FUNCTIONAL CAPACITY OF THE THYROID AUTOGRRAFT AND HETEROGRRAFT: AN EXPERIMENTAL STUDY

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Abstract: Background. The aim of this study was to investigate the functional capacity of thyroid autografts/heterografts in a guinea pig model.

Methods. A total of 24 guinea pigs were divided into 4 groups. Group A had only a thyroidectomy incision, and group B had total thyroidectomy. We performed autotransplantation in group C and heterotransplantation in group D. We monitored the guinea pigs for an 8-week period, with weekly measurements of free triiodothyronine (fT3), free thyroxine (fT4), and thyroid-stimulating hormone (TSH). At the final stage, the autografts/heterografts were histologically examined.

Results. In group B, fT3/fT4 showed a gradual decrease; however, an increase of TSH was noted. In groups C and D, fT3/fT4 showed a gradual decrease, followed by a gradual increase until euthyroid levels; an exact opposite was noted for TSH. In histologic examination, there were functional thyroid follicles in all animals of groups C and D.

Conclusions. The autotransplanted/heterotransplanted guinea pig’s thyroid tissue provides adequate thyroid function.

Keywords: thyroid; autotransplantation; heterotransplantation; postoperative hypothyroidism; total thyroidectomy

Thyroid diseases can be treated by antithyroid medications, radioactive iodine ablation, and surgery. Each therapy has its own advantages, disadvantages, and complications. The optimal procedure for the treatment of this disease depends on the situation of each specific patient.

Surgery for thyroid disease is less common than radioisotope and antithyroid drug (ATD) therapy, worldwide.1-4 However, it is considered the best therapy for certain patients, such as those with uncontrollable thyroid function in spite of prolonged ATD therapy, those with severe hyperthyroidism, those with a large diffuse goiter which is producing obstructive symptoms, those with a coexistent thyroid nodule, those under side effects of ATD, those with infiltrative ophthalmopathy, young women, and pregnant women.

On the other hand, the weak spots of isotope therapy can be followed as radiation exposure in young patients and late development of hypothyroidism. Likewise, weak spots of ATD therapy are the need for long-term medication and agranulocytosis, which is uncommon but potentially creates severe side effects. Then again, surgical therapy for thyroid disease has many advantages.5-11 It can normalize thyroid function in most of the patients who have their disease suddenly terminated and require far fewer physician visits and laboratory tests, compared with patients subject to ATD therapy or 123I radioisotope therapy.

Even though surgical therapy is chosen to avoid consuming medicine for a long period of time, approximately 10% of patients still require levothyroxin (l-T4) replacement therapy for the rest of their lives because of unexpected permanent postoperative hypothyroidism.12,13 Hypothyroidism, both overt and latent, has been associated with disruption of lipid metabolism14 and development of coronary heart disease.15

In 1909, for the first time, Halsted proposed the principle of autotransplantation of the endocrine glands, to avoid postoperative functional insufficiency after total or subtotal resection of the glands.16 In the following decades, this principle was applied on the parathyroid and pancreatic glands with great success.17,18 However, no similar success has been achieved for adrenal, testicular, or ovarian tissue.19-21

Overall knowledge concerning thyroid autotransplantation and heterotransplantation is relatively
poor and partially controversial. Furthermore, in
accord with the model of parathyroid autotransplantation, there are no heuristics or clinical studies that
could confirm the functional capacity of a thyroid
autograft and heterograft. In light of the above-men-
tioned facts, heuristics have intended to replace the
thyroid hormone of the patients with hormone
secreted from autotransplanted thyroid tissue that
had been cryopreserved in vivo\textsuperscript{22,23} and in vitro.\textsuperscript{24,25}

Although the results of some studies\textsuperscript{26–32} about
animal thyroid autotransplantation/heterotransplantation is encouraging, the achievability and the sequel
of animal thyroid autotransplantation and heterotransplantation have not been extensively investigated.

We performed this study to evaluate if animal thy-
roid tissue’s autografts and heterografts can survive
in a new environment (eg, muscle, in the absence
of their native blood supply) and to understand the
growth and the functionality of the transplanted tis-
sue. The benefits of this potential can be incorporated
into routine surgical practice by autotransplanting/
heterotransplanting thyroid tissue at an easily acces-
sible site, with the objective of reducing the rate of
postoperative hypothyroidism.

