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Salivary Flow and Composition in Lymphoma Patients Before, During and After Treatment with Cytostatic Drugs

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To investigate the effect of modern, intensive chemotherapy on salivary flow rate and composition, 79 patients suffering from Hodgkin's disease or non-Hodgkin lymphoma were studied before, during and after administration of cytostatic drugs. 49 patients (mean age 49.9 years, 30 men, 19 women) completed the 1-year study. All patients who received radiotherapy or medication other than cytostatics were excluded. The results showed no marked differences in stimulated salivary flow rates, buffering capacities and amylase and total protein concentrations between the beginning and the end of the 12 month trial. However, significant increases in albumin secretion into saliva and salivary lysozyme concentrations were observed. Total salivary IgG, IgA and IgM concentrations decreased significantly during the cancer therapy but values returned to the baseline levels after termination of treatment. Despite the well-known cytolytic effect of anticancer drugs, chemotherapy need not therefore be permanently detrimental to saliva.
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INTRODUCTION

A REDUCTION in salivary flow rate is a common side effect of many drugs but reports in the literature on effects of cytostatic drugs on salivary characteristics are few. Treatment with cytostatic drugs has been reported to cause hyposalivation [1, 2]. Variations in salivary flow are associated with alterations in the composition of saliva. Saliva is the principal defensive factor in the mouth [3, 4] but alterations in its composition can reduce its effectiveness. Hyposalivation can affect orodental health in particular of patients undergoing treatment for malignancies [5]

There are no data on the effects of intensive chemotherapy of lymphoma patients in relation to salivary flow rate and composition. Cytostatic drugs have a cytolytic effect because they disturb DNA and RNA synthesis [6]. All actively functioning cells, including salivary gland cells, are affected [7].

The aim of the one year study reported here was to investigate salivary flow rates and salivary compositions in lymphoma patients receiving combination chemotherapy. Saliva samples were taken before treatment, during induction of, and active treatment with cytostatic drugs, and 4–6 months after termination of medication.

PATIENTS AND METHODS

The patients

The study was conducted in 1989 and 1990 in the Department of Radiotherapy and Oncology, Helsinki University Central Hospital, Helsinki, Finland. The patients suffered from Hodgkin's disease or non-Hodgkin lymphoma. Treatment was intended to be curative. Informed consent was obtained before inclusion in accordance with the ethical guidelines of the Declaration of Helsinki [8]. Table 1 shows basic data relating to the patients. 5 patients died during the study, 6 were too ill to participate, 4 moved away, 3 received radiotherapy and 12 displayed lack of motivation.

Anticancer drugs

Patients with Hodgkin's disease received combinations of doxorubicin–bleomycin–velbe–dacarbazine (ABVD) or mustine–oncovine–procarbazine–prednisone (MOPP)-ABV hybrid chemotherapy. For non-Hodgkin lymphoma, combinations of methotrexate-bleomycin-doxorubicin/epiadriamycin–cyclophosphamide–oncovin–dexamethasone (M-BACOD or M-BECOD) were given. MOPPABV was given

Table 1. Patients' data

	Patients who completed study ($n=49$)	Patients who did not complete study ($n=30$)
Mean age (range) (years)	49.9 (22.5–81.7)	49.7 (19.1–66.7)
Sex (M/F)	30/19	9/21
Hodgkin's disease	13	3
Non-Hodgkin lymphoma	36	27
History of smoking (%)	33.3	32.1

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at intervals of 1 month for 6 months, ABVD at intervals of 2 weeks for 6 months, and M-BACOD or M-BECOD of 3-week intervals for 7 months. Subjects whose medical history and records revealed regular use of drugs other than those prescribed by the oncologists were excluded. No use of anticholinergics, antihypertensives or other drugs known to reduce salivary flow was allowed.

Dental check-ups

Patients visited the Department of Radiotherapy and Oncology in accordance with their individual treatment plans and medical conditions. Dental check-ups took place and saliva was sampled before cancer treatment was started, and then 2 weeks, 4 weeks, 6 weeks, 2 months, 4 months, 6 months and 12 months after the start of treatment. The first 4 weeks were regarded as a treatment-induction period and the following period up to 4 months as an active treatment period. The visit 12 months after the start of treatment was 4 to 6 months after receipt of the last dose of cancer medication.

