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Case Report

Squamous cell carcinoma (well differentiated): A case report
Nutan Tyagi, Rishi Tyagi

Research Article

Evaluation of salivary flow rate, pH, buffering capacity, calcium and total protein levels in caries free and caries active adolescence
Case Report

Squamous cell carcinoma (well differentiated):
A case report

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In India, cancer of the oral cavity is one of the five leading sites of cancer in either gender. More than
90% of the oral cancers occur in patients over the age of 45, with a male predilection. Squamous cell
carcinoma (SCC) is the most common neoplasm of the oral cavity. The current case highlights the
cautions need to be exercised in clinical assessment and differential diagnosis of SCC from
another chronic ulcer with similar features (tubercular ulcer). The treatment modalities of the two vary,
thus making correct judgement and diagnosis essential.

Key words: Squamous cell carcinoma, tubercular ulcer, differential diagnosis.

INTRODUCTION

Over the past two decades, there has been a surge in the
investigations conducted pertaining specifically to oral
cancer. A significant variation has been noted in the
incidence of oral cancer, with high rates reported in the
Indian subcontinent and parts of Asia. In India, cancer of
the oral cavity is one of the five leading sites of cancer in
either gender. More than 90% of the oral cancers occur in
patients over the age of 45, with a male predilection. The
incidence increases steadily with age until 65, when the
rates level off (Shafer et al., 2006).

Squamous cell carcinoma is defined as "a malignant
epithelial neoplasm exhibiting squamous differentiation
as characterized by the formation of keratin pearls and /
or presence of intercellular bridges" (Pindborg et al
1977). It is the most common neoplasm of the oral cavity.
The main cause of oral cancer has been attributed to the
use of tobacco in its various forms, especially when
associated with the use of alcohol (Shafer et al., 2006).

CASE REPORT

A 60 year old female reported to the department, with the chief

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tuberculous infection. Since the history did not reveal any detail on possible tubercular infection, the positivity of the test was judged to be due to vaccination or a subclinical infection, as prevalent in the Indian subcontinent. However, to rule out any current infection, an acid fast bacilli (AFB) stain ulcer and histoplasmosis was also given. Intraoral periapical radiograph of the 33 and 34 regions, haematological investigations and incisional biopsy were the investigations advised. The intraoral radiograph revealed bony erosion in the canine-premolar region. The patient was advised and the reports ruled out TB. Incisional biopsy was performed and the tissue submitted to the department of oral pathology. The tissue specimen was 10% formalin fixed, creamish white in colour, oval in shape, measuring 0.7 cm in length and 0.8 cm in width, with adequate connective tissue.

On microscopic examination, the section showed predominantly ulcerated, atrophic epithelium invading into the underlying connective tissue (Figure 2). The dysplastic epithelial cells were arranged in islands of varying size. Numerous keratin pearls (Figures 3 and 4) and few mitotic figures with cellular and nuclear pleomorphism and hyperchromatism were also seen. Minimal chronic inflammatory cells were seen in the intervening stroma between the tumor islands. Areas of necrosis were also present. The deeper margins of the tissue section were not found to be clear of dysplastic cells. A diagnosis of well-differentiated squamous cell carcinoma was given.

