Cytomorphometric analysis of keratinized round cells in human oral carcinoma

ABSTRACT

Aim: During the present investigation, two types of keratinized round cells (KRCs), large keratinized round cells (LKRCs) and small keratinized round cells (SKRCs), were observed in the exfoliated buccal smears of oral cancer patients. Therefore, the aim of the present study was to determine the practical utility of KRCs during difficult diagnosis and in the early detection of oral squamous cell carcinoma (OSCC) through cytomorphometric analysis.

Materials and Methods: In a hospital-based case control study, exfoliated scrape smears were collected from 136 patients clinically diagnosed as suffering from pre-cancerous lesions and OSCC and a parallel set of 136 samples were also collected from non-addicted and non-cancerous healthy individuals from different regions of Odisha, and this was considered as the control group. Wet-fixed smears were stained by adopting Papanicolaou’s staining protocol and counter-stained with Giemsa’s solution. One thousand cells were screened and keratinized round cells along with other cytological atypia were scored. Cytomorphometry was carried out using a computer-assisted Cat Cam 1.30 (1.3 Mega Pixel) microscope camera. The findings were statistically analyzed and interpreted with respect to oral sites, age groups and sexes.

Results: Cytomorphometrically, the nucleus to cytoplasmic ratio of the LKRCs was 1:4.7 in males and 1:4.3 in females, and in SKRCs it was calculated to be 1:4.6 in males and 1:5.2 in females.

Conclusion: Cellular keratinization, hyperchromasia and increased N/C ratios in both LKRCs and SKRCs indicates the state of malignancy and thus the present finding has a practical value in early detection and diagnosis of OSCC patients.

Key words: Cytomorphometric analysis; cytopathology; exfoliated scrape smears; large keratinized round cells (LKRCs); nuclear–cytoplasmic (N/C) ratio; oral squamous cell carcinoma (OSCC); small keratinized round cells (SKRCs)

Introduction

Oral cancer is one of the 10 most common cancers as stated by the World Health Organization (WHO) and each year 5,75,000 new cases and 3,20,000 deaths occur world-wide.[1] In India, oral cancer is a major health problem, which accounts for 30-40% of all cancers diagnosed and is the sixth common cause of death in males and seventh most common condition in females.[2] Approximately 90% of oral cancers are squamous cell carcinoma, and this is particularly common in the developing world.[3] The oral cavity is readily accessible for inspection and examination, even by the patient. But, it is unfortunate that patients still present with advanced tumors. Oral squamous cell carcinoma (OSCC), at an early stage, is generally asymptomatic and...
probably, due to this, diagnosis and treatment are all too often delayed until the cancer is malignant or has spread to cervical lymph nodes.\textsuperscript{[4,5]} Similarly, the cytopathologists very often encounter problems in detection and diagnosis of OSCC patients as most of the oral squamous cells appear to be either well differentiated or moderately differentiated and mimic to be benign and non-neoplastic. Contrary to that, many benign and non-neoplastic lesions appear to be malignant neoplasms.\textsuperscript{[6-8]} Therefore, the aim of the present investigation was to determine the practical utility of keratinized round cells (KRCs) during difficult diagnosis and in the early detection of OSCC through cytomorphometric analysis.

**Materials and Methods**

**Collection of samples**
Exfoliated scrape smears were collected from 136 patients clinically diagnosed to be suffering from pre-cancerous lesions and OSCC at the outpatient department (OPD) of the Government Cancer Hospital during May 2007-May 2009. Smearing was carried out on pre-cleaned and pre-coded microslides and the slides were fixed in aceto alcohol (1:3) fixative immediately. As per the International Classification of Diseases (ICD-10), two slides were smeared and prepared from each affected site of the patient. Prior to the collection of samples, case-history of the patients related to their age, sex, food, habits (addiction to tobacco, alcohol, etc.), oral hygiene and occupation were asked and recorded for detailed analysis. A parallel set of 136 samples were also collected from the non-addicted and non-cancerous healthy individuals from different regions of Odisha, which was considered as the control group.

