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Pathology

IMPRINT CYTOLOGY IN THE STUDY OF ORAL AND PHARYNGEAL MUCOSAL LESIONS WITH IMMUNOHISTOLOGICAL CORRELATION

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ABSTRACT **OBJECTIVE -** The oropharyngeal region represents the upper portion of the digestive tract. A number of non-neoplastic and neoplastic mucosal lesions of this region constitute a major health problem in developing countries. Carcinoma of this region is the commonest neoplasm in male in our country. Our study was done with the aim of early diagnosis of these lesions in a cost effective manner.

MATERIAL AND METHODS - Total 40 cases were selected having symptoms of ulceration and growth in these regions. The tissues were collected at the time of operation for imprint cytology of the epithelial cells. Thereafter, the tissues were processed routinely for histopathology and immunohistochemistry (p53 & PCNA).

RESULTS - Statistically significant difference was found between non-neoplastic, premalignant and malignant lesions. Cytological diagnosis was confirmed by histological findings along with immunohistochemistry. p53 expression and PCNA staining were more in malignant lesions.

CONCLUSION - The imprint cytology can be an important tool for early diagnosis and determination of therapeutic protocol in oral and pharyngeal lesions specially if they are correlated with histology and immunohistochemistry.

KEYWORDS : Oropharyngeal lesions, Imprint cytology, Histology, Immunohistochemistry.

INTRODUCTION

The oral cavity and pharynx are the parts of the aerodigestive tract, one of the main portals into our body. A number of non-neoplastic as well as neoplastic lesions are very frequent in these sites. Oral cancer is a major problem in India and accounts for 50 to 70 % of all cancers diagnosed [1] . The malignancies arising out of the mucosa of the oropharyngeal region are squamous cell carcinoma in more than 90% of the time [2]. The imprint cytology is one of the upcoming methods that can be used in the diagnosis of malignant and benign lesions in shorter period though the histopathology remains the gold standard[3]. Sometimes the distinction between some cases of premalignant dysplasia and carcinoma in situ or minimally invasive carcinoma is difficult in routine Haematoxylin and Eosin (H & E) stained sections. Application of immunohistochemistry has been found to be an important tool to resolve the problems in microscopic diagnosis of these grey zones in histopathology.

In this study we have considered the expression of p53 and PCNA as cell proliferative markers in different epithelial lesions.

MATERIALS AND METHODS

Total 40 cases were selected from the patients attending the department of Surgery as well as ENT with the complaints of pain inside mouth, difficulty in swallowing, foul smelling breath etc.. Only the lesions arising from the surface mucosa are taken in the study. After clinical diagnosis, the lesions were operated and each case was studied by imprint cytology, histopathology and immunohistochemistry. Immediately after obtaining the biopsy specimen, a direct imprint was prepared and the slides were immediately fixed in 95 % ethyl alcohol or air dried and then stained with Papanicolaou stain and MGG stain.[4] For the histopathology, the slides were stained by H&E staining and immunohistochemistry was done by p53 and PCNA nuclear staining. For staining by monoclonal antibody against p53 and PCNA, the kit literature of the manufacturer was followed [5,6,7]. H&E stained slides were examined thoroughly and a provisional diagnosis of each case was made. The final diagnosis was made after interpreting the results of immunohistochemistry with p53 staining was evaluated by the positive cases (the percentage of cases showing positive staining) and p53 positivity (the percentage of nucleus showing positive staining out of total nuclei counted).[14] PCNA staining was evaluated by PCNA labeling index (PCNA LI % - the percentage of nucleus showing positive staining out of total nuclei counted).[8] Statistical analysis was done by unpaired Student's 't' test and p values were obtained. A level of significance of 5% (p value <0.05) is chosen, for no better reason than that it is conventional [9,10].

RESULTS

Most of the patients with neoplastic lesions in our study were older males (>60 years.). (table 1).We see from the table 2 that 8 out of 9 cases(89%) cytologically diagnosed as inflammatory lesions were in consistent with the histopathological diagnosis. Out of 20 cases of dysplasia diagnosed on cytology, 7 cases were found to be carcinoma on provisional histopathological diagnosis and 2 were found to be hyperplasia without dysplasia. All cases (100%) of cytologically diagnosed carcinoma were in consistent with the results of histopathological diagnosis. Three of the five unsatisfactory cytological smears were found to be hyperplastic on histopathology and the rest 2 were carcinoma. Table 3 shows the interpretation of the p53 nuclear staining of the cases. The p values of them showing the statistically significant difference is given in the table 4. Table 3 also shows the interpretation of the PCNA nuclear staining of the cases. The p values of them showing the statistically significant difference is given in the table 4.

