

SALIVARY COMPONENTS OF TREATED CANCER PATIENTS AND PATIENTS WITH PRECANCEROUS LESIONS

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SUMMARY

Oral squamous cell carcinoma (OSCC) accounts for nearly 50 % of all newly diagnosed cancers in India. In Bulgaria OSCC reaches nearly 75% of malignant tumours in the oral cavity (Ugrinov, 2006). The prognosis of this cancer remains relatively unchanged for the past 30 years, despite advances in diagnosis and management.

Salivary analysis holds promise as a non-invasive approach to identify biomarkers for human oral cancer. Salivary levels of immunoglobulins and acute phase proteins may have significant prognostic value in early cancer diagnostics.

Key words: saliva, oral cancer patients, proteins

INTRODUCTION

Oral malignancy represents a serious public health problem. Oral squamous cell carcinoma is one of the leading causes for death in France. In Bulgaria OSCC reaches nearly 75% of malignant tumors in the oral cavity. Survival rates for OSCC patients have remained relatively unchanged for the past 30 years, despite advances in diagnosis and management.

AIM

This study aimed to investigate a large constellation of proteins taking part in immune mechanisms in oral neoplastic disease.

MATERIALS AND METHODS

Patients were recruited from the Specialized University Hospital for Active Treatment in Maxillofacial Surgery, Sofia for a period of 28 months. 35 patients with histologically confirmed OSCC (untreated OSCC patients), 10 patients with precancerous lesions, 15 treated OSCC patients and 31 healthy controls were included in the study. The demographic and clinical characteristics of the studied individuals are given in Table 1 and Table 2. None of the untreated OSCC patients had received any prior chemotherapy, radiotherapy, surgery or alternative treatment.

Table 1. Demographic data of the studied individuals

| | OSCC untreated | OSCC treated | Precancerous lesions | Controls |
|----------------|----------------|--------------|----------------------|------------|
| males | 25 | 10 | 5 | 9 |
| females | 10 | 5 | 5 | 22 |
| yrs | 56 (40-77) | 58 (42-76) | 46 (22-72) | 35 (18-75) |

Table 2. Clinical characteristics of the studied individuals

| | OSCC untreated | OSCC treated | Precancerous lesions |
|-----------------------|--|--|---|
| Characteristic | 12 - ca fundi cavi oris 11 - ca linguae 8 - ca gingivae 2 - ca labii oris 1 - ca oropharyngis 1 - ca buccae | 1 pt - chemotherapy 8 pts - radiotherapy 6 pts - surgery | 3 pts- lichen planus 7 pts - leukoplakia |

| | | | |
|------------------------|--|--|--|
| Differentiation | 14 pts - well 15 pts - moderate 6 pts - poor | 6 pts - well 6 pts - moderate 3 pts - poor | |
|------------------------|--|--|--|

The data of 31 disease free subjects were used as controls. Each healthy individual underwent a face and mouth examination by a surgeon and a dentist to ensure that suspicious mucosal lesions, as well acute and chronic parodontitis were not present. All control individuals had not received any medication one month prior to the study. None of the studied subjects had a history of any chronic disease, prior malignancy, immunodeficiency and autoimmune disorders. All patients and controls gave informed consent.

Collection of the samples

Whole unstimulated salivary probe was collected as described by Dawes and Weatherell.

Determination of IgA and IgG in saliva

The levels of IgA and IgG were assessed by radial immunodiffusion with high sensitivity by Manchini (Immunotest kits, Sofia, Bulgaria).

Determination of haptoglobin and CRP

The levels of haptoglobin and CRP in saliva were determined by immunoturbidimetric method. Salivary samples were centrifuged at 10 000 g for 10 min to avoid visible precipitates. Cobas Integra 400 - Roche Diagnostic analyser was used (lower detection limit for haptoglobin was 0.102 g/l and for CRP 0.85 mg/l).

Determination of total protein

Total protein was determined by colorimetric method using Cobas Integra 400 - Roche Diagnostic analyzer (lower detection limit 0.8 g/l).

Statistical analysis

Statistical analysis was performed using SPSS package. Mann-Whitney U test was used for comparison of the data between tested groups and values lower than 0.001 ($p < 0.001$) were considered statistically significant.

RESULTS

OSCC untreated patients:

• versus controls:

Statistically significant increase of salivary total protein, IgG, IgA, haptoglobin and CRP were found in untreated OSCC patients compared to control group (Table 3).

OSCC treated patients:

• versus controls:

In treated cancer patients only salivary level of total protein, IgG and IgA remain significantly higher ($p < 0.05$). Significant decrease of salivary haptoglobin level is observed (Table 3).

