Parathyroid Allotransplant With a New Technique: A Prospective Clinical Trial

Erhan Aysan,[1] Burcugul Altug,[2] Cilem Ercan,[3] Cemile Kesgin Toka,[1] Ufuk Oguz Idiz,[4] Mahmut Muslumanoglu[1]

Abstract

Objectives: **Parathyroid allotransplant is a valuable alternative in treating permanent hypoparathyroidism. However, it is a difficult process that requires several trained staff and advanced laboratory equipment, which makes the costs high. Here, we identify a new parathyroid allotransplant technique.**

Materials and Methods: After obtaining informed consent from patients, parathyroid cell suspensions obtained from 4 donors who had undergone a subtotal parathyroidectomy owing to chronic renal failure were transplanted in 10 patients with permanent hypoparathyroidism after short-term cell cultivation. Prednisolone were used as immunosuppressant for the first 10 days and discontinued thereafter.

Results: **Allograft function was observed in 7 patients (70%) at a mean follow-up of 12 months. Daily oral calcium and vitamin D supplementations discontinued totally in 7 patients. No major or minor complication was observed.**

Conclusions: **Our technique is simple, fast, and has a low cost, with a 70% success rate at a mean follow-up of 12 months. It requires few staff, minimal equipment, and short-term immunosuppressant use for maintenance. The technical developments of parathyroid allotransplant, as mentioned in this study, may be important in treating permanent hypoparathyroidism.**

Key words: *Parathyroid, Allograft, New technique, Clinical*

From the [1]Department of General Surgery, Bezmialem Vakif University, Faculty of Medicine; the [2]Department of Biology, Faculty of Medicine, Anadolu University; the [3]Department of Medical Biology, Faculty of Medicine, Bezmialem Vakif University; and the [4]Department of General Surgery Sisli Etfal Research and Training Hospital

Acknowledgements: *The authors declare that they have no sources of funding for this study, and they have no conflicts of interest to declare.*

Corresponding author: *Ufuk Oguz Idiz, Ihlas Marmara Evleri 1. Kisim A-18 Blok, Daire: 5, Beylikduzu, Istanbul, Turkey*

Phone: *+90 212 472 6712* ***Fax:*** *+90 212 531 0092* ***E-mail:*** *oguzidiz@yahoo.com*

Experimental and Clinical Transplantation (2016) 4: 431-435

DOI: 10.6002/ect.2014.0294

Introduction

Hypoparathyroidism is a clinical condition associated with hypocalcemia, hyperphosphatemia, and low parathyroid hormone levels (PTH). It often manifests as a complication of thyroid surgery.[1,2] Direct injury, ischemia, and unintended excision of parathyroid tissue are the most common causes of temporary or permanent hypoparathyroidism (PH). Temporary hypoparathyroidism (duration, < 6 mo) develops in 10% of patients after thyroid surgery, nearly half of whom develop PH (duration, > 6 mo).[1,3,4] Owing to the rapid increase in thyroid cancer cases in recent years, total thyroidectomy and neck dissection are performed now more frequently than before, resulting in an increase in the incidence of PH.[5]

As current standard treatments, oral or intravenous calcium preparations, active vitamin D3, and teriparatide are used to treat hypocalcemia caused by PH. However, this approach is symptomatic, and must be continued throughout the patient's lifetime. It entails high costs and is intended only to correct hypocalcemia. It has no beneficial effect on metabolic problems caused by PH.[6,7]

For these reasons, less costly and effective treatments have been sought for more than 30 years and parathyroid allotransplant (PA-t) was introduced as an alternative. Nonetheless, PA-t did not fully meet expectations.[8-16] The main problem with treating with PA-t is development of immune and inflammatory responses against the graft tissue, which shortens the graft's life and impairs treatment.[11] Various treatment methods (eg, immunosuppression, microencapsulation, and cultivation) have been used to overcome these problems. But despite these, convincing results have not yet been achieved.[8-15] Parathyroid allotransplant often present as case reports and only a few clinical series are available.[15,17]

We aimed to present the results of our new PA-t technique, which we performed in 10 patients

diagnosed as having permanent hypocalcemia after undergoing thyroid surgery. These patients did not benefit from standard medical therapy.