**DISCUSSION**

The case presented in a 60 years old, moderately built female showed an oval shaped, single ulcer on the mandibular ridge in the canine-premolar region, with irregular margins and undermined edges. The base and borders were firm on palpation. The floor of the ulcer was erythematous, with presence of bleeding points. It was tender on palpation, and bleeding was present on slightest provocation. Oral ulcerative lesions are common findings, although often of similar clinical appearance, their aetiologies can have a wide range such as immunological, traumatic, neoplastic or oral manifestations of systemic and dermatologic disease. Clinically, almost all the oral cancers, barring the early forms, have characteristic presentation in the form of a persistent ulcer with indurated margins (Shafer et al., 2006). Tubercular involvement of oral cavity is very rare; but when seen, can present in a variety of forms (ulcers, nodules, tuberculomas and peri-apical granulomas). These may be either primary (single, painless ulcer, with
Oral tuberculosis is difficult to differentiate from other conditions on the basis of clinical signs and symptoms alone. While evaluating a chronic, indurated ulcer, clinicians should consider both infectious processes such as primary syphilis and deep fungal diseases and non-infectious processes such as chronic traumatic ulcer and squamous cell carcinoma. Thus in such cases inspite of a positive Mantoux test (which may indicate previous BCG vaccination or latent infection) a biopsy for confirmatory diagnosis is mandatory (Dixit et al., 2008). The most common clinical presentation in oral squamous cell carcinoma is either an ulcer or an ulceroproliferative growth. Classically, a carcinomatous ulcer has an irregular papillary surface, elevated borders and a hard base on palpation. The lesions are almost always chronic and have indurated margins. The lateral border, the ventral surface of the tongue and the lips are the most commonly affected sites, followed by the floor of the mouth, the gingival, the alveolar mucosa and the palate (Shafer et al., 2006; Neville et al., 2008).

The epidemiology of squamous cell carcinoma of the head and neck (SCCHN) is complex due to the multigenic nature of the disease and the number of potential environmental agents to which individuals may have been exposed. The major etiological agents that have been implicated are the use of tobacco and alcohol abuse. Other risk factors include nutritional deficiencies, occupation, viral infection and dental irritation. These risk factors do not, however, adequately explain 5 ± 10% of SCCHN cases (Johnson, 2001; Jefferies and Foulkes, 2001; Mehrotra and Yadav, 2006). They can develop from precancerous lesions, such as leukoplakia and erythroplakia, or apparently normal epithelium (Shafer et al., 2006). The histopathologic picture of tuberculous ulcer...
depicts granuloma formation exhibiting foci of caseous necrosis (not seen always) surrounded by epitheloid cells, lymphocytes and occasional multinucleated giant cells (Shafer et al., 2006). The report of incisional biopsy in the current case confirmed a diagnosis of well differentiated squamous cell carcinoma, thus ruling out tuberculous ulcer.

Histopathologically, SCC is divided into 3 grades depending on the degree to which the tumor resembles the parent tissue and produces keratin. They are categorized as well-differentiated, moderately-differentiated and poorly differentiated. A well differentiated tumor is mature enough to closely resemble its tissue of origin, grows at a slightly slower pace and metastasizes later in its course. On the contrary one which shows much cellular and nuclear pleomorphism that is, immature and bears no resemblance to the tissue of origin is designated as poorly differentiated. The tumor that lies between these two extremes is labeled as moderately differentiated (Neville et al., 2008; Anneroth and Batsakis, 1987; Bryne et al., 1989). The five year survival rate studies have proven well differentiated SCC to be of a better prognosis as compared to the poorly differentiated variant (Shafer et al., 2006; Mehrotra and Yadav, 2006; Neville et al., 2008). Epidemiological surveys have revealed that, of the areas of the oral cavity the mortality rate is lowest for lip cancer (0.04 per 100,000) and highest for the tongue, particularly the base (0.7 per 100,000), in which metastases may be ipsilateral, bilateral, or contralateral owing to cross vascular and lymphatic drainage. Also, the incidence increases steadily with age until 65 years, when the rate levels off (Shafer et al., 2006; Neville et al., 2008).

Cases of persistent leukoplakia or oral submucous fibrosis, are established early predictors of possible change to SCC, with the rate of malignant transformation varying from 0.13 to 6%, and the risk increasing to 14% with dysplastic lesions (Shafer et al., 2006). Treatment of the intraoral SCC is guided by the clinical stage of the disease and consists of wide excision, radiation therapy or a combination. Usually, larger lesions require combined therapy. Those with lymph node metastases are advised a radical neck dissection and radiation therapy in amalgamation (Frazell and Lucas, 1962; Scully and Ward-Booth, 1995; Singh et al., 1996). It is clear that the necessity of early diagnosis of SCC is paramount and hence, the responsibility of a dentist considerable.

