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Parotid Saliva Composition During and After Irradiation of Head and Neck Cancer

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Parotid saliva composition was studied before, during and up to 18 months after the irradiation period in 16 cancer patients treated for malignancies in the head and neck region. Stimulated parotid saliva was collected prior to radiotherapy and, when possible, weekly during treatment. New samples were taken 2, 4, 6, 12 and 18 months after the end of radiotherapy. Nine of the 16 patients were treated with bilateral irradiation fields and 7 patients with unilateral irradiation fields, with a total dose not exceeding 52 Gy. During the entire irradiation period the fraction of glands producing measurable volumes of saliva decreased to 40%. In the postirradiation period the number of active glands gradually increased and saliva secretion rate returned to an average of 72% of the initial value 18 months after the end of irradiation. The concentrations of the measured variables increased already during the first week of radiotherapy and at the end of the treatment period the concentrations for total protein, salivary peroxidase, hexosamine and salivary IgA were significantly increased. The concentrations for total protein, salivary peroxidase and salivary IgA were still increased 6 months after the end of irradiation. At the 18-months observation all concentrations had returned to normal, as evaluated in a paired *t*-test. The majority of glands irradiated with 40–52 Gy recovered not only in secretion rates but also with respect to the components studied in this investigation.

Keywords: irradiation, saliva, salivary gland
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INTRODUCTION

SALIVARY GLANDS are often involved in the treatment volume when tumours in the head and neck region are subjected to radiotherapy. Because of the radiosensitivity of salivary glands [1–3], the radiation dose affecting the salivary glands and especially parotid glands is of crucial importance [4, 5]. It is reasonable to assume that the degree of damage occurring is related not only to the radiation dose but also to the fractions of salivary glands that are irradiated [6, 7]. Dryness of the mouth is a common early manifestation of radiotherapy [8, 9]. A partial recovery of secretion rates usually occurs when the total radiation dose to salivary glands is below 52 Gy, but is rarely seen following higher doses [10].

Together, the secretory components have several important functions in the mouth [11], such as lubrication of oral tissues and the bolus during chewing of food, facilitation of speech and swallowing of food. Saliva also contains biological systems important for the maintenance of oral health. Among these systems are constitutents with antibacterial properties regulating both bacterial adhesion and bacterial metabolism and other constituents involved in the maintenance of ionic equilibria at the tooth surface [12, 13]. In irradiation of malignant tumours in the head and neck region, a very marked hampering of salivary gland function measured as secretion rate occurs. The effects of irradiation on the composition and quality of saliva from different glands have been reported only in a few studies [14–17]. In the present paper we have evaluated the effect of irradiation on the secretion of parotid saliva and some of its constituents. The parotid saliva composition was followed longitudinally on nine occasions; before, during and up to 18 months after the irradiation period.

PATIENTS AND METHODS

Patients

Twenty-five adult males or females with a cancer in the head and neck region and no other diseases or medication, who were to receive irradiation treatment, were included in the study. The diagnoses of the patients were Hodgkin's disease (5 patients), malignant lymphoma (2 patients), hypopharynx carcinoma (1 patient), thyroid carcinoma (1 patient) and oral carcinoma (7 patients).

Experimental design

Due to permanent xerostomia, 9 of the 25 patients treated with full-dose irradiation were later excluded from the statistical calculations concerning saliva constituents. The other 16 patients were irradiated with doses between 40 and

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52 Gy, where at least the 95% isodose curve comprised the parotid glands. A linear accelerator 4 MV (Warian) or a BBC accelerator 6 MV were used and the irradiation was delivered with isocentric technique, opposed lateral and posterior-anterior oblique fields. Patients with lymphoma were treated with mantle field. The weekly dose was 7.7–11.0 Gy delivered in five fractions. The patients were examined before the start of radiotherapy and every week during the irradiation period and 1, 2, 4, 6, 9, 12 and 18 months after finishing the treatment period. All patients were checked by the same physician and dentist at each control. 9 of the 16 patients were treated with bilateral and 7 with unilateral irradiation fields. Thus 25 glands were irradiated with 40–52 Gy. Saliva composition was analysed in the secretion produced on the various collection occasions. Comparisons were then made with the initial value for the individual.