With this study, we have shown that the thyroid
autografts and heterografts can survive and can be
substituted completely by the thyroid function after
total thyroidectomy.

**MATERIALS AND METHODS**

This clinical study was approved by the ethics commit-
tee of Marmara University Animal Laboratory. The
study began with 32 guinea pigs; however, 8 guinea
pigs (2 guinea pigs for each group) died in the 5th and
6th postoperative weeks. These animals were excluded
from further evaluation since they did not complete the
8-week period of observation. The final analysis con-
sisted of 24 guinea pigs (\textit{Rattus norvegicus} of a Wistar
Albino strain) and all of them were females. They were
3 to 6 months old with an initial body weight of approxi-
ately 200 to 300 g. All animals were kept under the
same conditions during the study and received the
same amount of food and water every day.

Surgical anesthesia was achieved with an intra-
peritoneal injection of 100 mg/kg ketamine and 10
mg/kg xylazine hidroclorure. Before the operation
began, a bolus dose of 250 mg kefamandole was administered intramuscularly.

The animals were divided into 4 groups as follows:
group A (6 animals), sham group: Only a midline thy-
roidectomy incision of 3-cm length was performed
from the thyroid cartilage to the jugular line. Without
having any thyroidectomy, the skin was closed with
interrupted sutures of silk 4–0.

group B (6 animals), thyroidectomy group: After a
midline thyroidectomy incision of 3-cm length was
performed, we followed with total thyroidectomy.
However, we did not have any transplantation.

group C (6 animals), autotransplantation group:
After a 3-cm length midline thyroidectomy incision,
we performed total thyroidectomy. The sarcodorsalis
muscle was identified and the thyroid autograft was
placed intramuscularly. About 4 fragments were
implanted into 4 small pockets by splitting the dorso-
sacral muscle. The fascia was closed with nonabsorb-
able sutures.

group D (6 animals), heterotransplantation group:
After a 3-cm length midline thyroidectomy incision,
we performed total thyroidectomy. The sarcodorsalis
muscle was identified and the thyroid heterograft was
placed intramuscularly. About 4 fragments were
implanted into 4 small pockets by splitting the dorso-
sacral muscle of another guinea pig. The fascia was
closed with nonabsorbable sutures.

A midline incision of 3-cm length was performed
from the thyroid cartilage to the jugular line. After
the platysma was divided and the subhyoid muscles
were laterally displaced, the 2 thyroid lobes were
exposed that got through from the surrounding tis-
sues (Figures 1 and 2). The superior and inferior thy-
roid arteries were identified and ligated with Vicryl 5/

**FIGURE 1.** Preparation for surgery. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**FIGURE 2.** Thyroid lobes were determined. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
During resection of the thyroid, the upper parathyroid glands were identified and preserved. The skin was closed with interrupted sutures of silk 4-0.

After both of the 2 lobes were cleaned from the covering fibrous tissue, the excised gland was washed thoroughly in Ringer’s solution (Figure 3). The fibrous capsule was stripped away. The tissue to be transplanted was cut into small fragments measuring about \(2 \times 2 \times 2\) mm. The pieces of the thyroid tissue were preserved until implantation in gauze infiltrated with normal saline (Figure 4). We performed 4 incisions of 1-cm length at both the lateral side of the vertebral column and the sacrospinalis muscle was identified. About 4 fragments were implanted into 4 small pockets by splitting the sacrospinalis muscle (Figure 5). Each pocket in the muscle was closed with the transplanted tissue with a nonabsorbable suture to prevent displacement (Figure 6). Both side skin incisions were closed with nonabsorbable sutures (Figure 7).

The animals were observed for 8 weeks with weekly measurements of thyroid hormones postoperatively. Blood samples were drawn from the tail vein of the animals. Free triiodothyronine (fT3), free thyroxine (fT4), and thyroid-stimulating hormone (TSH) were measured preoperatively and periodically for 8 weeks after autotransplantation to use the microparticle enzyme immunoassay. After 8 weeks, the autografts and heterografts were removed for histopathologic examination; we euthanized the animals by administering a high dose (200 mg/kg) of pentobarbital via intraperitoneal access. During histopathologic examination, the presence of functional thyroid cells and their functional status (follicular size, quantity of colloid, height of epithelium) were evaluated.