**Conclusion**

Squamous cell carcinoma is the most common malignancy affecting the oral cavity characterized by a chronic non healing ulcer which has a range of provisional diagnosis. SCC occurs more frequently in males, usually in the 5 to 6th decade of life. The current case presented in an elderly female and the diagnosis was based on the clinical and histopathological examination. The classical presentation, with individual cell keratinization and keratin pearl formation, confirmed its resemblance to the parent tissue which validated its grading as a well differentiated tumor.

**REFERENCES**


Evaluation of salivary flow rate, pH, buffering capacity, calcium and total protein levels in caries free and caries active adolescence

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The present study was undertaken to compare salivary flow rate, pH, calcium, buffering capacity and total protein between caries free and caries active adolescence. Un-stimulated whole saliva was collected from one hundred healthy adolescents with age range of 15 to 17 years who were divided to four groups: caries free female, caries free male, caries active female, caries free male. Then, flow rate of saliva was determined and samples were analyzed for pH, calcium, buffering capacity and total protein. The date was analyzed using student’s t-test. The results showed that when all of these parameters were compared among caries free and caries active groups, buffering capacity of saliva decreased significantly in caries active group. Comparison of all of these parameters between girls and boys revealed the level of total protein and buffering capacity were significantly higher and pH was significantly lower in boys as compared to girls. Level of flow rate and composition of saliva were different between caries free and caries active adolescence. Moreover, buffering capacity decreased in caries active group. Notably difference in quantity and quality of saliva can contribute as an important causal factor in explaining sex difference in caries rate.

Key words: Dental caries, saliva, pH, buffers, calcium, salivary proteins.

INTRODUCTION

Dental caries is one of the most common, communicable and intractable infectious disease in human (Bowen et al., 2001; Amit and Robin Wendell, 2012). It remains the persistent and important oral health problem internationally, and particularly among developing countries (Garcia-Godoy and Hicks, 2008; Tickle et al., 2011). It is also profoundly affected by many factors like oral hygiene and saliva (Preethi et al., 2010). Saliva is a biologic fluid in the oral cavity, composed of a mixture of secretory products from the major and minor salivary glands (Lima et al., 2010). Saliva plays key roles in lubrication, mastication, taste perception, prevention of oral infection and dental caries (Lima et al., 2010; Pink et al., 2009; Chiappini et al., 2007). By constantly bathing the teeth and oral mucosa with saliva functions as a cleansing solution, a lubricant, a buffer and ion reservoir of calcium and phosphate which are essential for re-mineralization of initial carious lesions (Preethi et al., 2010). Many studies discussed about salivary flow rate, pH, buffer capacity and total protein in relation to dental caries, but there are differences in obtained results between the studies in different regions. Hence, evaluation of these factors in saliva that may increase risk of dental caries is important. Therefore, the aim of this study was to evaluate the
Table 1. Salivary parameters in caries active and caries free adolescence.

<table>
<thead>
<tr>
<th>Group</th>
<th>Flow rate</th>
<th>pH</th>
<th>Calcium (mmol/L)</th>
<th>Total protein (mg/ml)</th>
<th>Buffering capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caries active (n= 50)</td>
<td>0.730±0.878</td>
<td>7.624±0.354</td>
<td>1.115±0.807</td>
<td>8.878±6.104</td>
<td>41.224±16.99</td>
</tr>
<tr>
<td>Caries free (n=50)</td>
<td>0.692±0.255</td>
<td>7.737±0.365</td>
<td>0.891±0.504</td>
<td>7.873±6.777</td>
<td>54.540±54.125</td>
</tr>
<tr>
<td>P value</td>
<td>0.774</td>
<td>0.117</td>
<td>0.099</td>
<td>0.438</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

*Significant.