Table 3. Alterations of salivary proteins in untreated OSCC versus control subjects and OSCC treated patients versus control subjects. Results are expressed by median

| Parameters/ Patients | Total protein (g/l) | IgG (mg/l) | IgA (mg/l) | Haptoglobin (mg/l) | CRP (mg/l) |
|----------------------------------|------------------------------|-----------------------------|------------------------------|-----------------------------|--------------------------------|
| OSCC untreated (n=35) | 2,8 p=0,002 | 38 p=0,000 | 152 p=0,000 | 30 p=0,006 | 0,157 p=0,004 |
| Controls (n=31) | 1,2 | 22 | 83 | 15 | 0,030 |
| OSCC treated | 2,1 p=0,044 | 90 p=0,001 | 130 p=0,037 | 0 p=0,121 | 0,140 p=0,360 |

• versus OSCC untreated patients:

Haptoglobin in saliva was significantly higher in OSCC untreated patients, compared to OSCC treated patients. No significant differences were found in the other salivary components (Table 4).

Table 4. Alterations of salivary proteins in OSCC untreated versus OSCC treated. Results are expressed by median

| Parameters/ Patients | Total protein (g/l) | IgG (mg/l) | IgA (mg/l) | Haptoglobin (mg/l) | CRP (mg/l) |
|-----------------------------|------------------------|---------------|---------------|-----------------------|---------------|
| OSCC untreated (n=35) | 2,8 | 38 | 152 | 30 | 0,157 |
| p | p=0,293 | p=0,432 | p=0,375 | p=0,007 | p=0,144 |
| OSCC treated | 2,1 | 90 | 130 | 0 | 0,140 |

Patients with precancerous lesions

• **versus controls:**

IgG and IgA were significantly increased in patient with precancerous lesions (**Table 5**).

Table 5. Alterations of salivary proteins in in patients with precancerous lesions versus untreated OSCC patients and control subjects. Results are expressed by median

| Parameters/ Patients | Total protein (g/l) | IgG (mg/l) | IgA (mg/l) | Haptoglobin (mg/l) | CRP (mg/l) |
|---------------------------------|------------------------|------------------|------------------|-----------------------|---------------------|
| OSCC untreated | 2,8p=0,221 | 38p=0,795 | 152p=0,623 | 30p=0,021 | 0,157p=0,017 |
| Precancerous lesions | 2 | 70 | 168 | 0 | 0,110 |
| Controls | 1,2p=0,205 | 22p=0,018 | 83p=0,043 | 15p=0,474 | 0,030p=0,434 |

• **versus untreated OSCC:**

The salivary levels of haptoglobin and CRP were significantly higher in untreated OSCC. The other parameters did not differ significantly between these two groups (Table 5).

DISCUSSION:

According to Watanabe et al. the mucosal immune system, with its local mechanisms, appears to be independent from systematic immunity. It represents the first line of defense against the uptake of macromolecules and infectious agents in intestines, respiratory tract and genitourinary system. However, its relation to the development and control of neoplasia is not well understood, despite the fact that the most human malignancies derive from epithelial tissues and appear at sites where the secretory immune system is vigorously functioning.

In the present study the salivary levels of IgA and IgG in patients with oral squamous cell carcinoma were significantly increased, which is in accordance with the data

of Brown et al. and Shpitzer et al. Although a direct transudation of IgA and IgG from the blood cannot be fully excluded, one could suppose that these findings reflect the local inflammation, accompanying the neoplastic process in oral cavity. This fact is confirmed by the high levels of the tested parameters in treated patients. On the other hand, it could be considered as being local defense mechanism against the tumor development.

Serum elevation of CRP has been reported to be an indicator of the unfavorable outcome in patients with some malignant tumors (Hirasaki et al.). It has been shown that a high CRP serum level in patients with squamous cell carcinoma is associated with tumor progression and poor survival (Gockel et al.). In the present study salivary CRP levels in patients with precancerous lesions and OSCC untreated patients were significantly elevated.

Elevated concentrations of haptoglobin are seen in various malignant diseases, including oesophageal squamous cell carcinoma (Chen et al.). Increased salivary levels of haptoglobin could be related to direct transudation from the blood.

The elevated level of total protein in OSCC untreated patients, OSCC treated and patients with precancerous lesions and reflects, at least partly, the tendency of the above proteins to increase. This suggests a local origin of total protein in saliva in our patients.

CONCLUSION

The investigation of salivary proteins in the present study suggests the idea of their local production as an answer to the malignant process.

The practical usefulness of this constellation of proteins in saliva deserves further evaluation with a view of screening for early oral cancer, possible recurrent disease and individuals with high risk of oral malignant disease.

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