Towards Digital Diagnosis of Oral Cancer: A Study on Optimum Preferences of Histopathological Techniques and Features

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Abstract

Accurate diagnosis is dependent on various factors in the pathological domain, like types of slides used and features scrutinized. Sometimes a diagnosis is evident due to clear symptoms. But under adverse constraints, like improper acquisition etc., it is very difficult to give a quick and clear diagnosis. The study aims to conduct a survey from the well-known histopathologists of the country to gather an understanding of the techniques preferably used by them for diagnosis of the disease and summarize it, for arriving at optimum options which may be adopted for automation. This was a cross-sectional study conducted from March 2018 to May 2018 using a pre-tested structured questionnaire of multiple answer choices. The study subjects comprised of resident histopathologists of the hospitals covering states/locations all over the country, by selective sampling. The hospitals were selected based on the availability of on-site pathology centres and wide coverage. The analysis of the data was done using Ms-Excel and SPSS. The Non-Parametric Friedman Test was conducted to test for significance of the responses. Oral Squamous Cell Carcinoma (OSCC) of buccal mucosa with both moderately and well-differentiated grades were reported. For diagnosis, H&E stain for the slides of 4μ thickness is mostly used. Further, invasion of basement membrane was the most important architectural feature and increased nucleo-cytoplasmic ratio the most important cytological feature. This type of survey will help in carrying out a directed diagnosis or further research for automated diagnosis using the results.

Keywords: *Oral cancer, Diagnosis techniques, Feature study, Preference, Survey*

Introduction

Healthcare costs will need to reduce if we are to treat more people. The area of diagnosis offers possible significant cost reduction while improving accuracy by using technology. Recent times have witnessed an increase in the incidence of cancer.[1] According to the World Health Organization, 6,57,000 new cases of cancers of the oral cavity and pharynx are estimated each year and more than 3,30,000 demises.[2] This is mainly because of urbanization and industrialization which leads to a major change in lifestyle and exposure to environmental pollution. It is well known that cancer incidence in urban population is nearly double that of the rural population. Further other important factors like demographics, increased life expectancy and access to healthcare contribute to the cause. Morbidity due to cancer is almost double in the rural population than the urban population.[3] Clear causes are poverty, illiteracy, ignorance, myths of the disease and access to cancer facilities. Oral and oropharyngeal carcinomas still continue to hold the sixth most common cancers in the world.[4] Surveillance, Epidemiology, and End Results (SEER) 9 registries of the National Cancer Institute of the United States Public Health Service, 2018 report shows the following statistics.[5]

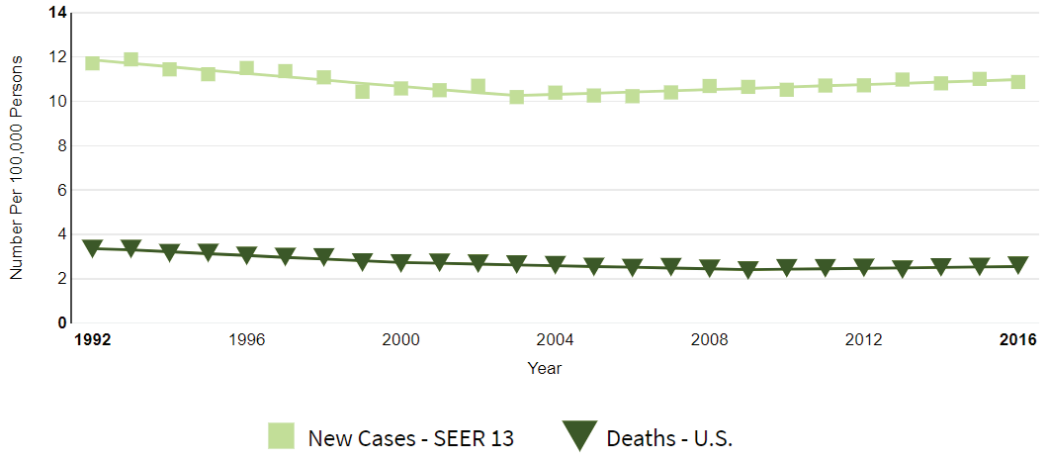

Figure 1: Trends in incidence rates of cancer of the oral cavity and pharynx from 1975-2015

Head and neck cancers are a major concern worldwide and especially in North-East India. National Cancer Registry Programme declares that, in India, oral cancer is the most common cancer amongst men (16.1 % of all cancers) and the second most common cancer amongst women (10.4 % of all cancers). According to Globocan 2018 (it is an online database providing estimates of incidence and mortality in 185 countries for 36 types of cancer, and for all cancer sites combined) data, new cases registered for oral cancer is 1,19,992. The total number of demises in men and women together is 72,616.[6]

Figure 2 shows the proportion of mouth, tongue and other oral cancers incident in patients of North-East India, as per latest report of Dr. B. Borooah Cancer Institute (BBCI), a grants-in-aid institute of Department of Atomic Energy, Government of India, and a unit of Tata Memorial Centre, Mumbai, and the premier institute of cancer treatment in the region.