Table 2. Salivary parameters according to sex.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Flow rate</th>
<th>pH</th>
<th>Calcium</th>
<th>Total protein</th>
<th>Buffering capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (n=50)</td>
<td>0.783±0.872</td>
<td>7.783±0.375</td>
<td>0.998±0.572</td>
<td>5.992±5.247</td>
<td>37.326±12.632</td>
</tr>
<tr>
<td>Male (n=50)</td>
<td>0.639±0.258</td>
<td>7.577±0.306</td>
<td>1.008±0.778</td>
<td>10.75±6.676</td>
<td>58.360±23.977</td>
</tr>
<tr>
<td>P value</td>
<td>0.264</td>
<td>0.004*</td>
<td>0.938</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD; *Significant.

salivary flow rate, buffer capacity, pH, total protein and calcium in two caries free and caries active groups.

MATERIALS AND METHODS

Subjects

One hundred healthy high school students (50 females and 50 males) with age range of 15 to 17 years were randomly selected and participated in this study. Written informed consent was obtained from all subjects. Exclusion criteria were: having systemic disease, using medication, smoking, having periodontal diseases and poor oral hygiene. Subjects were divided to four groups as follows: caries free females (CF), caries active females (CA), caries free males, caries active males, each group consisted of 25 subjects.

Saliva sampling

Un-stimulated whole saliva specimens were collected in the morning, and it was asked from all selected students that brush their teeth and do not use any oral stimulation such as eating and drinking for 90 min prior to collection (Navazesh, 1993). Students were in sitting and anterior head protrusion position. Whole saliva samples were obtained by expectorating into polypropylene tubes within 5 min. The saliva samples were first weighed and reweighed again, then immediately were put on to ice and were stored at 4°C and transferred to the laboratory for up to 20 min and then were kept at -80°C until the analysis.

Clinical examination

All clinical examination was carried out by single examiner. Caries detection was based only on clinical caries observed with dental mirror and explorer and radiographic examination was not performed. CA group were selected within the subjects that had at least five clinical caries surface. CF group contained students that did not have any caries and filling and sign and symptom of sensitivity of teeth (Decayed/Missing/Filled Teeth (DMFT)=0). All selected groups had same age range.

pH and buffer capacity measurement

pH of saliva samples was determined using a pH meter (Hana Italy). To measure buffering capacity of saliva, after measuring pH, 1 ml of 0.1 N HCl was added to 1 ml of saliva and pH was recorded (Ericsson method) (Tulunoglu et al., 2006). Buffer capacity was calculated according to changes in pH.

Total protein assay

The protein content of saliva samples was measured using Bradford method (Nicholas, 2009).

Calcium assay

Saliva total calcium concentration was measured using spectrophotometric method (Pars Azmoon Kit, Iran).

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS; ver. 13). Statistical comparisons were performed using Student’s t-test. The values are expressed as mean ± standard deviation (SD). A P value of <0.05 was considered statistically significant.

RESULTS

The results of the studied parameters are presented in Table 1. The obtained data showed that flow rate, calcium and total protein were slightly increased in caries active group as compared to caries free group, but pH of saliva decreased. Buffering capacity of saliva was decreased significantly in caries active group as compared to caries free (P=0.002). The difference in these parameters between two genders is presented in Table 2. The mean value of flow rate was slightly increased in girls as compared to boys, but the difference was not significant (P=0.264). The mean value of saliva
Table 3. Sex difference in salivary parameters in caries active and caries free groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Flow rate</th>
<th>pH</th>
<th>Calcium (mmol/L)</th>
<th>Total protein (mg/ml)</th>
<th>Buffering capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caries active</td>
<td>Female</td>
<td>0.846±1.212</td>
<td>7.668±0.365</td>
<td>0.976±5.49</td>
<td>7.740±6.281</td>
<td>34.39±11.004</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.783±0.872</td>
<td>7.783±0.375</td>
<td>0.998±0.572</td>
<td>5.992±5.247</td>
<td>37.32±12.632</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.356</td>
<td>0.383</td>
<td>0.226</td>
<td>0.19</td>
<td>0.005*</td>
</tr>
<tr>
<td>Caries free</td>
<td>Female</td>
<td>0.721±0.271</td>
<td>7.898±0.358</td>
<td>1.020±6.04</td>
<td>4.244±3.287</td>
<td>40.14±13.646</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.664±0.241</td>
<td>7.575±0.276</td>
<td>0.762±0.354</td>
<td>11.502±7.451</td>
<td>68.94±23.786</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.438</td>
<td>0.001*</td>
<td>0.071</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*Significant.

