Effect of Radioiodine Therapy on Several Hematological and Immune Parameters in Patients with Differentiated Thyroid Carcinoma

Metin Özata* Hakkı Ergun* Gökhan Özişik* Aysel Pekel** Ferit Arcu*** Erol Bolu* Zeynel Beyhan*
Çağatay Öktenli* Ali Şengül** Nuri Aslan**** Atilla Yalçın*** Çağlayan Özdemir*

Gülhane School of Medicine, Ankara
* Department of Endocrinology and Metabolism
** Department of Immunology
*** Department of Hematology
**** Department of Nuclear Medicine

Although several reports describe depression of circulating cellular elements in the blood of patients treated with radioactive iodine (RAI) for differentiated thyroid carcinoma (DTC), little is known about changes in platelet aggregation, cytokines and complement levels after RAI treatment. We selected 14 patients with DTC (8 females and 6 males; mean age: 34±10 yr) who received the same dose of 131I therapy for thyroid cancer. Fourteen sex-and age-matched normal subjects (mean age: 36±5 yr, 8 female and 6 males) were enrolled as controls. All patients were in class III and none had accompanying thyroiditis. Serum samples for IL-6 and TNF-α were collected 1 day before and at 2, 4 and 6 months after the radioactive treatment. At each visit, peripheral blood count, platelet aggregation, immunoglobulins, complement levels and lymphocyte subpopulations were evaluated. WBC counts, hemoglobin, platelet and total lymphocyte count and TNF-α levels did not change significantly after RAI administration. CD19 counts decreased significantly at 2 months. There were no other significant changes in lymphocyte subpopulations. IL-6 levels increased significantly 2 months following treatment. A significant fall in C3c and C4 levels was observed 4 months after treatment. No significant changes were found in pre- and after-treatment immunoglobulin levels. Platelet aggregation induced with ADP, collagen and epinephrine also did not show any difference before and after treatment. We conclude that RAI administration in DTC patients associated with a significant increase in circulating IL-6 levels and a reduction in CD19 count and complements.

Key words: Radioiodine therapy, differentiated thyroid carcinoma, immune parameters, hematological parameters

Introduction

Radioactive iodine (RAI) has been used for nearly fifty years for the treatment of hyperthyroidism and thyroid cancer. Although several reports describe a depression of circulating cellular elements in the blood of patients treated with RAI for thyroid carcinoma (1-4), little is known about platelet function after RAI treatment.

It is also known that RAI treatment can induce an alteration in immune response to thyroid antigens in Graves’ disease (5). Thus it is also possible that thyroid antigens can be released into the circulation after destruction of thyroid cells by RAI in patients with thyroid cancer. Evidence for this comes from our previous study which showed that serum thyroid peroxidase was detectable after RAI treatment in
most patients with thyroid cancer (6). Thus, RAI treatment in thyroid carcinoma may cause some alterations in cytokines and other immune system parameters. No previous study has reported cytokine alterations after RAI treatment in patients with thyroid carcinoma.

The present study was designed to evaluate the effects of RAI treatment on platelet aggregation and immunological parameters in patients with differentiated thyroid carcinoma.

**Patients and Methods**

To evaluate the effects of radioiodine treatment on various hematological and immune parameters, we selected 14 patients with differentiated thyroid cancer (DTC) (8 females and 6 males; mean age: 34±10 yr) who received the same dose of $^{131}$I therapy (150 mCi) for ablation of a postsurgical thyroid remnant or treatment of metastatic lesions and were in the same clinical class. None of the patients had received radioiodine therapy or chemotherapy before enrolment in the study. Histological examination showed papillary cancer in 11 patients and follicular cancer in 3 and none of the patients had thyroiditis. Fourteen sex- and age- matched normal subjects (mean age: 36±5 yr, 8 female and 6 males) were selected as controls. Goiter or symptoms suggestive of thyroid disorders or other autoimmune diseases were absent in all normal subjects at the time of study. In addition, family histories of healthy subjects were essentially negative for thyroid disorders and other autoimmune disease.