In this study, Statistical Package for Social Sciences (SPSS for Windows 15.0) software was used for statistical analysis of the results. Data values are expressed as mean, SD, median, minimum, and maximum. For the quantitative data comparison of the independent groups, the Mann–Whitney U test was used for dual comparisons, and the Kruskal–Wallis test was used for multiple comparisons. For the quantitative data comparison of the dependent groups, the Wilcoxon test was used for dual comparisons, and the Friedman test was used for multiple comparisons. Differences were considered significant at \(p < .05\).
RESULTS

We did not have any major intraoperative complications during the procedure. Twenty-four of 32 animals from the study groups survived for 8 weeks (survival rate was 75%). Eight guinea pigs (2 guinea pigs for each of the 4 groups) that were excluded from further evaluation expired during the 5th and 6th postoperative weeks.

There was no change in the levels of the thyroid hormones in the sham group (group A) during 8 weeks. In the control group (group B), a gradual decline of fT4 and fT3 to undetectable levels has been observed by the end of the 3rd and 4th weeks, followed by becoming zero on the 5th week. A gradual decline at the levels of the thyroid hormones was found in 3 of the study groups (groups B, C, and D), which were already significant in terms of statistics by the end of the 1st week. However, the minimal values for fT4 and fT3 were recorded within the 4th week (group C: fT3: 1.25 ± 0.01 pg/mL, fT4: 0.29 ± 0.02 ng/dL; group D: fT3: 1.24 ± 0.03 pg/mL, fT4: 0.29 ± 0.02 ng/dL, respectively) for groups C and D.

Consequently, a gradual increase in the levels of fT4 and fT3 was noted, and euthyroid levels were reached by the 8th week for groups C and D. At the end of the 8th week, the levels of fT3 (2.28 ± 0.07, 2.28 ± 0.08 pg/mL, respectively) and fT4 (0.91 ± 0.03, 0.91 ± 0.02 ng/mL, respectively) did not differ significantly from the levels of groups C and D before the experiment started. The levels of fT3 and fT4 during postoperative 8 weeks and preoperative period for all groups is shown in Tables 1 and 2.

There was no change in the levels of the TSH in the sham group (group A) within 8 weeks. In the control group (group B) a continuous rise of TSH levels was noted, which reached 8 times of the levels before total thyroidectomy by the end of the 8th week (before total thyroidectomy: 0.08 ± 0.01 IU/mL; after total thyroidectomy: 0.65 ± 0.02 IU/mL). Preoperative TSH level of groups C and D was 0.07 ± 0.01 IU/mL, although TSH levels showed a significant rise that was already distinctive by the end of the 1st week for groups C and D (0.15 ± 0.02 IU/mL, 0.14 ± 0.01 IU/mL, respectively). The maximal values for groups C and D were recorded at the 4th week of the experiment (0.38 ± 0.03 IU/mL, 0.39 ± 0.02 IU/mL, respectively). Afterward, a gradual decline of TSH levels was noted. At the end of the 8th week, TSH levels were approximately 3-fold greater than those when the time experiment started (0.38 ± 0.03 IU/mL, 0.39 ± 0.02 IU/mL, respectively) (Table 3).

No statistically significant differences were noted during the weekly measurements among those 2 autotransplantation and heterotransplantation groups (groups C and D).

During the histologic examination, we found the presence of functional thyroid follicles and cuboid-to-cylindrical epithelial cells for all cases of the 2 autotransplantation and heterotransplantation groups (groups C and D) (12/12). Furthermore, an increase of small follicles and colloid was noted at several regions of reactive hyperplasia (Figures 8 and 9).

Table 1. Free triiodothyronine levels.