Two points are noteworthy regarding oral cancer control and treatment management. First, as per WHO recommended priorities and strategies for prevention and control of Oral (Mouth and Pharynx) cancer, Primary prevention is very effective and early detection is partly effective.[7]

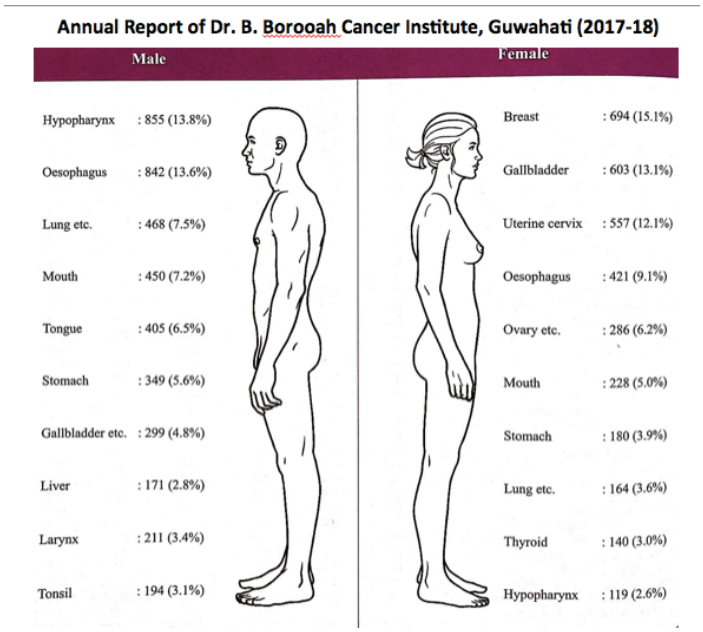

Figure 2: Distribution of various types of cancers by site in male and female

Hence, coupling the two together gives a direction for activating control programmes. Secondly, more than 80% of the diagnosis of oral cancers is done on the basis of microscopy. Accurate and timely diagnosis is the major strategy, as well as a challenge for lowering the incidence of the disease and follow-up clinical treatment. It itself is a by-product of various inputs, starting from the type of slide to type of stains used and types of features inspected and analysed. This stimulates us to conduct a survey in this direction to gather some information from the well-known histopathologist of the region, so as to make a bucket list of the most important parameters required for generating a fast yet accurate diagnosis. For the survey, we have designed a questionnaire consulting with highly experienced pathologists. The queries placed through the questionnaire carries significance as it gives us ample insight into understanding the techniques used by the pathologist for the diagnosis of the disease. Our aim is to utilize the information hence collected, for development of algorithms for automated oral cancer diagnosis, so that the software may be as realistic as possible and may relate well with the process followed in the region.

Materials and Method

Study Population and study design

This survey was conducted from March 2018 to May 2018. The study subjects comprised of resident histopathologists of the hospitals who were sent the questionnaires by post and contacted either by phone or email for intimation. The study does not involve any ethical clearance as no experiments were done on the participants. This is a cross-sectional study covering states/locations (Assam, Meghalaya, Manipur, Kolkata and Kerala) all over the country, by selective sampling. In total, 110 questionnaires were sent to the histopathologists of different hospitals and well-known diagnostic centres of India. The hospitals were selected based on the availability of on-site pathology centres and wide coverage.

The Questionnaire

All information was obtained by using a pre-tested structured questionnaire with multiple answer choices (**Annexure 1**). Then data were compiled in an excel sheet and calculations and data analysis was done with the help of SPSS-14 version. The questionnaire consisted of two parts. The first part contained the questions regarding preferred broad techniques related to the detection of oral cancer from biopsy sections. In the second part, some additional questions were put forward to understand in detail the common features observed by the pathologists for detection of malignancy.

It was also queried "What is the maximum time taken to investigate one slide?" Finally, pathologists were asked whether any automated imaging system was used for diagnosis. Response category for this question was "Yes" and "No". For each query, the respondents were offered to give all their options, with priority if so entailed.