In all of the 14 patients with DTC, near total or total thyroidectomy was accomplished. Six to eight weeks following surgery standard total body scans were performed at 48 and 72 hr after the administration of 5 mCi of $^{131}$I. As the postoperative 5 mCi scan indicated residual disease or thyroid tissue, an empirically determined dose of $^{131}$I was given with the intent to ablate post surgical residual thyroid tissue in the thyroid bed and/or treatment of metastatic lesions. $^{131}$I total body scans were performed again in all patients 6 months after $^{131}$I therapy when the patients were off thyroxine (T$_4$) suppressive therapy.

All patients were given suppressive therapy with levothyroxine (L-T$_4$) in an amount adequate to suppress TSH to low levels depending on the patients’ disease status after initial radioiodine therapy. L-T$_4$ therapy was stopped 6 weeks before scans and patients were replaced on triiodothyronine (T$_3$). Two weeks before the follow-up scan, T$_3$ was stopped.

Patients were classified for the extent of disease at diagnosis according to DeGroot’s classification (7). All patients were in class III.

All patients and the control subjects were informed about the aim and procedure of the study and gave their consent. The studies were approved by the local ethical committee of Gulhane School of Medicine.

Serum samples for IL-6 and TNF-α were collected 1 day before and at 2, 4 and 6 months after the radioiodine treatment. At each visit, peripheral blood count, platelet aggregation, immunoglobulins, complement levels (C3c and C4) and lymphocyte subpopulation were studied. Patients were hypothyroid when blood samples were collected before radioiodine therapy and at 6 months when follow-up scans were obtained. At 2 and 4 months after $^{131}$I therapy, patients were on L-T$_4$ suppressive therapy. All serum samples obtained from patients during follow-up and from normal subjects were stored at -70°C until analysis, and all sera were run in the same assay.

Peripheral blood count was measured on Cell-Dyn 1600 hemocounter (Santa Clara, USA).

Immunoglobulins, C3c and C4 were measured using antibodies and the BN-100 nephelometer (Behring Merck AG, Germany).

Lymphocyte subpopulations were analyzed by Flow cytometry (FACS Calibur) using antibodies (Becton Dickinson, Sanjose, USA).

Platelet aggregation was measured using Sigma platelet aggregating reagents (Sigma Diagnostic Corporation, St. Louis, USA and Cod No 885/A. For ADP: 885/3, fr collagen: 885/1, for epinephrine: 885/5). Blood samples were centrifuged (250xg, 10 min) to isolate platelet rich plasma (PRP) contained in the supernatant. The remainder of the blood was centrifuged again (1500xg, 10 min) to prepare platelet poor plasma (PPP). The PRP was diluted with the PPP to yield test PRP with a final
Results

All parameters except platelet aggregation are shown in Table 1. WBC count was significantly lower in the patient group than the controls. There was no significant change in WBC, Hb, Htc, and total lymphocyte count after RAI treatment. CD4 and CD8 counts were significantly lower in the patient group when compared to those in the control group. CD3, CD4 and CD8 counts did not change significantly after treatment. CD19 counts decreased significantly at 2 months.

Pretreatment IL-6 levels were not significantly different from controls. Despite a significant increase at 2 months, IL-6 levels were significantly lower at 4 months when compared to pretreatment values. TNF-α levels did not change significantly after RAI therapy.

count of 260±50 x10⁹/L. Platelet aggregation was studied on a Lumi-Dual platelet aggregometer (Chrono-Luog Corporation Model 450). Measurements were made for adenosine diphosphate (ADP), collagen and epinephrine; the final concentrations in sequence were 20 mmol, 0.2 mg/L-1 and 10 mmol. Maximum aggregation responses were evaluated according to this formula: (90 - distance to upper range of graph paper/80) x 100.