Collection and analyses of saliva

Paraffin wax stimulated whole saliva was collected over a 5 min period, always between 1 and 3 p.m., 2 h after a meal, during prescheduled dental appointments at the hospital. Smoking was not allowed for 1 h before sampling. Saliva secreted during the first 30 s was discarded. The rest was collected into ice-chilled tubes. Saliva sampling was always supervised by the same oral surgeon (P.L.). Portions of saliva were taken immediately after collection for microbiological analysis, and determination of salivary flow rate, buffering capacity and lysozyme concentrations. The rest of each sample was centrifuged (15 min, 18 000 *g*, 4°C) and deep-frozen (−70°C).

Stimulated flow rate was recorded as ml/min. Salivary buffering capacity was assessed using Dentobuff kits (Orion Diagnostica, Espoo, Finland). Lysozyme assays were performed using Quantiplate Lysozyme Test Kits, based on Micrococcus diffusion plates (Kallestad Laboratories Inc., Chaska, Minnesota). Lyophilized human urine was used as a reference material.

All samples were thawed and analysed within 3 months. Amylase was determined using the Phadebas method (Pharmacia, Uppsala, Sweden). Total protein was determined using a colorimetric method [9]. Albumin was determined using a single radial immunodiffusion method (Albumin Test Kit, Kallestad Laboratories Inc., Austin, Texas) and human serum albumin as a reference. Immunoglobulin (IgG, IgA, IgM) concentrations were determined using the enzyme immunoassay of Lehtonen *et al.* [10].

Statistical methods

The significance of differences between means was determined using the rank-sum test. Analysis of variance was used for continuous data. All P values reported are based on two-tailed tests of significance. The analyses related to differences between baseline values, the end of the induction-period values (4 weeks after entry into the trial), active treatment period values (2 and 4 month values), and follow-up values (12 month results).

RESULTS

Salivary flow rate and buffering capacity

No differences from baseline values were observed in stimulated salivary flow rates after starting cancer treatment (Table 2). In men, salivary flow rates were found to be reduced at the 12 month appointment, 4–6 months after termination of cytostatic drug treatment ($P<0.05$). In general, patients with low salivary secretion rates (<0.8 ml/min) at baseline had low secretion rates throughout the study.

Table 2. Stimulated salivary flow rate [ml/min (S.D.)]

Time of Measurement	Men	*n*	Women	*n*
Baseline	1.7 (0.6)	36	1.1 (0.7)	34
4 weeks (induction period)	1.7 (0.8)	34	1.3 (0.6)	25
2 months (of active treatment)	1.6 (0.9)	35	1.2 (0.6)	31
4 months (of active treatment)	1.6 (0.7)	33	1.1 (0.6)	24
12 month follow-up	1.4 (0.8)*	30	1.0 (0.5)	19

*$P<0.05$ when compared with baseline.

Numbers of men and women with low salivary buffering capacities (end pH < 4.0) decreased significantly ($P<0.05$) during the period of induction of cancer treatment. Otherwise, buffering capacity stayed similar throughout the study (Table 3).

Total protein and albumin

Figure 1 shows that cancer treatment in general had no effect on total protein concentration in saliva. In women, however, a statistically significant increase in salivary total protein was observed between the 2 and 12 month values($P<0.05$).

Salivary albumin concentrations are shown in Table 4. The percentage of patients whose whole saliva contained albumin had increased significantly on completion of cytostatic drug treatment seen at the 12-month time point. Every woman had albumin in her 12 month saliva sample. In women, albumin concentrations were significantly higher in the 12 month samples than at the start of the trial. In men, the opposite was true, the highest albumin values were found during induction of cancer treatment.

Lysozyme and amylase

Salivary lysozyme concentrations increased after termination of cytostatic drug treatment. The increase was significant in men ($P<0.01$). During the induction and active treatment periods, however, lysozyme concentrations did not change significantly. No statistically significant differences were observed in salivary amylase concentrations in relation to the various periods of the trial (Fig. 1).