Table 4. Salivary parameters in caries active and caries free adolescent in girls and boys of group.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group</th>
<th>Flow rate</th>
<th>pH</th>
<th>Calcium (mmol/L)</th>
<th>Total protein (mg/ml)</th>
<th>Buffering capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girl</td>
<td>Caries active (n=25)</td>
<td>0.846±1.212</td>
<td>7.66±0.37</td>
<td>0.976±5.49</td>
<td>7.754±6.248</td>
<td>34.39±11.004</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.618</td>
<td>0.030*</td>
<td>0.79</td>
<td>0.017*</td>
<td>0.112</td>
</tr>
<tr>
<td>Boy</td>
<td>Caries active (n=25)</td>
<td>0.614±0.277</td>
<td>7.58±0.339</td>
<td>1.255±9.95</td>
<td>5.861±1.172</td>
<td>47.78±19.248</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.497</td>
<td>0.953</td>
<td>0.024*</td>
<td>0.433</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Significant.

pH was increased significantly in girls as compared to boys (P=0.004), but the mean level of buffering capacity was decreased significantly in girls when compared with those of boys (P=0.001). There was a slight but non-significant increase in calcium in boys. The mean value of total protein and buffering capacity were increased in boys and the difference as compared to those of the girls was statistically significant (p=0.000).

In caries active group, the mean level of flow rate and pH were increased in girls as compared to boys, but these difference was not significant (p=0.356, p=0.190, respectively). Furthermore, the mean level of calcium and pH were lower in girls in this group as compared to boys, but the difference was not statistically significant (P=0.226, P=0.383, respectively). The mean level of buffering capacity of saliva was decreased in girls in this group and the difference was statistically significant (p=0.005). In caries free group, the mean level of flow rate and total protein were decreased in boys and these differences were statistically significant (P=0.000) (Table 3). There was a higher but non-significant level of flow rate and total protein in caries active group as compared to caries free group (P=0.618 and 0.112, respectively). Comparing the mean of pH between caries active and caries free groups showed significantly lower level in caries active group (P=0.03). However, calcium and buffering capacity were decreased in caries active group as compared to caries free group; the difference was not statistically significant (P=0.790, P=0.112, respectively). The mean level of flow rate and total protein were decreased; however, non-significant in caries active group when compared with caries free group. The mean level of buffering capacity was lower in caries active group when compared with caries free group; the difference was not statistically significant (P=0.001). In caries active group, the mean levels of pH and calcium were decreased as compared to caries free group, but the difference was not statistically significant (Table 4).

DISCUSSION

The obtained data in this study indicated no significant difference in salivary flow rate between two groups of caries active and caries free subjects. Lumikari and Loimaranta (2000) reported no correlation between salivary secretion and decay, which was parallel with the findings of this research. Also, Leone and Oppenheim (2001) showed that diseases such as Sjögren's syndrome as well as taking certain drugs can lead to hyposalivation, and lower salivary flow rate to the pathological levels dramatically elevates risk of caries.
Since all subjects included in this study were completely free from systemic or local disease and they took no particular medications which affect salivary secretion, the statistically non-significant difference between salivary flow rate in the two groups: caries active and caries free, in our study therefore can be justifiable. In relation to pH of saliva, this study showed that average pH of both groups was over 7, thus indicating no significant difference between individuals with active and free caries activity. Since dissolution of enamel minerals start to dissolve when the pH falls below critical pH (<5.5) (Lumikari and Loimaranta, 2000), thus the saliva pH in these two studied groups had not reached the critical limit to cause demineralization of inorganic substance of the tooth (Kleinberg et al., 1973).