Analysis

The analysis of the data was done using Ms-Excel (Ver 2016) and SPSS (14). The data were coded and entered in SPSS where the Non-Parametric Friedman Test was conducted to test for significance of the responses.[8] The Friedman test is most suitable for the study as the responses are all 'ordinal', that is being scaled according to preferences stated by the respondents. It is further appropriate because the data may not be normally distributed. To calculate the percentage of preference for the questions with priority choice, weights were assigned. Highest weight was given to the first choice, and so on in reverse order. The study tested for the perception of pathologists about the response to different questions. Thus null hypotheses formed for each response were "there is a difference in perception or preference between the pathologists regarding the responses".

Findings

75 responses were received (68.18 %) out of 110 questionnaires mailed. All the pathologists (100%) viewed squamous cell carcinoma as the most common histological type of oral cancer diagnosed in their laboratories. The number of choices per question is shown in the following table [**Table 1**].

From the study, it was observed that 43.5% opted for buccal mucosa as the most common site of occurrence followed by 17.4% for gingivobuccal sulcus, 13% for tongue, 7.2% for alveolus, 5.8% each for lip and base

of the tongue, 4.2% for gingiva and 2.9% for retromolar trigone. The percentage of choices were calculated and are depicted in **Figure 3**.

Further, as per the survey, both well-differentiated grades and moderately differentiated grades have been found to be in the leading position with 48 cases. Poorly differentiated grade was in the second position with 11 cases. Regarding preference of thickness of biopsy section/sample used for preparing a slide, most of the laboratories 43.9% preferred 4µ thickness, followed by 17.1%, 12.2% and 7.3% preferred for 3µ, 5µ and 6µ respectively.

All 75 laboratories (100%) uses Hematoxyline and Eosin stain for a routine examination. 31 pathologist (22.7%) considers the invasion of basement membrane as the most important architectural feature to diagnose malignancy. 22 pathologists agreed on the invasion of sub-epithelial tissue/other tissue as the second most important architectural feature to diagnose malignancy and 20 put loss of polarity of basal cells in third place. A maximum number of pathologists (22.7%) considers increased nucleo-cytoplasmic ratio as the most important cytological feature to diagnose cancer followed by hyperchromasia (19.2%) and atypical mitotic figures (17.4%).

Response to the question "Maximum time taken to investigate one slide" varies from 1 minute to 1 hour. Further, no histopathologist reported uses of any automated imaging system for diagnosis in their laboratories.

The test-statistic of Freidman test, viz. Chi-Square is like a variance over the mean ranks: it's 0 when the mean ranks are exactly equal and becomes larger as they lie further apart. Also, *p* is the probability of finding our sample differences, i.e. if the population distributions are equal. The mean ranks of the responses, chi and respective *p*-values of the test are shown in the last two columns of **Table 1**.

Table 1: Response of respondents on general observations

Question	Response choices	No. of responses	Mean ranks	Chi (p)
most common histological type of oral cancer diagnosed	Squamous cell carcinoma	75	5.00	132.000 (.000)
	Verrucous carcinoma	Nil	2.50	
	Minor salivary gland carcinomas	Nil	2.50	
	Lymphomas	Nil	2.50	
	Other	Nil	2.50	
Most common site of occurrence	Lip	7	4.64	66.860 (.000)
	Gingiva	7	4.50	
	Gingivobuccal sulcus	32	5.73	
	Alveolus	11	4.77	
	Buccal mucosa	52	6.83	
	Tongue	30	5.29	
	Base of tongue	11	4.62	
	Retromolar trigone	5	4.36	
	Other	Nil	4.26	
maximum reported grades of OSCC	Well-differentiated	48	2.20	12.071 (.002)
	Moderately differentiated	48	2.20	
	Poorly differentiated	11	1.61	

Cont... **Table 1: Response of respondents on general observations**

Thickness of biopsy section/sample used for preparing slide (in µ)	3	16	3.03	29.108 (.000)
	4	36	3.86	
	5	16	2.88	
	6	7	2.73	
	Other	Nil	2.50	
Stain used for routine examination	Hematoxyline and Eosin	75	2.00	33.000 (.000)
	Other	Nil	1.00	