IL-6 and TNF-α were measured by immunoassay using commercially available kits from Biosource International (Camarillo, California, USA).

Wilcoxon test was used for comparison of the parameters in the patient group. Parameters of the patient and control groups were compared by Mann Whitney U test. p<0.05 was considered significant. All results are expressed as means ±SD.

### Table 1. Peripheral blood counts, lymphocyte subpopulations, and immune parameters before and after ¹³¹I treatment in patients with DTC and in controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before ¹³¹I</th>
<th>2 Months after ¹³¹I treatment</th>
<th>4 Months after ¹³¹I treatment</th>
<th>6 Months after ¹³¹I treatment</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10⁹/L)</td>
<td>5.5±1.571¹</td>
<td>4.96±0.739</td>
<td>5.42±0.942</td>
<td>5.32±1.268</td>
<td>7.05±3.1³</td>
</tr>
<tr>
<td>Hb (gr/dl)</td>
<td>12.47±2.08</td>
<td>12.03±1.08</td>
<td>12.23±1.3</td>
<td>13.14±1.65</td>
<td>13.28±0.9</td>
</tr>
<tr>
<td>Htc (%)</td>
<td>36.65±5.4</td>
<td>35.59±3.4</td>
<td>36.34±3.5</td>
<td>38.3±4.1</td>
<td>39.9±2</td>
</tr>
<tr>
<td>Platelet (10⁹/L)</td>
<td>235.71±58.73</td>
<td>231.60±28.935</td>
<td>235.30±65.1</td>
<td>229.21±58.169</td>
<td>223.61±42.10</td>
</tr>
<tr>
<td>Lymphocyte count (10⁹/L)</td>
<td>1.756±0.571</td>
<td>1.539±0.330</td>
<td>1.799±0.753</td>
<td>1.703±0.541</td>
<td>2.42±1.55</td>
</tr>
<tr>
<td>CD3 (10⁹/L)</td>
<td>1.234±0.382</td>
<td>1.103±0.262</td>
<td>1.250±0.641</td>
<td>1.171±0.376</td>
<td>1.649±0.50</td>
</tr>
<tr>
<td>CD19 (10⁹/L)</td>
<td>0.141±0.068²</td>
<td>0.079±0.02³</td>
<td>0.115±0.064</td>
<td>0.137±0.063⁵</td>
<td>0.206±0.08⁴</td>
</tr>
<tr>
<td>CD4 (10⁹/L)</td>
<td>0.733±0.30²</td>
<td>0.659±0.37²</td>
<td>0.726±0.303</td>
<td>0.662±0.308</td>
<td>0.993±0.30²</td>
</tr>
<tr>
<td>CD8 (10⁹/L)</td>
<td>0.568±0.24¹</td>
<td>0.525±0.126</td>
<td>0.599±0.426</td>
<td>0.568±0.215</td>
<td>0.836±0.33³</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>52.46±19.4</td>
<td>80.2±28.3¹</td>
<td>35.29±11.9⁴</td>
<td>54.23±13.3</td>
<td>51.66±15.4</td>
</tr>
<tr>
<td>TNF-a (pg/ml)</td>
<td>12.87±9.4</td>
<td>11.49±8.4</td>
<td>18.31±10.7</td>
<td>18.58±11.5</td>
<td>15.67±9.0</td>
</tr>
<tr>
<td>C3c (gr/l)</td>
<td>1.0±0.2⁵</td>
<td>1.07±0.12</td>
<td>0.77±0.11³</td>
<td>0.98±0.244</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>C4 (gr/l)</td>
<td>0.30±0.19⁶</td>
<td>0.27±0.16</td>
<td>0.2±0.14r</td>
<td>0.39±0.6</td>
<td>0.18±0.3⁵</td>
</tr>
<tr>
<td>IgG (gr/l)</td>
<td>11.5±3.7</td>
<td>12.6±2.1</td>
<td>14.5±7.8</td>
<td>12.2±4.3</td>
<td>13.7±2.2</td>
</tr>
<tr>
<td>IgA (gr/l)</td>
<td>2.32±1.03</td>
<td>2.2±0.49</td>
<td>2.46±1.1</td>
<td>2.02±0.69</td>
<td>2.19±0.6</td>
</tr>
<tr>
<td>IgM (gr/l)</td>
<td>1.75±2.1</td>
<td>2.1±1.4</td>
<td>2.18±1.3</td>
<td>1.7±1.3</td>
<td>1.7±0.4</td>
</tr>
</tbody>
</table>