<table>
<thead>
<tr>
<th>Value</th>
<th>Group A</th>
<th>Group B</th>
<th>p value*</th>
<th>Group C</th>
<th>Group D</th>
<th>p value*</th>
<th>BG (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg ± SD</td>
<td>Avg ± SD</td>
<td></td>
<td>Avg ± SD</td>
<td>Avg ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>2.36 ± 0.05</td>
<td>2.34 ± 0.05</td>
<td>—</td>
<td>2.31 ± 0.03</td>
<td>2.34 ± 0.03</td>
<td>—</td>
<td>.225</td>
</tr>
<tr>
<td>1st week</td>
<td>2.36 ± 0.03 ± 0.08</td>
<td>1.58 ± 0.06 ± 0.08</td>
<td>.028</td>
<td>1.65 ± 0.04 ± 0.08</td>
<td>.027</td>
<td>1.59 ± 0.03 ± 0.08</td>
<td>.028</td>
</tr>
<tr>
<td>2nd week</td>
<td>2.37 ± 0.03 ± 0.08</td>
<td>0.80 ± 0.04 ± 0.08</td>
<td>.027</td>
<td>1.44 ± 0.04 ± 0.08</td>
<td>.027</td>
<td>1.46 ± 0.03 ± 0.08</td>
<td>.028</td>
</tr>
<tr>
<td>3rd week</td>
<td>2.38 ± 0.02 ± 0.08</td>
<td>0.28 ± 0.03 ± 0.08</td>
<td>.028</td>
<td>1.33 ± 0.03 ± 0.08</td>
<td>.027</td>
<td>1.34 ± 0.03 ± 0.08</td>
<td>.027</td>
</tr>
<tr>
<td>4th week</td>
<td>2.38 ± 0.02 ± 0.08</td>
<td>0.00 ± 0.00 ± 0.08</td>
<td>.027</td>
<td>1.25 ± 0.01 ± 0.08</td>
<td>.027</td>
<td>1.24 ± 0.03 ± 0.08</td>
<td>.028</td>
</tr>
<tr>
<td>5th week</td>
<td>2.39 ± 0.01 ± 0.08</td>
<td>0.00 ± 0.00 ± 0.08</td>
<td>.028</td>
<td>1.55 ± 0.04 ± 0.08</td>
<td>.028</td>
<td>1.54 ± 0.03 ± 0.08</td>
<td>.028</td>
</tr>
<tr>
<td>6th week</td>
<td>2.37 ± 0.02 ± 0.08</td>
<td>0.00 ± 0.00 ± 0.08</td>
<td>.028</td>
<td>1.71 ± 0.04 ± 0.08</td>
<td>.027</td>
<td>1.74 ± 0.03 ± 0.08</td>
<td>.028</td>
</tr>
<tr>
<td>7th week</td>
<td>2.37 ± 0.01 ± 0.08</td>
<td>0.00 ± 0.00 ± 0.08</td>
<td>.028</td>
<td>1.99 ± 0.08 ± 0.08</td>
<td>.028</td>
<td>2.05 ± 0.08 ± 0.08</td>
<td>.028</td>
</tr>
<tr>
<td>8th week</td>
<td>2.38 ± 0.01 ± 0.08</td>
<td>0.00 ± 0.00 ± 0.08</td>
<td>.028</td>
<td>2.28 ± 0.07 ± 0.08</td>
<td>.248</td>
<td>2.28 ± 0.08 ± 0.08</td>
<td>.248</td>
</tr>
</tbody>
</table>

Abbreviations: BE, comparison between experiments; BG, comparison between groups; Avg ± SD, average ± standard deviation.

*Different from group A.
†Different from group B.
‡Different from group C.
§Different from group D.

Note: Levels are measured in pg/mL.
**DISCUSSION**

The aim of autotransplantation of thyroid tissue, after total or subtotal thyroidectomy, is the preservation of postoperative thyroid function and the avoidance of substitution therapy with oral intake of thyroid hormones for a lifetime. Another advantage of this method could be the preservation of the inner autoregulatory mechanism of thyroid hormone production, in accord with the needs of the human organism. In our study at the end of the 8th week, in the autotransplanted group, we also detected the euthyroid level indicating thyroid function.

Implantation of thyroid cells can occur during the manipulations of thyroidectomy. Eickhoff et al. described 2 cases of thyroid nodule development in the subcutaneous tissue of neck after total thyroidectomy. In the literature, there are sporadic reports about thyroid autotransplantation in infants, in cases of thyroid ectopia, with relatively good results. In our animal research, we also got relatively good results in the autotransplanted group.

