Sialochemical and oxidative analyses in radioactive I\textsuperscript{131}-treated patients with thyroid carcinoma

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Abstract

Background: I\textsuperscript{131} in relatively high doses has been shown in the past to cause damaging salivary effects and oral discomfort in patients. Although lower dosage is now widely accepted, I\textsuperscript{131} may still be the source of salivary damage over the long-term and subsequent harmful effects on both the oral cavity and the gastrointestinal tract, into which the saliva is swallowed. This study examined the effects of radioactive I\textsuperscript{131} on salivary gland activity, saliva composition and oxidative profile, and related oral discomfort complaints following thyroidectomy due to carcinoma of thyroid gland.

Methods: Out of 40 consenting female post-thyroidectomy patients, 23 (mean age 50±4 years old) were treated with I\textsuperscript{131} while 17 (mean age 46±4) were not. Whole saliva from all subjects was analyzed for antioxidant and biochemical composition and flow rate.

Results: The salivary flow rates of both groups were similar but their composition differed considerably. Salivary superoxide dismutase enzyme (SOD), total protein, and albumin concentrations were significantly reduced in the treated patients by 40, 25, and 18% respectively ($P<0.05$), as were all other salivary antioxidants. Oral discomfort complaints were far more prevalent in the I\textsuperscript{131}-treated patients.

Conclusions: I\textsuperscript{131}-dependent damage to the salivary glands was evidenced by a broad spectrum of compositional alterations and oral complaints. Reduction in salivary antioxidant status, SOD enzyme, and the uric acid molecule leaves the oral cavity less protected against oxidative stress. This is the first report of radioactive I\textsuperscript{131} treatment being harmful to salivary glands due to compromised salivary compositional and oxidative profile and oral discomfort complaints.

Introduction

In recent years, it has become common to administer radioactive I\textsuperscript{131} as part of the therapeutic regime in patients with thyroid carcinoma following the surgical procedure of total or partial thyroidectomy. Ideally, I\textsuperscript{131} treatment should ablate residual micro/macrophagic tumor cells in the neck and/or distant metastasis but not affect surrounding healthy tissues such as the salivary glands. Lower doses of I\textsuperscript{131} are considered especially valid as patient complaints of salivary gland pain or swelling are fewer in the past when higher doses of I\textsuperscript{131} were routinely administered (1, 2). However, if salivary ‘sub-lethal’ damage occurs following the administration of the radioactive I\textsuperscript{131}, it may have harmful effects over the long term on both the oral cavity and the gastrointestinal tract (into which the saliva is swallowed).

The purpose of the current study was to analyze the salivary flow rate and composition, including salivary antioxidants in radioactive I\textsuperscript{131}-treated patients, and to concomitantly monitor salivary-related complaints.

Materials and methods

Study design

In the current study, 40 consenting female patients, who had undergone surgery for thyroid carcinoma (either a total or a sub-total thyroidectomy) 8.4±7 years prior to the study, were enrolled. The post-surgical iodine absorption was 1±0.018%. Out of these 40 patients, 23 (mean age 50±4 years) were treated with I\textsuperscript{131} post-operatively (study group), while the other 17 (mean age 46±4 years) did not receive I\textsuperscript{131} at all (control group). The difference in the age of both groups was not significant. None of the patients smoke and they used general medications as required which are typical to their age group (such as for hypertension) but not any medication that is known to affect saliva. There was no significant difference in the percentage of the post-menopausal individuals in both groups (4 out of 17 of the non-iodine-treated group and 5 out of 23 of the iodine-treated group). There were no differences with respect to their TSH levels and only one...
post-menopausal patient of each group used hormone replacement therapy. The treated patients received \(^{131}\)I therapy at least 1 year prior to the study: 20 women received 100 mC, 2 women 150 mC, and 1 obese patient with neck lymph node metastasis received 200 mC. In all subjects, salivary flow rates were measured, and saliva samples were studied for their biochemical and antioxidant composition. Concurrently, the patients were questioned about possible accompanying oral complaints. The biochemical analysis included various parameters reflecting the normal composition of human saliva (3): calcium (Ca), inorganic phosphate (P), magnesium (Mg), total protein (TP), albumin (Alb), lactate dehydrogenase (LDH), and amylase (Amy). The salivary oxidative analysis included the main salivary antioxidant molecule, such as uric acid (UA), the superoxide dismutase (SOD) enzyme, the peroxidase enzyme, and the total antioxidant status (TAS). The oral complaints monitored included mouth dryness, difficulty in swallowing, and changes in taste. All of these are known to be related to salivary alterations and are based on an established questionnaire previously used (4, 5).