**Staining protocol and scoring**
Wet-fixed smears were stained by adopting Papanicolaou’s staining protocol and counter-stained with Giemsa’s solution. One thousand cells were screened and keratinized round cells along with other cytological atypias were scored.

**Statistical analyses**
Of 1000 screened cells from each stained slide, the numbers of KRCs were scored and the morphometry of the respective cell was performed simultaneously. The mean diameter (in the form of length and breadth) of the KRC was taken into account as the diameter of the respective KRC as each cell was measured at three different regions using a computer-assisted Cat Cam 1.30 (1.3 Mega Pixel) microscope camera from Catalyst Biotech\textsuperscript{8} (Maharashtra, India). The measured values were tabulated. The nuclear–cytoplasmic ratio (N/C) was calculated after taking the area of the cytoplasm (C) and nucleus (N) of the respective cell. The findings were statistically analyzed and interpreted with respect to oral sites, sexes and nature of addiction.

**Results**
In comparison with the normal oral squamous cells (NOSCs) [Figure 1a and b], two types of KRCs, large keratinized round cells (LKRCs) and small keratinized round cells (SKRCs), were observed under the microscope [Figure 1c and d]. The cells are reported to be moderately differentiated and keratinized and exhibited hyperchromasia.\textsuperscript{[9]} The age group and site-specific enumeration of the KRCs gives a clear picture of occurrence. \textit{In toto}, 326 LKRCs (male-155 and female-171) and 404 SKRCs (male-183 and female-221) were scored. During scoring, an increasing trend from 30-49 to 50-69 years and then a decreasing trend was observed toward 70-89 years [Table 1]. A detailed account of scoring and morphometric analysis of the LKRCs and SKRCs, along with the NOSCs, is discussed below.

**NOSC**
Exfoliated NOSCs of the oral cavity are mostly angulated and polyhedral in shape. Generally, the thickness of the squamous cell was negligible in relation to its length and breadth. These are seen in smears as flat and polyhedral cells with a centrally placed rounded or oval nucleus in each. Variations occur in staining of the exfoliated mature normal cells. But, mostly, the cells are basophilic and appear to be sky blue in color in Papanicolaou’s staining. A total of 1000 normal squamous cells were taken into account from different sites of the oral cavity in each sex. Recently, in a comparative account, it was reported that the N/C ratios of NOSCs were calculated to be 1:34.5 in males and 1:34.4 in females.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A comparative account of normal oral squamous cells and keratinized round cells. (a) Normal oral squamous cells (Pap, ×400). (b) Normal oral squamous cells (Giemsa’s stain, ×400). (c) Large keratinized round cells (LKRCs) (Pap, ×400). (d) Small keratinized round cells (SKRCs) (Pap, ×400)}
\end{figure}
LKRC

The LKRCs appeared to be swollen and enlarged, acquiring almost a rounded contour. The cytoplasm was keratinized from orange green to orange red in color. Hyperchromasia state and enlargement of the nucleus in LKRCs was mostly observed.

Age is an important factor during carcinogenesis in general and triggers in the field of cytological pleomorphism in oral carcinoma. The recorded age of the patients in our study ranges from 30 to 87 years and so were grouped into three broad groups: 30-49, 50-69 and 70-89 years. The highest number of LKRCs (18) was recorded from the sample of the floor of the mouth in the age group of 50-69 years in males and the lowest, i.e. nil, in the age group of 70-89 years in the samples from carcinoma of the tongue and palate. In case of females, the highest number of LKRCs was scored from lip and floor of the mouth in the age group of 70-89 years, which is found to be 22 and the lowest, i.e. only 2, was recorded in the age group of 30-49 years from palatal cancer.