p=0.0004 for a vs b, p=0.005 for c vs d, p=0.005 for d vs e, p=0.048 for c vs f, p=0.004 for g vs h, p=0.001 for i vs j, p=0.004 for k vs l, p=0.009 for k vs m, p=0.004 for n vs o, p=0.016 for p vs r, p=0.004 for p vs s

### Table 2. Maximum platelet aggregation before and after ¹³¹I treatment in patients with DTC and in controls.

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Before ¹³¹I</th>
<th>2 Months after ¹³¹I treatment</th>
<th>4 Months after ¹³¹I treatment</th>
<th>6 Months after ¹³¹I treatment</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (20 µmol)</td>
<td>73.20±7.80</td>
<td>72.63±4.71</td>
<td>75.26±5.71</td>
<td>73.83±6.74</td>
<td>74.01±6.43</td>
</tr>
<tr>
<td>Collagen (0.2 mg/ml)</td>
<td>73.22±7.65</td>
<td>72.62±4.54</td>
<td>74.92±6.30</td>
<td>74.50±6.62</td>
<td>75.58±6.32</td>
</tr>
<tr>
<td>Epinephrine (10 mmol)</td>
<td>73.74±7.72</td>
<td>71.35±5.25</td>
<td>73.38±6.93</td>
<td>74.52±7.14</td>
<td>72.23±6.5</td>
</tr>
</tbody>
</table>
Significant falls in C3c and C4 levels were observed at 4 months following treatment.

No significant changes were observed in pre- and after-treatment immunoglobulin levels. Platelet aggregation induced with ADP, collagen and epinephrine also did not show any difference before and after treatment (Table 2).

**Discussion**

Radiation depresses erythropoiesis via interfering with iron incorporation into erythrocytes. The impact of radiation on hematopoiesis is related to dose and duration of exposure. Erythroid cells are more vulnerable to radiation than myeloid precursors and megacaryocytes (8). Van Nostrand et al. demonstrated that Hb levels fell to as low as 12 g/dla at 4 and 6 weeks after treatment and reached pretreatment levels by 8-10 weeks (9). Haynie and Beierwaltes, reported subnormal hemoglobin levels in 35 % of 155 patients with thyroid carcinoma who received a mean dose of 207 mCi radioiodine (1). Our results demonstrated a non-significant trend for falls in Hb at 2, which returned to the normal range at 6 months.

We observed no significant change in WBC count after treatment. Van Nostrand et al. a significant decrease of 35 % in leukocyte count after one month, which returned to pretreatment levels at 3 months (9). Other studies have shown that lowest leukocyte counts were observed at 4 and 6 weeks; followed by a rise at 8 weeks (2-4, 10). We could not observe a similar decrease in WBC since first WBC counts were performed at 8 weeks following treatment in our study.

Although total lymphocyte counts did not change significantly, we observed a non-significant decrease of 14 % at 2 months. M’Kacher et al. reported that lymphocyte counts decreased by 4 to 21 % in patients with thyroid cancer after exposure to 100 mCi radioiodine (11). Teng et al. also reported a drop of 20 % in patients treated for Graves’ disease (12).

A significant fall in CD19 counts was found at 2 months following treatment. Non-significant decreases in CD3, CD4 and CD8 cells were found after treatment. Soliman et al. reported no significant alterations in CD4 and CD8 following radioiodine treatment in patients with autoimmune thyroid disease (13). B cells appear to be more vulnerable to radiation than T cells.