**Sialometrical analysis**

Saliva was collected using the widely accepted procedure previously described (3), controlled to avoid drooling or swallowing. Whole saliva was collected in resting conditions in a quiet room during the morning, between 0900 and 1200 h, at least 1 h after food intake. Subjects were asked to collect saliva in their mouths and to spit it into a wide-mouthed test tube for 5 min. Saliva flow rates were estimated by measuring the volume of saliva thus collected, which was then centrifuged and kept at 4°C until analyzed within 1 week of collection. Previous studies have shown that saliva remains stable during this time period with respect to the parameters analyzed in such conditions.

**Salivary biochemical analysis**

The P concentration was measured spectrophotometrically, and Ca and Mg concentrations were measured by atomic absorption (6, 7). TP was measured as previously described (3) while amylase was measured by the Phadebas amylase test (Pharmacia Diagnostics). Albumin was measured by the radial immunodiffusion method (8), using an Oxford viewer for measuring the diameters of the precipitation rings (Mancini plates purchased from Binding Site, Birmingham, UK.) The diameter of the ring formed is quantitatively related to the concentration of various parameters analyzed. The LDH was measured at 37°C by an optimized standard method using pyruvate as the substrate with the Hitachi 911 automated clinical chemistry analyzer using reagents purchased from Roche Diagnostics. The assay coefficient of variation (CV) was 2.1%. Amylease was measured at 37°C using 4.6-ethylidene (G7)-p-nitrophenyl (G1)-z,D-maltoheptaoside as substrate, as previously described (9, 10); the assay CV was 3.4%.

**Salivary oxidative analysis**

**Uric acid (UA)** UA concentration was measured in the saliva of the patients using a kit supplied by Sentinel CH (Milan, Italy), as previously described (3). UA is transformed by uricase into allantoin and hydrogen peroxide that, under the catalytic influence of peroxidase, oxidizes the chromogen (4-aminophenazone/N-ethylmethylanilin propan-sulfonate sodic) to form a red compound whose intensity of color is proportional to the amount of UA present in the sample and is read at a wavelength of 546 nm.

**Peroxidase activity** Peroxidase activity was measured in the patients’ saliva according to the NBS assay, as previously described (11). Briefly, the calorimetric change induced by the reaction between the enzyme and the substrate, dithiobis-2-nitrobenzoic acid in the presence of mercaptoethanol, was read at a wavelength of 412 nm for 20 s.

**Superoxide dismutase (SOD) activity** The total salivary activity of the SOD enzyme (Cu/Zn- and Mn-SOD) was measured using the method of xanthine oxidase/XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt). The method is a modification of the NBT assay, in which XTT is reduced by superoxide radical generated by xanthine oxidase. Formazan is read at 470 nm. The SOD enzyme inhibits this reaction by scavenging superoxide radical. One unit of the enzyme is defined as the amount needed for a 50% inhibition of the absorption (12).

**Total antioxidant status (TAS)** TAS was assessed in the saliva samples, as previously described (3). Briefly, this assay is based on a commercial kit supplied by Randox (USA) in which metmyoglobin in the presence of iron is turned into ferrylmyoglobin. Incubation of the latter with the Randox (County Antrim, United Kingdom) reagent ABTS results in the formation of a radical colored blue-green, which can be detected at 600 nm.