During scoring, a total of 40 LKRCs from five males and 36 cells from six females were estimated in carcinoma of lip. The relative frequencies of LKRCs were calculated to be 25.8% in males and 20.9% in females. In cases of lingual carcinoma, 25 LKRCs from 11 males and 21 cells from seven females were scored, having relative frequencies of occurrence of 16.2% and 12.3%, respectively. In carcinoma of alveolus and gingiva, the estimated numbers of LKRCs were 18 from 16 males and 31 from six females. The average frequency of occurrence was calculated to be 11.6% in males and 19.9% in females.

The number of LKRCs in palatal cancer was found to be very less in comparison with the other sites. A total of eight cells from six males and six cells from three females were recorded. Their relative frequencies were calculated to be 5.2% and 3.6%, respectively. In a total of 155 LKRCs from 82 male and 171 LKRCs from 54 female samples were recorded from all sites during the course of scoring [Table 1].

Cytometrically, the mean diameter of the LKRCs in males [Table 2] was measured to be $98.258 \mu m (\pm 18.574 \mu m)$ and thus its cytoplasmic area was calculated to be $7589.95 \mu m^2 (\pm 271.07 \mu m^2)$. Similarly, the mean diameter of the nucleus of the LKRCs was measured to be $46.745 \mu m (\pm 8.058 \mu m)$ and the nuclear area was calculated to be $1715.39 \mu m^2 (\pm 64.468 \mu m^2)$. Hence, the N/C ratio of the LKRCs was 1:4.7 in males. In case of females, the mean diameter and area of the LKRCs were measured to be $96.354 \mu m (\pm 22.123 \mu m)$ and $7294.976 \mu m^2 (\pm 384.515 \mu m^2)$, respectively. The nuclear

### Table 1: Oral site, age group and sex-wise enumeration of atypical keratinized round cells

| Oral sites (ICD-10) | Age groups in years | No. of samples | LKRCs scored | Total LKRCs scored | SKRCs scored | Total SKRCs scored |
|---------------------|---------------------|----------------|--------------|-------------------|--------------|-------------------|
| Lip                 | 30-49               | 2 Male 4 Female| 12 Male 15 Female | 40 (25.8) Male 36 (20.9) Female | 10 Male 7 Female | 34 (18.6) Male 18 (8.1) Female |
| Lip                 | 50-69               | 2 Male 4 Female | 15 Male 14 Female | 25 (16.2) Male 21 (12.3) Female | 11 Male 14 Female | 30 (16.4) Male 44 (19.9) Female |
| Tongue              | 30-49               | 5 Male 2 Female | 12 Male 8 Female | 18 (11.6) Male 34 (19.9) Female | 8 Male 24 Female | 32 (17.5) Male 52 (23.5) Female |
| Alveolus and gingiva| 30-49               | 4 Male 3 Female | 1 Male 14 Female | 18 (11.6) Male 34 (19.9) Female | 8 Male 24 Female | 32 (17.5) Male 52 (23.5) Female |
| Floor of the mouth  | 30-49               | 4 Male 1 Female | 6 Male 8 Female | 30 (19.3) Male 46 (26.9) Female | 24 Male 15 Female | 58 (31.6) Male 77 (34.8) Female |
| Palate              | 30-49               | 4 Male 1 Female | 6 Male 2 Female | 8 (5.2) Male 6 (3.6) Female | 4 Male 3 Female | 14 (7.7) Male 8 (3.7) Female |
| Buccal mucosa       | 30-49               | 14 Male 4 Female | 12 Male 5 Female | 18 (11.6) Male 34 (19.9) Female | 6 Male 3 Female | 15 (8.2) Male 22 (10.0) Female |
| Total               | 30-49               | 82 Male 54 Female | 155 Male 171 Female | 183 Male 221 Female | 183 Male 221 Female |
diameter and area of the LKRCs were found to be 46.452 μm (± 12.431 μm) and 1695.405 μm² (± 121.396 μm²), respectively. Hence, the N/C ratio of the LKRCs was calculated to be 1:4.3 in females.