The intraoperative implantation of thyroid tissue after subtotal thyroidectomy in an adolescent, who developed hypothyroidism 6 months after the operation, was first performed by Tsarikovskoj and Tkatsov in 1975. However, they proposed the autotransplantation after total or subtotal thyroidectomy in children. In contrast to this study, we followed the autotransplanted guinea pigs for 8 weeks and at the end of this time we measured the euthyroid level.

Sheverdin published the only report of the results of thyroid autotransplantation in adults after subtotal thyroidectomy for benign pathology of the thyroid in 1992. He reported 3.2% postoperative hypothyroidism. However, there was no report of the size of the remnant of thyroid tissue after subtotal thyroidectomy. Instead of subtotal thyroidectomy, we performed total thyroidectomy in our research and we did not report any postoperative hypothyroidism (0%) as the results of thyroid autotransplantation. We performed total thyroidectomy to achieve a better...
evaluation of the thyroid autograft without the possible interference of the thyroid remnant function.

Past studies have proven that thyroid tissue can be cryopreserved. Shimizu et al. showed that cryopreserved thyroid tissue is capable of surviving and functioning after autotransplantation similar to freshly excised tissue. The autotransplantation of cryopreserved thyroid tissue appears to be a promising treatment option for patients with postoperative hypothyroidism to obviate the need for lifelong thyroxine supplementation. An added benefit is that excess transplanted tissue can easily be excised in the event of postoperative recurrent hyperthyroidism, whereby the risks of recurrent neck surgery are avoided. In our study, we chose fresh thyroid autograft (in group C) instead of cryopreserved thyroid tissue. However, we got the same results with Kitamura et al. and Shimizu et al. which turned out to be a promising treatment option for patients with postoperative hypothyroidism to obviate the lifelong need for thyroxine supplementation.

In the autotransplantation group, the levels of the thyroid hormones showed a gradual decline between the 1st and 4th weeks after the implantation, which was followed by a gradual increase and an establishment of euthyroid levels at 8th weeks respectively. There was a gradual increase in the levels of TSH until the 4th week, followed by a gradual decrease until the end of the 8th week. These results indicated that the autograft assumed functioning in the 3rd to 4th weeks after implantation. It seems that there is a latent period between thyroidectomy and initiation of the function of the autograft, which may be necessary for the vascular supply of the autograft from the surrounding tissues. During this period, a transient hypothyroidism can develop.

As compatible with the research of Papaziogas et al. and O’Malley et al., the functional thyroid follicles were found in all autografts and heterografts by the end of the 8th week. There is the presence of cuboid or cylindrical follicular cells, as expected by the rise of TSH, proving the preservation of the autoregulatory mechanism between the hypothalamus, hypophysis, and grafts.

Total thyroidectomy led to the maximal rise of TSH, which served as the main trigger for the survival or, in some cases, led the hyperplasia of the autograft. Kasuga et al. studied development of the levels of thyroid hormones after xenotransplantation of human thyroid tissue on immunocompromised guinea pigs, and found that the establishment of euthyroid levels occurred at the 3rd week after implantation. We also studied development of the levels of thyroid hormones after heterotransplantation of thyroid tissue in guinea pigs (group D), and found that the establishment of euthyroid levels occurred at the 8th week (instead of 3rd week) after implantation.

Shupnik et al. and Ross et al. reported that serum TSH values were remarkably reduced after daily T4 administration following radiothyroidectomy in guinea pigs. Their results with the serum TSH in guinea pig were comparable with our own: the fT4 and fT3 that autograft or heterograft secrete caused the expected feedback-induced decline in TSH.

**CONCLUSIONS**

In conclusion, our study proved that the thyroid autograft and heterograft could survive and restore thyroid function within 8th weeks after total thyroidectomy. Although the observation period in our experimental study was relatively short, the presence of vital thyroid follicles along with the restoration of euthyroidism and the absence of any immune response to the

**FIGURE 8.** Photomicrograph shows a low magnification of the autotransplanted rat thyroid tissue. The gland is organized into follicles that are lined by simple cuboidal, or sometimes columnar epithelium. Each follicle contains colloid. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**FIGURE 9.** Photomicrograph shows a low magnification of the heterotransplanted rat thyroid tissue. The gland is organized into follicles that are lined by simple cuboidal, or sometimes columnar epithelium and containing colloid. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
autograft and heterograft could be the evidence of long-term efficacy of the thyroid autografts and heterografts.

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