DISCUSSION

In our study, we did the imprint cytology of the biopsied specimen or surgically resected specimens from total 40 patients. Among the non - neoplastic inflammatory lesions the male: female ratio was 1.2: 1 and among the neoplastic lesions the ratio was 4.8: 1 (Table 1).

The cytological examination had revealed 9 cases (22.5%) of inflammatory smears of which 1 case was diagnosed as dysplasia and the rest were hyperplasia without dysplasia on histopathology (Table 2). The only false negative case in our study may be inherent in the procedure. Out of 20 cases (50%) of dysplasia on cytology, 11 were dysplasia , 2 were hyperplasia without dysplasia and 7 were carcinoma on provisional histopathological diagnosis . This is because of difficulty in differentiation of epithelial cell dysplasia and well differentiated carcinoma on cytology as Wellman ML found in his study[11]. Moreover MR Hussein et al. said that one of the disadvantages of the imprint cytology is that it can not differentiate between in situ and invasive lesions [12]. All cases of cytologically diagnosed carcinoma (15 %) were consistent with the results of histopathological diagnosis. The cause of two false positive cases (dysplasia in cytology but hyperplasia without dysplasia in histopathology) in our study may be due to interpretation error. The unsatisfactory specimen in the

cytology (12.5%) may be due to scant cellularity, air drying or distortion artifact obscuring blood or inflammation as found by Foet K et al. in their study [13].

From the results of our study (Table 2) we see that the sensitivity & specificity of detecting malignancy with imprint cytology was 96% and 80% respectively. M.R.Hussein et al. found the sensitivity and specificity were 88% and 92% in their study.[12]

Both nuclear positivity for p53 and mean p53 positivity of each positive case was gradually increasing from hyperplasia to dysplasia to carcinoma(Table 3). A statistically significant difference is found between different groups (Table 4). Kerdpon Det et al. in their study showed that positive nuclear staining was found in 36 % cases of hyperplasia, 85% cases of dysplasia and 94% cases of carcinoma.[14] S.C.Sharma et al. showed both the percentage of positive cases & p53 positivity showed a corresponding increase in values with increase degree of dysplasia and carcinoma.[15].

The mean PCNA labeling index is the lowest in hyperplasia without dysplasia and highest in carcinoma (Table3) which corroborates with the finding of the study done by Kurokawa H et al.[16] A statistically significant difference is found between different groups (Table 4).

TABLES

Table 1 – showing age and sex distribution of the cases (n=40, as per final diagnosis)

Diagnosis	<20		20-40		41-60		61-80	
	M	F	M	F	M	F	M	F
Inflammatory	1	1	8	2	1	-	-	-
Neoplastic	-	-	4	1	9	1	11	1

Table2: Distribution of cases according to histological and cytological diagnoses

Cytological Diagnosis	Histological diagnosis	Number of cases in histopathological Diagnosis
Inflammatory lesions (9)	Hyperplasia without dysplasia	8
	Dysplasia	1
Dysplasia(20)	Dysplasia	11
	Carcinoma	7
	Hyperplasia without dysplasia	2
Carcinoma(6)	Carcinoma	6
Unsatisfactory(5)	Hyperplasia without dysplasia	3
	Carcinoma	2

Table3- Results of IHC with staining by monoclonal antibody against p53 and PCNA

Final histological diagnosis	P53 nuclear positivity(%)	Positive cases(%)	PCNA labeling index
Hyperplasia without dysplasia	5.5+/-.95	20	3.58+/-0.36
Dysplasia	30+/-5.5	85	29.5+/-1.00
Carcinoma	80.8+/-15.2	95	34.0+/-0.79

Table 4 - p-values showing the significance of differences in P53 nuclear positivity(%) and PCNA labeling index between different categories

Final histological diagnosis	P53 nuclear positivity(%)	PCNA labeling index
Hyperplasia without dysplasia & Dysplasia	.000234425	3.90038×10^{-8}
Hyperplasia without dysplasia & carcinoma	.000104823	1.10967×10^{-9}
Dysplasia& carcinoma	.000146996	6.40021×10^{-5}

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