SKRCs
A type of pleomorphic atypical cells that appear to be rounded in structure with keratinized cytoplasm and nearly 1/4th of the nuclei of the cell were distinct, enlarged and deeply stained (hyperchromasia). SKRCs were found more commonly than LKRCs. The highest number of SKRCs was recorded in the age group of 30-49 years at the floor of the mouth in males. But, in case of females, the highest number of SKRCs was found in the 50-69 years age group from the same region. Interestingly, the lowest number of SKRCs was estimated from the buccal mucosa in the age group of 70-89 years. In carcinoma of the lip, the total estimated number of SKRCs from five males and six females was 34 and 18, respectively, having relative percentages of 18.6 and 8.1. The total number of SKRCs scored from all the three age groups having the same site, but are found in malignant lesions of the oral cavity. Garcia[6] has emphasized the useful discriminating criteria between non-neoplastic conditions, benign neoplasms that may mimic malignancy and malignant neoplasms that may pose as benign entities. He has indicated that increased nuclear size can stimulate malignant change, but it is a benign feature when the cytoplasm is increased proportionately. Parida[7] has reported that a practical difficulty arises with such well-differentiated squamous cell carcinomas in cytologic examinations as, in many of these cases, nuclear characteristics of malignancy, such as hyperchromatism and an increased N/C ratio are not marked. McKinley[8] stressed on nuclear hyperchromasia and increased N/C ratio as the key factors for discriminating neoplastic cells from benign or non-neoplastic ones. Mohanta et al.[9,10] have reported that both LKRCs and SKRCs are observed to be mostly moderately differentiated, keratinized and hyperchromatic.

Cytometrically, it was observed that the mean diameter of the SKRCs in males [Table 2] was 20.073 μm (± 5.122 μm) and the cytoplasmic area was 316.498 μm² (± 20.613 μm²), whereas the mean diameter and area of the nucleus was found to be 9.324 μm (± 2.92 μm) and 68.289 μm² (± 6.693 μm²). Hence, the N/C ratio of SKRCs was calculated to be 1:4.6 in males. In females, the mean diameter of the SKRCs was found to be 20.522 μm (± 5.320 μm), with area of the cell of 330.815 μm² (± 22.237 μm²). The nuclear diameter was measured to be 8.976 μm (± 2.950 μm) and its area was calculated to be 63.306 μm² (± 6.827 μm²). Thus, the N/C ratio was calculated to be 1:5.2 in females.

### Discussion

Transformation of the angulated, polyhedral normal squamous cells to the KRCs is considered to be an important cellular change during oral carcinogenesis. These are not seen either in normal mucosal smears or in benign lesions at the same site, but are found in malignant lesions of the oral cavity. Garcia[6] has emphasized the useful discriminating criteria between non-neoplastic conditions, benign neoplasms that may mimic malignancy and malignant neoplasms that may pose as benign entities. He has indicated that increased nuclear size can stimulate malignant change, but it is a benign feature when the cytoplasm is increased proportionately. Parida[7] has reported that a practical difficulty arises with such well-differentiated squamous cell carcinomas in cytologic examinations as, in many of these cases, nuclear characteristics of malignancy, such as hyperchromatism and an increased N/C ratio are not marked. McKinley[8] stressed on nuclear hyperchromasia and increased N/C ratio as the key factors for discriminating neoplastic cells from benign or non-neoplastic ones. Mohanta et al.[9,10] have reported that both LKRCs and SKRCs are observed to be mostly moderately differentiated, keratinized and hyperchromatic.
The round cell tumors are a heterogeneous group of malignant neoplasms. These consist of discrete cells that are round to oval rather than fusiform. Because the histologic pattern of these neoplasms is often similar, cytologic examination of touch imprints or fine needle aspirations of tumors often can be a valuable adjunct to rendering a definitive diagnosis. The differential diagnosis of round cell tumors by histologic examination without concomitant cytologic characterization may, in some instances, depend more on age of animal, growth rate, location of tumor, number of tumors and lymph node involvement rather than histologic criteria.[11] In addition to the above routine protocol, immunohistochemistry has a significant role in the identification of tumors in diagnostic pathology. In recent years, molecular testing is used as one of the most valuable tools for difficult diagnosis.[12]