Immunoglobulins

Salivary total IgG concentrations decreased significantly during induction of cancer treatment (Fig. 2). A year later concentrations were similar to those at the start of the trial. IgM concentrations also decreased significantly after therapy was begun but the 12 month values were similar to these at the start of the trial (Fig. 2). In women, an increase was observed which was statistically significant. In men, the differences between the 12 month concentrations and the 4 week, 2 month and 4 month concentrations were all significant at the $P<0.01$ level.

A statistically significant decrease was observed in salivary IgA concentrations in men after induction of cytostatic treatment ($P<0.001$). In women the significance of the decrease

Table 3. Salivary buffering capacity. Percentage distribution of Dentobuff scores.

Time of measurement	Men Low (%)	Men Moderate (%)	Men High* (%)	Men n	Women Low (%)	Women Moderate (%)	Women High* (%)	Women n
Baseline	23	49	28	39	47	31	22	32
4 weeks (induction period)	12	44	44	34	32	52	16	25
2 months (of active treatment)	27	52	21	33	53	33	14	30
4 months (of active treatment)	25	41	34	32	50	23	27	22
12 month follow-up	21	48	31	29	42	42	16	19

*Low = pH < 4.0; Moderate = pH 4.5–5.5; High = final pH > 6.0.

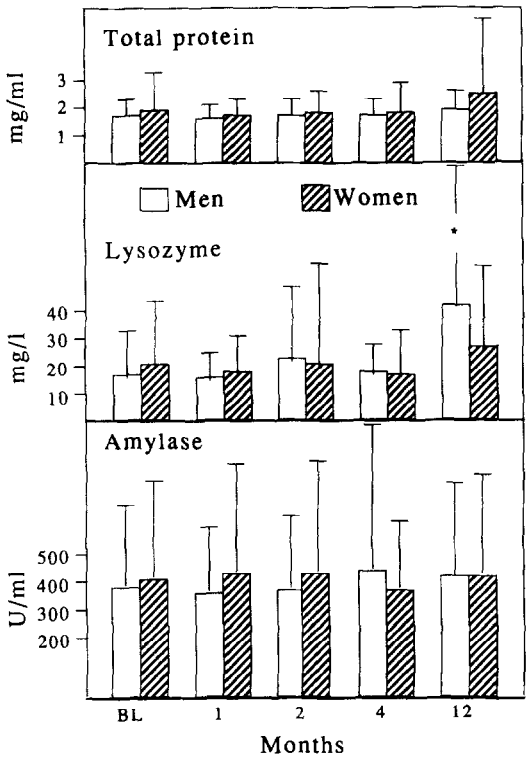

Fig. 1. Mean salivary total protein, lysozyme and amylase concentrations (S.D.). The asterisk indicates statistically significant difference between baseline (BL) and 12 month results ($P < 0.05$).

Table 4. Salivary albumin [μg/ml (S.D.)]

Time of measurement	Albumin concentration	Patients with albumin in saliva (%)
Men		
Baseline	150.2 (102.4)	53
4 weeks (induction period)	178.1 (95.3)*	46
2 months (of active treatment)	152.2 (46.3)	49
4 months (of active treatment)	111.1 (72.8)	56
12 month follow-up	125.4 (73.7)†	78
Women		
Base	133.5 (55.4)	54
4 weeks (induction period)	150.8 (103.8)*	76
2 months (of active treatment)	131.8 (63.2)	55
4 months (of active treatment)	206.5 (394.0)	68
12 month follow-up	229.3 (387.7)‡	100

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

was less ($P < 0.05$) (Fig. 2). Mean salivary IgA concentrations in both sexes returned to values not significantly different from those at the start of treatment. In men, the mean 12 month concentration was significantly different from the 4 week, 2 month and 4 month concentrations ($P < 0.01$). In women, the only significant difference was between the 4 week and 12 month values ($P < 0.01$).

DISCUSSION

In contrast to previous findings [11, 12] we did not observe stimulated salivary flow rate to be significantly affected by cytostatic drug treatment. One explanation for the discrepancy may be that we took great care not to include patients who received radiotherapy in addition to anticancer drugs. No previous studies of the effects on saliva of the chemotherapy used in the study reported here have been conducted.