Collection of saliva

Collection of parotid saliva was made with Lashley cups placed over the orifice of Stenson's duct. Stimulation was carried out with a mildly acid saliva stimulating tablet (SST, Salix Pharma, Sweden), placed on the tongue. Saliva was collected from each parotid gland into ice-chilled separate test-tubes. The sampling was made before noon and the collection time never exceeded 20 min. Secretion rates were determined. When only one gland was irradiated, saliva collected from the non-irradiated gland was used as control.

Salivary analysis

Calcium, sodium and potassium were analysed with atomic absorption spectroscopy (Varian Techtron AA6, Varian Associates, California, U.S.A.). Concentration of total protein was determined with the Coomassie brilliant blue method [18] using bovine serum albumin as a standard (Sigma, U.S.A.). Hexosamine was analysed after lyophilisation of the hydrolysate with a modification of the method used by Blix [19] and sialic acid was determined according to Warren [20]. The activity of amylase was measured using the Phadebas kit (Pharmacia, Sweden). Furthermore, the activity of salivary peroxidase (SPO) was measured from the spectrophotometrical curve describing the oxidation of 0.5 M pyrogallol by 1 M H_2O_2 [21]. The enzyme activity was expressed as ΔA_{400} per min and ml. The activity of a bacteria aggregating glycoprotein (BAGP) was analysed by measurement of the decrease in turbidity of a bacterial suspension after addition of saliva [22]. The activity is expressed as the value m which relates to the slope of the turbidity curve in the point of inflextion. Secretory IgA was measured with the Immuno-Fluor Technique (Bio-Rad Laboratories, Richmond, California, U.S.A.).

Statistical analysis

Differences were tested with a two-tailed paired t-test [23].

RESULTS

Twelve months following the completion of full-dose irradiation (65–74 Gy), 11 of 14 irradiated parotid glands could not produce any saliva. The three glands producing measurable volumes of saliva had a mean secretion rate of 0.1 ml/min.

The secretion rates of 25 glands receiving 40–52 Gy, as well as the fraction of glands not producing saliva on the various occasions of collection are shown in Fig. 1. The secretion rates decreased rapidly during the first week of irradiation but 72% of all glands irradiated with the low dose still produced some saliva. After the whole irradiation period of 5 weeks, the fraction of glands producing measurable volumes of saliva had decreased to 40%. In the postirradiation period the number of secreting glands gradually increased. After 12 and 18 months, 80 and 88% of the parotid glands, respectively, produced measurable volumes of saliva. The secretion rates increased gradually during the follow-up period and returned to a mean of 72% of the pre-irradiation value after 18 months. However, large interindividual variations were observed and in a paired t-test the decrease was significant up to 1 year after the end of treatment.

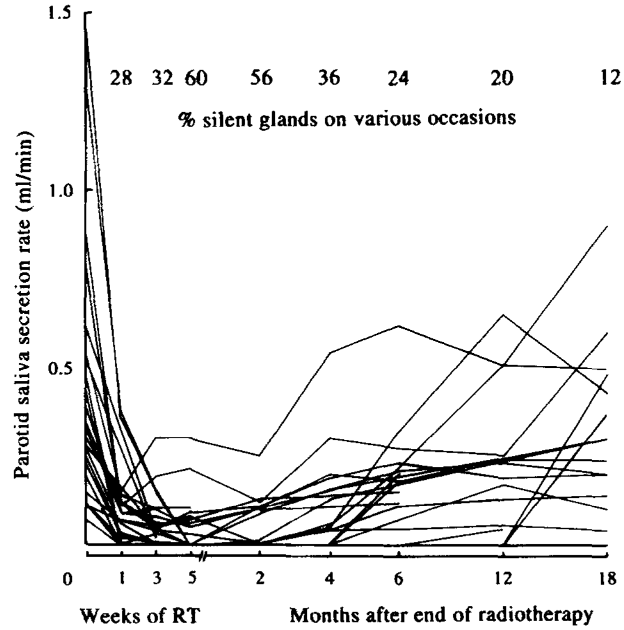

Fig. 1. Stimulated parotid saliva secretion rate from individual glands before, during and after irradiation with doses between 40 and 52 Gy. The numbers in the figure show the fraction of glands not producing any saliva on various occasions.