Materials and Methods

A prospective clinical trial was planned, and ethical approval was obtained from the local ethics committee of Istanbul University. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration, and written informed consent was obtained from all subjects. All transplants in Turkey were performed with permission from the Organ, Tissue Transplantation and Dialysis Committee of the Ministry of Health. In October 2012, Bezmialem Vakif University applied to the Ministry of Heath for permission to perform PA-t. In May 2013, Turkey's first and only PA-t unit was officially allowed to perform the transplant (Clinical ID: NCT02134483).

Between July 2013 and January 2014, ten women (mean age, 41.4; range, 20-61 y) who had been admitted to the internal medicine, endocrinology, general surgery, and endocrine surgery outpatient clinics of Bezmialem Vakif University were enrolled. All patients were using oral calcium and vitamin D preparations at various dosages. Between July 2013 and December 2013, five patients with parathyroid hyperplasia owing to chronic renal failure were referred from the nephrology outpatient clinic to the general surgery outpatient clinic, and were identified as donors. A complete blood count; PTH, calcium, and phosphorus levels; and potential infection with human immunodeficiency virus, hepatitis B and C viruses, *cytomegalovirus*, and Epstein-Barr virus were evaluated for either the donor or recipient before transplant. No pathological findings that could have contraindicated transplant were identified.

Cell cryopreservation and cultivation protocol

Because of our protocol for secondary hyperparathyroidism, all donors underwent subtotal parathyroidectomy (3.5 gland resection). Half of all resected glands were sent for histopathologic evaluation; the rest were sent to the parathyroid tissue transplant unit immediately in a culture medium composed of 4 mL of AmnioMAXII Complete Medium (catalog No. 11269-016; Gibco Life Technologies, Carlsbad, CA, USA) with 20% inactivated fetal bovine serum (catalog No. 10500-064; Gibco) and 1% penicillin-streptomycin (catalog No. 15140-122; Gibco). After histopathologic confirmation of hyperplasia, cryopreservation was performed in a biohazard safety cabinet (L.02131262, Mars Safety Class 2, SCANLAF, Denmark) under sterile conditions. Tissues in melting ice medium were washed 5 times with the same medium.

The collected tissues were isolated from blood vessels, gland capsule, connective, and fatty tissues via a sterile filter and were mechanically disintegrated in 1X phosphate buffered saline solution (catalog No. AM9624; Ambion Life Technologies, Carlsbad, CA, USA) supplemented with 5% inactivated fetal bovine serum. The solution was filtered into a 15-mL tube using a sterile cell strainer (100 μm, catalog No. 352360, Falcon, BD Biosciences, Franklin Lakes, NJ, USA) and mixed with 600 μL deoxyribonuclease I (from bovine pancreas, A3778-0010, AppliChem, Darmstadt, Germany).

After centrifugation at 1600 rpm for 5 minutes at room temperature, the supernatant was removed and the pellet was rapidly suspended in 1 mL of culture medium. Cell viability was assessed by Muse Cell Analyzer (catalog No. 0500-3115 Merck Millipore, Darmstadt, Germany). The cells that mixed with 5% dimethyl sulfoxide (catalog No. sc-200262, UltraCruz) were then suspended in cryotubes and transferred to flasks containing isopropanol. Afterwards, cells were kept at -80°C for 8 to 16 hours and transferred to liquid nitrogen tank for storage. When the recipient was matched for transplant, cells in nitrogen tank removed and were cultivated into flasks (sc-200262,UltraCruz, Santa Cruz Biotechnology, Dallas, TX, USA), and kept in the incubator (CCL-170B-8, ESCO, Singapore) at 37°C, 5% CO2-containing humidified atmosphere for 24 to 48 hours. After incubation, cell solutions in flasks were transferred to 15-mL tubes. Cells that attached to flasks were washed with 4-mL phosphate buffer saline and then 300 μL trypsin (25300-054, Gibco, UK) was added to maintain detachment and incubated for 5 minutes. After centrifuging at 1600 rpm for 5 minutes, the supernatant was removed and the pellet was suspended in 1 mL of cell culture media. After cell count, 50×10^6 cells were suspended in 1 mL of the recipient's blood serum.