Combined large cell neuroendocrine carcinoma of the lungs with giant cell carcinoma is extremely rare, with a high malignant potential.[13] It has already been reported that malignant small round cell tumors are generally characterized by small, round and relatively undifferentiated cells.[14] This morphologic class of tumors contains representatives from almost all major categories of neoplasms, including carcinomas, sarcomas, lymphomas and neuroendocrine–neuroectodermal tumors. The light microscopic similarity of many of these tumors often makes their diagnosis in routine histologic examination extremely difficult. The ongoing plethora of publications devoted to the correct identification of this class of neoplasms reflects the enduring diagnostic challenge of undifferentiated small round cell tumors.[15-18]

Using minimal markers to identify the true identity of round cell tumors, of 80 cases, Patel et al.[19] have reported 32.50% non-Hodgkin lymphoma, 18.75% germ cell tumor, 12.50% sarcoma, 8.75% each of melanoma and blastoma, 6.25% neuroendocrine tumor, 5% carcinoma, 2.50% plasma cell neoplasm, 1.25% mesothelioma and 3.75% undifferentiated round cell tumor and concluded that immunohistochemistry is a valuable adjunct to routine hematoxylin and eosin staining for adequate and accurate categorization of round cell tumors. In their independent study, Bashyal et al.[20] and Ahmed et al.[21] have found lymphoma as the most common tumor type (52.5% and 65.30%, respectively) in round cell tumors. Bianchini et al.[22] found lymphoma and carcinoma as the most common tumor types (36.36% each) in head and neck cancer.

In the present study, except for a few cases, in most cases, the maximum number of KRCs were seen in the age group of 50-69 years, which indicates that age plays a vital role in the genesis of oral round cell tumors in both sexes. Not only that but also the oral sites like lip and floor of the mouth were found to be more prone to KRCs than the other sites. Our finding reveals that the highest relative percentages of LKRCs are recorded to be 25.8 from lip in males and 26.9 from floor of the mouth in females. Similarly, the relative percentage of SKRCs was found to be highest in carcinoma of floor of the mouth: 31.6 in males and 34.8 in females. On the other hand, the lowest relative percentage of LKRCs and SKRCs was recorded to be 3.6 and 3.7 from palate in both sexes. The possible cause may be assigned to khaini and snuff dipping on the floor of the mouth and under the lower lip as both these cells were mostly scored from the samples of tobacco (khaini) chewers and snuff dippers in the respective sites. Cytomorphometrically, the overall N/C ratios were also found not to be in normal proportion, but in an increasing trend in comparison with those of NOSCs in both sexes, which indicate the state of malignancy.

**Conclusion**

Cytological pleomorphism is a unique feature in human oral carcinoma. Occurrences of both LKRCs and SKRCs in varied proportions at different sites of the oral cavity indicate that cytologic examination is valuable in the diagnosis of round cell tumors. Both the cells are observed to be well differentiated or moderately differentiated, highly keratinized and hyperchromatic. A higher number of KRCs scored in the age group of 50-69 years than the other two age groups in the different oral sites indicate that there is an increasing trend in the genesis of round cell tumors from 30-49 years to 50-69 years, followed by a decreasing trend toward the 70-89 years age group. Morphometrically, the N/C ratios in either of the KRCs were also found to be in an increased state in both sexes, which indicates the state of malignancy. Thus, the present finding will help the cytopathologists in difficult diagnosis and early detection of OSCC patients.

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**Conflicts of interest**

There are no conflicts of interest.
Mohanta, et al.: Cytomorphometric analysis of keratinized round cells

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