Since low salivary buffering capacity usually accompanies a low salivary secretion rate, our finding of fairly stable values for buffering capacity throughout the study was not unexpected.

Salivary total protein concentrations also remained stable throughout the study. The protein concentrations found were similar to those observed in studies in other patient groups, e.g. diabetics [13].

Albumin in saliva is regarded as a plasma ultrafiltrate. It is usually detectable in saliva in only small amounts [14]. Radiotherapy to the head and neck has been shown to increase secretion of albumin into saliva obviously because of injury to salivary gland tissue [15]. Immediately after cytostatic drug treatment began, a significant increase in albumin concentration was observed. During induction of anticancer treatment, the patients' conditions were highly compromised, which would also have affected the salivary glands. Lymphomas can also affect salivary glands. This would explain the high baseline values observed. By the end of the follow-up period, most of our patients were secreting albumin into their saliva, suggesting the possibility of injury [16]. Albumin in the whole saliva examined may well be also from mucosal damage or change in permeability and not only from salivary glands [17].

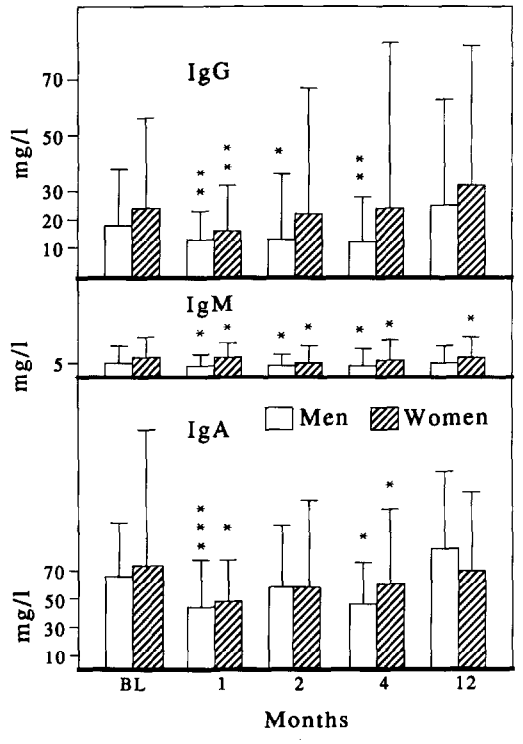

Fig. 2. Mean salivary IgG, IgM and IgA concentrations (S.D.). Statistically significant differences between follow-up recordings and baseline (BL) are indicated by asterisks (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

Amylase concentrations reflect functional capacities of salivary glands [18]. We found no impairment in amylase secretion during or after the various phases of anticancer treatment. Secretion of amylase remained essentially constant. Any tissue injury affecting membrane transport which may have occurred therefore did not impair the acini where amylase is synthesised.

We determined salivary lysozyme levels as indicators of the innate, unspecific defense factors in saliva. Lysozyme may also derive from inflammatory cells entering the oral cavity [18].

Immunoglobulin concentrations in saliva decreased consistently during the anticancer treatment. This was not unexpected because all immunoglobulins derive from plasma cells, which are known to be affected by anticancer treatment. Cytostatic drugs are mutagenic, teratogenic and immunosuppressive [19]. After treatment, as bone marrow function improves, immunoglobulin concentrations return to normal. However, improvement in bone marrow function can extend over 5–15 years [19, 20]. Our findings of a rapid increase of immunoglobulins in saliva after termination of cytostatic treatment could be a result of the new treatment used in our patients. It should be also noted that in the whole saliva examined most salivary antibody (IgA) is produced locally within the glands, where the effect of immunosuppression may not be as severe as in the bone marrow.

To conclude, our results, showing no marked decrease in stimulated salivary flow rate and amylase secretion despite administration of cytostatic drugs give new hope from the dental point of view in relation to treatment of patients with modern cancer chemotherapy. The finding that immunoglobulin concentrations returned to the sort of levels found at the start of the trial after treatment ended suggests that the side effects of the chemotherapy tested need not be permanent. However, the increase in secretion of albumin observed indicated that effective anticancer drug therapy used did cause ultrafiltration of this serum component to the mouth.

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