Tulunoglu et al. (2006) reported no correlation between pH and caries activity in spite of age and gender. Also, as shown by Weiler et al. (1967), the acidic tendencies in resting plaques in children with rampant and moderate caries, even after eating nothing for hours indicates inherent tendencies toward caries in these children. Leone and Oppenheim (2001) research pointed out that pH reduction independent of buffering capacity of saliva is not a strong relationship to caries experience. In this research, salivary buffering capacity in individuals with active caries was lower than those of caries free group and this difference was in fact statistically significant. Similar to this finding, Pretthi et al. (2010) showed that the buffering capacity of the saliva was lower in a group including children with active caries as compared to caries free children, but the difference was not statistically significant.

The results of this study were different from the observations reported by Ericsson who showed that the buffering capacity of saliva was negatively correlated with caries (Pretthi et al., 2010). Leone and Oppenheim (2001) reported that eleven studies showed a correlation between low salivary buffering capacity and caries. Dreizen and Mann (1946) pointed out a strong correlation between buffering capacity and the chemical and bacteriological compound of the saliva with caries, and caries activity increases with lower salivary buffering capacity. Salivary buffering factors help keep salivary pH at a normal level as fast as possible. Although, these factors can elevate salivary pH; there is no conclusive evidence of their protective role against dental caries (Tenovuo, 1997).

In the present research, in caries active group, the mean calcium concentration was higher than those of caries free, but the difference was not statistically significant. Furthermore, Pretthi et al. (2010) study showed (in contrast to our study) a lower mean of calcium concentration in individuals with caries active children as compared to caries free. Nevertheless, Leone and Oppenheim (2001) reported that the findings of seven studies indicated a moderate correlation between the low level of calcium and phosphate concentration in the saliva with caries susceptibility, whereas there was no such correlation in these two studies. In this study, the total proteins level increased in individuals with caries activity as compared to caries free group; however, there was no significant difference between the two groups. Pretthi et al. (2010) also found that the total proteins level in the saliva was increased in the group with active caries as compared to the caries free, which is in line with the findings of this research. Leone and Oppenheim (2001) reported that fourteen studies examined the correlation between caries and salivary proteins and found no correlation between them. Furthermore, Akyuz et al. (1995) reported that in diabetic children, protein level of saliva elevated, which leads to elevated saliva viscosity and reduced saliva quantities. Numerous studies pointed out a significant higher number of caries teeth in females as compared to males (Akyuz et al., 1995; Lukacs and Largaespada, 2006). In this study, the saliva flow rate was a little lower in girls than boys, although the difference was not statistically significant. Lukacs and Largaespada (2006) reported that women had a significantly lower mean saliva flow rate than men; for stimulated and un-stimulated parotid saliva. Perhaps female sexual hormones, specifically estrogen, have a significant role in the suppression of saliva flow (Lukacs and Largaespada, 2006; Temple, 2011). This study showed that the buffering capability of the saliva and also its total protein level was higher in the boys' group than in the girls' and the difference between the two genders was statistically significant, whereas in relation to pH, it is decreased in boys as compared to girls and the difference was statistically significant. Three factors can explain female's prevalence of caries as compared to males: (1) earlier eruption of teeth in girls, which causes longer exposure to cariogenic environment in the mouth, (2) women consume more snacks and candy, and (3) pregnancy and hormonal influence (Lukacs, 2011). Also, the role of genetic effects, as well as the kind of nutrition greatly influences differences in caries prevalence between the two genders (Leone and Oppenheim, 2001).

Conclusion

Saliva is one of the most important factors in prevention of dental caries. Therefore, physical and chemical changes in saliva composition and particularly changes in its buffering capability play an important role in development and progression of caries.

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REFERENCES


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