Transplant

A 250-mg dose of methylprednisolone (Prednol 250 mg Ampul, Mustafa Nevzat Co, Istanbul, Turkey) was administered intravenously 30 minutes before transplant. In 1 mL of the recipient's

blood serum, 50 million cells were suspended, and then injected in the left deltoid muscle. The patients were followed for 3 days in the general surgery outpatient clinic. Parenteral prednisolone dosages were reduced gradually. After the last parenteral administration, patients were discharged with 5-mg/day oral prednisolone (Deltacortril 5-mg tablet, Pfizer Co, New York, NY, USA) for 7 days. Blood PTH and calcium levels were assessed weekly during the first month and then once a month thereafter.

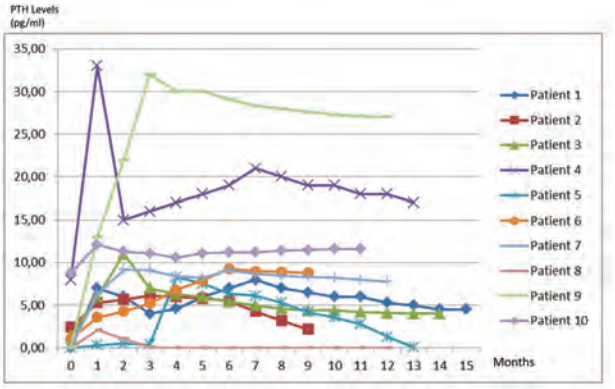
Results

Before PA-t, patients received the calcium preparation (Calcium Sandoz efervesan tablet, Eczacibasi Co, Istanbul, Turkey), vitamin D preparation (Cal-D-Vita, Bayer Türk Kimya San. Tic. Ltd. Şti., İstanbul, Turkey), and calcitriol (Rocaltrol, Roche, Basel, Switzerland) at the following dosages: 4350 ± 3118 mg (range, 1500-12 000 mg), 840 ± 478 IU (range, 0-1600 IU), and 0.8 ± 0.42 μg (0-1.5 μg). The blood groups of the donors were 0 Rh (+), AB Rh (+), AB Rh (+), AB Rh (-), and B Rh(+). The blood groups of the recipients were A Rh (+) in 6 patients, 0 Rh (+) in 3 patients and B Rh (-) in 1 patient. The donor and the recipients were not matched in 6 of 10 transplants. Mean cell viability was 90.8% (range, 87.1%-94.1%).

During a median follow-up of 12 months (range, 9-15 mo), allograft function was observed in 7 patients, whereas no endocrine activity was observed in 3 patients. The mean PTH level (reference range, 9.8-74.9 pg/mL) of the patients before transplant was 2.19 ± 3.37 pg/mL, and the mean calcium level (reference range, 8.610 mg/dL) before transplant was 7.58 ± 0.79 mg/dL. The mean PTH and calcium levels after transplant were 8.3 ± 8.45 pg/mL and 8.05 ± 0.61 mg/dL.

Only 1 patient (patient 5) underwent retransplant in the 10 weeks of follow-up because of a non-functioning allograft. The PTH levels increased to 8 pg/mL during the second week of retransplant. The PTH levels of the patients after transplant are shown in Figure 1. Seven of 10 patients had no need for administering intravenous calcium during follow-up. The daily calcium and vitamin D supplementation was reduced and then totally discontinued in 7 patients (Table 1). In all patients, no major or minor complications were observed during follow-up.

Figure 1. Serum PTH Levels of the Patients After Transplant



Abbreviations: PTH, parathyroid hormone levels

Two of 3 patients with no endocrine activity were not ABO-matched, and 1 of 3 patients was ABO-matched. The cost of medical treatment of secondary hypoparathyroidism per patient is USD $300/year (0.25-μg calcitriol, $0.16; 1500-mg calcium, $0.12; 400-IU vitamin D, $0.11; and hospital outpatient clinic fee, $4), and the cost of transplant using our technique is USD $130 (centrifuge tube, $1; cell strainer, $10; DNase, $5; cryotube, $1; petri, $1.5; pastor pipette, $1.5; liquid nitrogen transporter, $32; phosphate-buffered saline: $0.8, dimethyl sulfoxide: $0.3, 99% ethyl alcohol, $3; and 20-cc syringe, $1.5; patient hospitalization fee, $25/day).