**Statistical analysis**

For categorical variables, frequencies, percentages, and distribution were calculated. Distribution for categorical variables with small numbers of observations (at least one cell with less than five observations) was compared by Fisher–Irwin exact test. For continuous variables, ranges, medians, means, s.d., and s.e.m. were calculated. The results of age and time were compared by two-sample
t-test for differences in means. Due to the large in-born variability of parameters in saliva, median values were calculated and compared by Wilcoxon rank-sum test (pairs of subgroups).

Results

Salivary sialometrical and biochemical analysis

The salivary flow rate of both control and I131-treated groups was similar (0.29 ±0.04 and 0.32 ±0.04 ml/min respectively). However, the salivary composition of both groups was different. The median salivary Ca, P, and Mg concentrations in the control group were 2.7, 12.3, and 1.2 mg/dl respectively. In the I131-treated group, these values were lower by 26% (P = 0.07), 14% (P = 0.14), and 9% (P = 0.15) respectively. The median salivary total protein and albumin concentrations were 70 and 59.4 mg/dl respectively, while the median enzyme activity of salivary LDH and amylase were 17 and 792 IU/l respectively. All four were lower in the saliva of the I131-treated group by 25% (P = 0.007), 18% (P = 0.05), 30% (P = 0.45), and 31% (P = 0.45) respectively (Table 1).

Salivary oxidative analysis

The median salivary enzyme activity of the SOD in the control group was 0.61 U/ml, and in the I131-treated group it was 0.37 U/ml, a significant reduction of 40% (P = 0.009). The median salivary TAS concentration of the control group was 0.47 mmol/l, and in the I131-treated group it was lower by 22% (P = 0.34). The median UA concentration and peroxidase activity were lower by 5% in the I131-treated group, though these reductions did not reach statistical significance (Table 2).

Salivary-related complaints

The rate of complaints of dry mouth was higher in the I131-treated patients when compared with the controls (33% vs 18%). Five of the treated patients complained of difficulty in swallowing and three complained of taste disturbances, while in the non-treated patients there were no such complaints. Thus, the complaint of difficulty in swallowing was found to be significantly more prevalent in the I131-treated group (P = 0.05; Table 3). No significant correlations were found between any of the salivary changes and any of the subjective symptoms reported.

Discussion

The results obtained in the current study clearly indicate the I131-dependent damage to the salivary glands in patients who did not exhibit reduction in the salivary flow rate. This is the first such report, as patients who receive I131 following partial or full thyroidectomy have been considered safe from adverse salivary effects in contrast with those patients in the past who received higher doses of I131 and had reduction in the salivary flow, as previously reported (1, 2, 13). Whole saliva was collected at rest. The submandibular glands contribute significantly more

Table 1 Saliva parameters by study groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 17)</th>
<th>I131 (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (mg/dl)</td>
<td>Range (19–138) (4–115) P = 0.007</td>
<td>Median 70.0 53.0</td>
</tr>
<tr>
<td>ALB (mg/dl)</td>
<td>Range (18.6–386.7) (10.6–306.3) P = 0.22</td>
<td>Median 59.4 48.8</td>
</tr>
<tr>
<td>LDH (IU/l)</td>
<td>Range (7–332) (3–1081) P = 0.07</td>
<td>Median 17.0 12.0</td>
</tr>
<tr>
<td>Amy (102 IU/l)</td>
<td>Range (108–4878) (135–3823) P = 0.45</td>
<td>Median 792 549</td>
</tr>
</tbody>
</table>

