evaluation of minor salivary gland tumours cover two steps: specimen adequacy and factors inherent to the salivary glands like diverse morphology, complex histology, and a broad range of tumours arising from the salivary glands, diverse morphology of tumours with overlapping patterns. The first step is sampling the material and it is the first possible source of inaccurate diagnosis of the tumour of minor salivary gland: it is very import to point the right place of the lesion for getting the adequate sample. In our series of 132 aspirates there were 11 (8.3%) inadequate specimens that are slightly better than the results of Roland and associates who performed FNAC of 92 major salivary glands in the similar manner as we do and had 14 (14.4%) inadequate samples for assessment[20]. FNAC is very often in many countries performed by clinicians and the smears are sent to cytologist or cytopathologist. Al-Marzooq and associates compared proportions of unsatisfactory aspirates obtained by pathologists *versus* surgeons from different organs and got these results:

29.5% inadequate samples obtained by surgeon in comparison with 15.5% inadequate samples obtained by pathologist[36]. Both results are twice to almost 4 times worse than ours.

Cytological diagnosis is like a puzzle – if we have not all the pieces we shall not be able to put the accurate diagnosis. Therefore at our hospital we have the protocol that the fine needle aspiration cytology is always performed by a cytologist who is responsible for adequacy of aspirate and choice of staining and ancillary techniques that are necessary for precise diagnosis of tumours together with the surgeon who is responsible for clinical data concerning the tumour and for showing the target for the FNAC. It is very important that the cytologist examines the patient, gets informations about therapy that can change morphology of cells. It is also important for the cytologist to see the lesion, macroscopic appearance of aspirated material, consistency of tumour, colour and appearance of aspirate (liquid, needy, sticky, purulent), to see whether the surface is with intact mucosa or not… And the last puzzle's piece of the cytological diagnosis is microscopic analysis of the smears. This step depends of cytologist's skill and knowledge of cytopathology. In such a manner the team achieved specificity of 95.1%, sensitivity of 97.6% and diagnostic accuracy of 96.3% that is much better than the results of Zbaren and associates who presented FNAC of parotid gland that is easier to analyse and achieved specificity of 95%, sensitivity of 64% and diagnostic accuracy of 86%[37].

We can conclude that the team work of cytologist and surgeon performing FNAC of minor salivary gland tumours achieves the accurate preoperative diagnosis. FNAC is a minimally invasive technique that can provide useful preoperative information about lesions arising in the minor salivary gland but we must be very careful about the limitations of cytology thinking about many common and rare tumours that can cause diagnostic confusion. If FNAC is performed properly, it may prompt or postpone the decision for surgical intervention. Therefore we recommend that FNAC of minor salivary gland tumours should be considered in the first line of diagnostic procedure using our protocol.

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VAŽNOST TIMSKOG RADA CITOLOGA I KIRURGA U PREOPERACIJSKOJ DIJAGNOSTICI TUMORA MALIH ŽLIJEZDA SLINOVNICA U USNOJ ŠUPLJINI

S A Ž E T A K

Tumori malih žlijezda slinovnica u usnoj šupljini često uzrokuju dijagnostičke i terapijske dileme zbog preklapanja kliničkih i morfoloških karakteristika. Cilj ovog rada je utvrditi vrijednost aspiracijske citologije u razlikovanju dobroćudnih od zloćudnih tumora kao i određivanje točne vrste tumora. Željeli smo utvrditi da li timski rad kirurga i citologa može poboljšati dijagnostičku točnost citološke dijagnoze. Dva koraka su važna za točnost. Prvi je citološka punkcija koja se treba raditi timski, citolog zajedno s kirurgom, a drugi je citološka mikroskopska analiza razmaza punktata za što je potrebno veliko iskustvo. Ovo istraživanje uključuje 132 pacijenta s tumorom malih žlijezda slinovnica u usnoj šupljini i adekvatni materijal se dobio od njih 121 (91.7%) u razdoblju od 2002. do 2011. godine. Citološku punkciju tumora obavlja citolog zajedno s maksilofacijalnim kirurgom na citološkom odjelu što je važno zbog pripreme citoloških uzoraka odmah nakon punkcije, a naročito za hitnu mikroskopsku analizu punktatu. Na taj način citolog može odmah provjeriti adekvatnost punktata i odlučiti da li su potrebne dodatne tehnike, te ponoviti punkciju. Operirana su 82 pacijenta, 40 sa zloćudnim i 42 s dobroćudnim tumorom. Preoperacijska citološka dijagnoza je evaluirana patohistološkom dijagnozom nakon operacije. Najčešći benigni tumor je bio pleomorfni adenom, a od malignih tumora je najčešći bio adenoidni cistični karcinom. Najveći broj tumora je bio smješten u slinovnicama na nepcu. Timski rad kirurga i citologa ostvario je specifičnost 95,1%, osjetljivost 97,6% i dijagnostičku točnost 96,3%. Premda je citološka subklasifikacija pojedinih vrsta tumora malih žlijezda slinovnica manjkava, aspiracijska citologija je neprocjenjiva za trijažu pacijenata i zbog toga je neophodno kod svih lezija malih žlijezda slinovnica učiniti citološku punkciju u timu s kirurgom.