We found no significant alterations in immunoglobulin levels following radioiodine treatment. No previous study has investigated the effects of radioiodine on immunoglobulin levels. On the other hand Lucas et al (14) demonstrated tumor specific deposition of immunoglobulin G in papillary thyroid carcinoma (PTC).

No previous study investigated the effects of radioiodine treatment on the complement system. Our study demonstrated that C3c and C4 levels fall significantly at 4 months followed by a rise at 6 months, restoring the value to pretreatment levels. The fall in complement levels suggests activation of the immune system by release of thyroidal antigens. The interaction between complement and these antigens may cause lowered levels of C3c and C4. Lucas et al. (14) and Yamakawa et al. (15) suggested that a tumor specific immune response in PTC was associated with activation of the classical complement cascade. Alternatively, the consumption of complement could be due to changes in the alternate pathway without involving antigens/antibodies. On the other hand, pretreatment and 6-month levels (when the patients were hypothyroid) were higher than in euthyroid controls both for C3 and, even more evident, for C4, whereas values at 4 months (when euthyroid) were superimposable to those of the control group. Thus, it is possible to consider that variations in C3 and C4 levels might be related to restoration of euthyroidism. Thus further studies are needed to clarify the effect of radioiodine treatment on the complement system.

IL-6 is secreted by monocytes, macrophages, fibroblasts, endothelial cells, osteoblasts, osteocytes and thyroid follicular cells (16, 17). We observed a modest rise in IL-6 levels at 2 months followed by a fall at 4 months. The effects on IL-6 are not unexpected, as IL-6 is released after a number of inflammatory insults to the thyroid. Elevated IL-6 levels may be caused by release from either destroyed residua or metastatic thyroid tissue after radioiodine treatment (18-20). Bartelena et al. found that IL-6 levels peaked 10 minutes after ethanol administration into the thyroid, and a similar peak
was also observed 24 hours after radioiodine administration (19). Thus IL-6 is a marker for thyroid cell destruction. Elevated IL-6 levels may also be due to increased release from T-cells, associated with a decrease in lymphocyte counts at 2 months (21, 22). The increase in IL-6 to pretreatment levels at 6 months also suggests that T-cell function is not impaired following radioiodine administration.

TNF-α is released by various cells including macrophages, monocytes and epithelial cells, and causes, in vivo, necrosis of tumor tissue but not normal tissue (16). TNF-α gene expression has been demonstrated in thyroid cancer (23-25). We observed no significant change in TNF-α levels following radioiodine treatment. No previous study has evaluated the effects of radioiodine treatment on TNF-α levels. TNF-α enhances platelet aggregation by inducing increased release of phospholipase A and platelet activating factor from vascular endothelial cells (26-28). Thus, lack of alteration in platelet aggregation is a finding consistent with unaffected TNF-α levels following radioiodine treatment.

Van Norstand et al. reported that platelet counts were not affected by radioiodine treatment (9). This observation is consistent with our finding. Furthermore, we report that platelet aggregation is also not affected by radioiodine treatment.

In this study, basal data were collected in hypothyroidism and then compared with data collected 2 and 4 months later while patients were under LT₄ therapy and at 6 months while patients were “off” of LT₄ therapy. Although it is known that hypothyroidism can influence some of the parameters analyzed, most parameters such as WBC, Hb, lymphocyte count, immunoglobulins, platelet counts and stimulated platelet aggregation were not significantly different in both “off” LT₄ and “on” LT₄. Moreover, Chopra et al (17) demonstrated that there was no difference between TNF alpha levels in hypothyroidism and hyperthyroidism. However, the effects of thyroid status on C3 and C4 levels should not be underestimated.

In summary, radioiodine administration is associated with a significant decrease in CD19 count, C3c and C4 and a significant increase in IL-6 levels. Platelet counts and stimulated platelet aggregation were not influenced by radioiodine treatment.

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References