Table 2 Saliva parameters by study groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 17)</th>
<th>I131 (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UA (mg/dl)</td>
<td>Range (0.85–6.96) (0.46–5.71) P = 0.92</td>
<td>Median 2.92 2.77</td>
</tr>
<tr>
<td>Peroxidase (O.D.)</td>
<td>Range (0.5842–0.8627) (0.5235–0.8192) P = 0.087</td>
<td>Median 0.75 0.71</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>Range (0.41–3.71) (0.19–1.31) P = 0.009</td>
<td>Median 0.61 0.37</td>
</tr>
<tr>
<td>TAS (mmol/l)</td>
<td>Range (0.2–0.88) (0.03–0.85) P = 0.34</td>
<td>Median 0.47 0.37</td>
</tr>
</tbody>
</table>

TP, total protein; ALB, albumin; LDH, lactate dehydrogenase; Amy, amylase.}

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than the parotids to salivary volume when the glands are at rest. Clinically, it is known that the submandibulars, probably because of the mucous elements, are more resistant to the effects of $^{131}${I}. These facts may explain why there was no reduction in the flow rates in the $^{131}${I}-treated patients. The $^{131}${I}-dependent damage, manifested by a broad spectrum of compositional alterations, may be of clinical merit especially as it is accompanied by various oral complaints. Moreover, the reduction in salivary TAS, the SOD and peroxidase enzymes, and the UA molecule is not only novel but it is also of significant clinical merit as the oral cavity and gastrointestinal tract remain less protected against oxidative stress. The observation of the wide spectrum reduction of various salivary functions is of special importance, as this enzyme is a pivotal one in the salivary antioxidant system and more so in the parotid gland that is known to be mostly sensitive to irradiation damage (12, 14). Moreover, the fact that not all decreases in the salivary parameters were statistically significant may be explained by the relatively small number of individuals analyzed, the high variability known to characterize saliva composition, and the relatively marginal effect of the radioactive $^{131}${I} (15). Nevertheless, even if relatively marginal, the decreases in the salivary parameters may well have a long-term widespread debilitating effect on the saliva and its surroundings.

Regardless of the specific mechanism by which $^{131}${I} affects the salivary glands, the data presented indicate damage to the glands induced by the therapy. Accordingly, administration of supportive therapy should be considered, based on agents including antioxidants, anti-inflammatory drugs, and saliva substitutes to the oral cavity of treated patients.

### Acknowledgements

The authors thank Mrs S Gan for her assistance in the statistical analysis of this paper.

### References


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**Table 3** Oral sensorial complaints by study groups.

<table>
<thead>
<tr>
<th>Group/difficulty in swallowing</th>
<th>A patients (%)</th>
<th>$^{131}$I patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Difficulty in swallowing by study groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0)</td>
<td>5 (22)</td>
</tr>
<tr>
<td>No</td>
<td>17 (100)</td>
<td>18 (78)</td>
</tr>
<tr>
<td>Total</td>
<td>17 (100)</td>
<td>23 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group/mouth dryness</th>
<th>Control patients (%)</th>
<th>$^{131}$I patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. Mouth dryness by study groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (18)</td>
<td>8 (35)</td>
</tr>
<tr>
<td>No</td>
<td>14 (82)</td>
<td>15 (65)</td>
</tr>
<tr>
<td>Total</td>
<td>17 (100)</td>
<td>23 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group/impulse to wash the mouth</th>
<th>A patients (%)</th>
<th>$^{131}$I patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Impulse to wash the mouth by study groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (12)</td>
<td>5 (22)</td>
</tr>
<tr>
<td>No</td>
<td>15 (88)</td>
<td>18 (78)</td>
</tr>
<tr>
<td>Total</td>
<td>17 (100)</td>
<td>23 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group/changes in sense of taste</th>
<th>A patients (%)</th>
<th>$^{131}$I patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Changes in sense of taste by study groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metal taste</td>
<td>0 (0)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>No</td>
<td>17 (100)</td>
<td>20 (87)</td>
</tr>
<tr>
<td>Total</td>
<td>17 (100)</td>
<td>23 (100)</td>
</tr>
</tbody>
</table>

$P=0.05$ is statistically significant. $P=0.21$, $P=0.35$, $P=0.18$. 


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