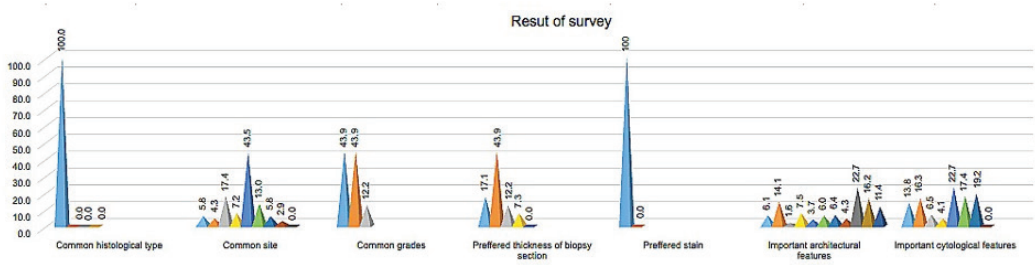

Figure 3: The percentage of choices of different responses

Discussion

According to the SEER (Surveillance, Epidemiology, and End Results program of the National Cancer Institute of the United States Public Health Service) registries in the USA between 1973 and 1987, of all the oropharyngeal malignancies reported, more than 95% were squamous cell carcinomas.[9] Other studies have corroborated that OSCC is the most common oral malignancy, representing up to 80–90% of all malignant neoplasms of the oral cavity.[10,11] The incidence of OSCC remains high.[12,13] 90% of all oral malignancies involve with OSCC.[13-16] As per the latest report of Dr B. Borooah Cancer Institute, 95% of head and neck cancers are squamous cell carcinoma. Likewise, in our study, we got 100% responses for OSCC. Other epidemiological studies found that tongue, lip, and floor of the mouth were the most frequent sites of lesions of OSCC.[17-19] Studies reported buccal mucosa and mandibular alveolus as most common sites too[20,21] and that the lip and tongue were the most and second most common site of OSCC.[21,22] The report of the findings is presented in **Table 2**.

Table 2: Response of respondents on features for the diagnosis

Question	Response choices	No. of responses	Mean ranks	Chi (p)
Most important architectural feature to diagnose malignancy	Irregular epithelial stratification	11	5.55	54.336 (.000)
	Loss of polarity of basal cells	20	7.42	
	Drop-shaped rate ridges	2	4.18	
	Increased number of mitoses	13	6.05	
	Abnormally superficial mitoses	7	5.00	
	Premature keratinization in single cell	9	5.14	
	Intraepithelial keratin pearls	10	5.56	
	Loss of intercellular attachment	8	5.36	
	Invasion of basement membrane	31	7.67	
	Invasion of sub-epithelial tissue/other tissue	22	6.82	
	Lymphatic and vascular invasion	18	7.26	

Cont... **Table 2: Response of respondents on features for the diagnosis**

Cytological features observed	Abnormal variation in nuclear size	18	3.97	78.049 (.000)
	Abnormal variation in nuclear shape	23	4.85	
	Abnormal variation of cell siz	12	3.92	
	Abnormal variation of cell shape	8	3.56	
	Increased nucleo-cytoplasmic ratio	32	6.67	
	Atypical mitotic figures	28	4.56	
	Hyperchromasia	30	5.92	
	Other	Nil	2.55	

As evident from the Friedman analysis, all the *p* values are highly significant and hence all the null hypotheses are rejected. It is henceforth summarized that there is a marked difference in perception of the pathologists about the histological methods and features used for evaluation of slides. Further, if choice is to be made for best 5 features for digitization, then that would be, in descending order of priority.

i) Invasion of basement membrane, Invasion of sub-epithelial tissue/other tissue, Loss of polarity of basal cells, Lymphatic and vascular invasion, and Increased number of mitoses for architectural analysis, and

ii) Increased nucleo-cytoplasmic ratio, Hyperchromasia, Atypical mitotic figures, Abnormal variation in nuclear shape, Abnormal variation in nuclear size for cytological analysis respectively

Conclusion

North-East India is considered to have the highest occurrence of oral cancer across the country. We have conducted this nation-wide survey to gather some useful information about the diagnosing techniques used by the pathologists and to summarize them, so as to identify the optimum ones. This information may be adequately used to incorporate into algorithms for automated detection and diagnosis of oral dysplasia, for researchers who want to carry out research in the area of digital pathology.

Acknowledgement The authors thank all the pathologists who had contributed in deriving a significant inference out of this survey with their valuable inputs. The first author would like to acknowledge the Department of Science and Technology (DST), Government of India for proving fund under Women Scientist-A scheme (SR/WOS-A/MS-11/2013 date 20.08.2014) for this study.

Ethical Clearance: Not applicable

Source of Funding: Department of Science and Technology (DST), Government of India

Conflict of Interest: None

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