Ten of the 25 glands receiving 40–52 Gy (daily target dose fraction 1.6–2.2 Gy) could not produce a volume of saliva which allowed analysis of the saliva variables at a sufficient number of collection times and were, therefore, excluded from comparison of saliva composition. Four of these 10 glands could not be followed during the check-up period since follow-up treatment was carried out in hospitals near to their homes. One of the remaining six glands had regained 37% of the baseline value after 12 months, two had returned to normal after 18 months and three were still silent after 18 months.

The 15 glands, which could be followed longitudinally, either secreted saliva during the whole study period or regained some saliva secretion 6 months after the end of irradiation (Table 1). The concentrations for total protein, sialic acid, potassium and calcium and the activity for salivary peroxidase increased in the first week of radiotherapy. The values for these variables, with the exception of calcium, were still significantly increased at the end of the treatment period. Total protein, sIgA, salivary peroxidase and potassium were increased 6 months after radiotherapy. Twelve months after irradiation therapy, amylase activity and calcium and sodium

Table 1. Concentrations (mean ± SD) for variables in parotid saliva before radiotherapy and changes (↑, ↓, →) following head and neck irradiation

Variables	Initial values n=15	One week of RT n=13	Five weeks of RT n=16	Six months after RT n=15	12 months after RT n=10	18 months after RT n=12
Secretion rate (ml/min)	0.53±0.40	↓***	↓***	↓**	↓*	→
Protein (mg/l)	545±284	↑*	↑**	↑*	→	→
Amylase (U/ml)	554±406	→	→	→	↓*	→
sIgA (mg/l)	32±28	→	→	↑***	→	→
SPO (ΔA_{400}/min ml)	2.1±0.8	↑*	↑*	↑**	→	→
BAGP (m-value)	0.15±0.05	→	→	→	→	→
Hexosamine (mg/l)	95±52	→	↓*	→	→	→
Sialic acid (mg/l)	19±12	↑**	↑*	→	→	→
Sodium (mmol/l)	14±11	→	→	→	↓*	→
Potassium (mmol/l)	24±4	↑***	↑*	↑*	→	→
Calcium (mmol/l)	0.8±0.2	↑*	→	→	↓*	→

Significant increase or decrease compared with the initial value for the individual in a paired *t*-test, are indicated as $*P<0.05$, $**P<0.01$, $***P<0.001$.

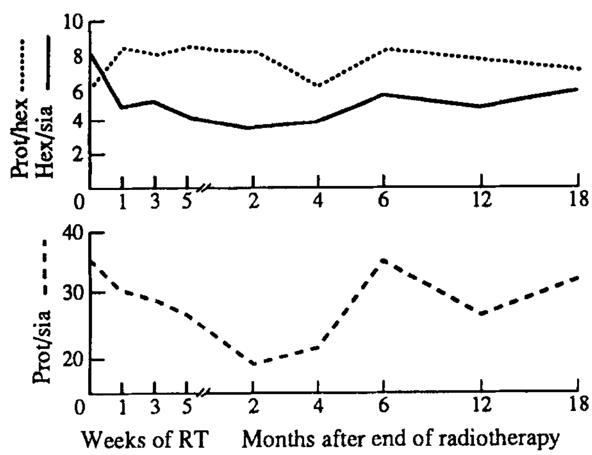

Fig. 2. Ratios between protein and hexosamine (·········), hexosamine and sialic acid (———) and protein and sialic acid (– – – –) before, during and after iradiation.

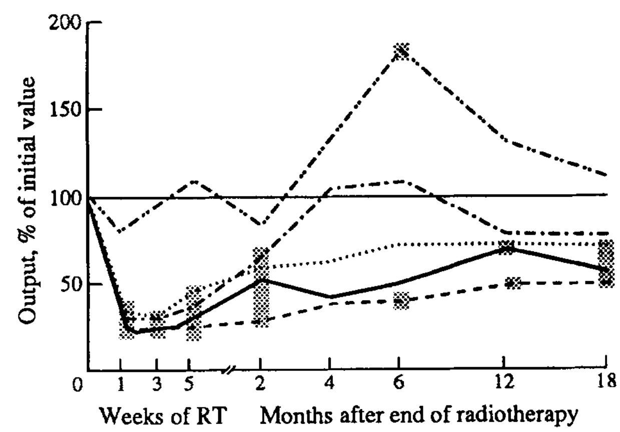

Fig. 3. Output of sIgA (– – ·· – – ··), salivary peroxidase (· – · – · – ·), total protein (·········), hexosamine (———) and amylase (– – – –) before, during and after irradiation, expressed as per cent of initial value. Each value is the mean of the individual changes. ▒ shows significant changes tested with a two-tailed paired *t*-test.

concentrations were significantly decreased. At the 18-months observation all concentrations had returned to levels which were not significantly different from the baseline values. The ratios between protein, hexosamine and sialic acid did not follow the changes in secretion rates (Fig. 2).

The outputs per minute of protein, peroxidase, hexosamine, amylase, potassium and calcium are significantly decreased during radiotherapy and up to 6 months after the end of treatment (Fig. 3). The output of peroxidase, potassium and calcium had returned to normal 18 months after radiotherapy whereas total protein, hexosamine and amylase were still significantly decreased. At the observations at 4, 6, 12 and 18 months there is an increased output of sIgA but significance is observed only at the 6-month control.

DISCUSSION

It is well known that the salivary flow rate decreases during irradiation. Depending on irradiation dose and the patient's sensitivity to radiation, the problem with dry mouth can become permanent [10, 24, 25]. Patients subjected to bilateral treatment fields of the same magnitude as in this study suffer from higher discomfort than these treated with unilateral irradiation [26]. Most of the patients reported discomfort and dry mouth problems during irradiation and for a long period after the end of treatment. Although the problem of reduced salivary flow has been emphasised, a rather limited interest has been denoted to the effects of irradiation on the different constituents in saliva.

The amount of total protein and hexosamine as well as the size and water binding capacity of the carbohydrate moiety including sialic acid are important for the lubrication function of saliva. Increased concentrations of such substances and low secretory rates, at least during the first year after radiotherapy, may explain the observation that the saliva became thick, sticky and ropy. Patients often have a considerable difficulty in handling these tenacious secretions. The recovery of secretion from the major glands was not experienced as an improvement until late in the control period. The combination of dry mouth problems and the sticky saliva may appear as contradictory but may be explained by a minor influence of the secretions from the major glands on the experience of dry mouth [27]. Possibly a major malfunction of the minor glands may occur in parallel with changes in secretion from the major salivary glands.

Evaluating our results we conclude that not only the secretion of water but also the biosynthesis and secretion of proteins has changed (Table 1). Looking, for example, at the significant increase in total protein and the nearly significant decrease in amylase it appears as a selective effect on secretion and release. Therefore, the rather general use of amylase activity as an indicator of salivary gland function may have to be questioned. This is further demonstrated by the observation presented in Fig. 2 in which the proportion of glycoprotein markers is described.

In conclusion, this longitudinal study of parotid gland function and saliva composition in patients subjected to irradiation of head and neck cancer demonstrated specific alterations in some of the constituents, however, great interindividual variations were seen. Radiation doses of about 40–52 Gy caused reversible changes in 60% of the glands with an almost restored function in some cases within 6–18 months following the end of radiotherapy. The variations in the clinical outcome of irradiation can not be predicted. Therefore, each irradiated patient must be considered individually and must have the full attention of a dentist with an interest in caries prevention.

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