Discussion

The medical insufficiency of the standard approach, adverse effects, and complications such as gastritis, urolithiasis, and nephrolithiasis led to the application of PA-t in treating PH.[6,18] Despite the introduction of

Table 1. Sample Size and Exclusions

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4
Patient 1	12000	4500	3000	1500	-	800	400	400	-
Patient 3	3000	1500	-	-	-	800	400	-	-
Patient 4	6000	3000	1500	-	-	800	400	-	-
Patient 6	4500	1500	-	-	-	800	400	-	-
Patient 7	1500	-	-	-	-	1600	800	400	-
Patient 9	3000	1500	-	-	-	1600	800	400	-
Patient 10	3000	1500	1500	-	-	-	-	-	-

PA-t more than 30 years ago, it had not been used in daily clinical practice, nor was it presented as a research issue or case series.[16] The major reason is the undeserved prejudice against PH, which has not been viewed as an important clinical problem requiring a difficult costly procedure that requires several trained staff, advanced laboratory equipment, and lifelong immunosuppressant use. The techniques described in the literature clearly require advanced laboratory equipment and several staff.[13,17,19,20] A significant advantage of PA-t is its applicability without using immunosuppressant (as in corneal allotransplant) because parathyroid cells do not express MHC class II antigens, which are responsible for graft rejection, and express a small amount of MHC class I antigens.[21]

Various techniques have been used to perform PA-t without immunosuppression. Wozniewicz and associates have observed the separation of parathyroid cells from other cells by using specific antibodies and cultivating in vitro conditions. The disadvantages of this technique are its costliness, its limited proliferation ability of the parathyroid cells in vitro, and viability restriction 6 weeks after in culture medium.[13] Tolloczo and associates used the above-mentioned technique and reported a clinical series of 18 patients who underwent a transplant. They considered compliance of ABO blood groups, unlike Wozniewicz and associates. The obtained graft survival was 14 months, which is longer than that in the series reported by Wozniewicz and associates.[17] Hasse and associates transplanted human parathyroid cells in microcapsules in 40 rats using a micro-encapsulation technique. All the rats were normo-calcemic up to week 16, and 27 of the 40 rats were normocalcemic at 30 weeks.[19] Development of the microencapsulation technique is still in its preclinical stage. No clinical series exist in the literature, except for a few human case reports. Cabane and associates performed PA-t using microencapsulation in a patient who developed permanent hypocalcemia after undergoing thyroid surgery; they followed with the patient for 21 months. Intravenous calcium administration was unnecessary during follow-up, but the serum PTH levels gradually decreased.[18] The microencapsulation technique is costly, and requires technical equipment and well-trained staff. Compared with other techniques, it has no significant advantage for graft survival.

Flechner and associates performed PA-t in a patient with PH, and the patient was normocalcemic during the 8-month follow-up.[22] Such PA-t cases with lifelong immunosuppressant use are rarely reported in the literature. While long-term use and related complications of calcium and vitamin D supplementation are prevented, patients are exposed to the risk of adverse events because of the use of immunosuppressants.

The largest series has been reported by Nawrot and associates, in which 116 PA-t procedures were performed between 1990 and 2004 in 85 patients with 20 different donors. Parathyroid cells obtained from donors were cultivated for ≥ 6 weeks. Parathyroid cells that did not express HLA class II antigens were isolated using specific antibodies. A total of 20 to 30 million cells with a viability > 85% was transplanted without any immunosuppressant use via injection under the fascia of the nondominant arm of the patient. The mean graft survival was reported as 6.3 months.[15] This also is a costly technique that requires long-term cultivation, advanced laboratory equipment, and a well-trained staff.

Timm and associates used the posttransplant short-term immunosuppression technique to avoid the adverse effects of immunosuppressants in rats. They administered cyclosporine before and after 7 days of transplant to 1 group and only after 13 days posttransplant in the second group. They concluded that short-term immunosuppressant use does not have a statistically significant lengthening effect on graft survival.[23] However, a transplant was performed without any cell cultivation in their study.

Before this study, we had reported this allotransplant technique in 2 case reports. In these case reports, during follow-up, patients had no complications that could indicate rejection, and clinical symptoms completely resolved with no drug supplementation.[24,25] In this study, we presented this hybrid system that covers the advantages of either the clinical or laboratory aspects of parathyroid allotransplant for a 10 patient's series. With the exception of 10 days of short-term immunosuppressant use (via 3-day parenteral and 7-day oral routes) in the clinical aspect and short-term cell cultivation, in addition to the standard cell isolation procedures in the laboratory, we did not perform any additional process that requires additional cost, equipment, or staff.

While medical treatment of PH costs $300/year in Turkey, transplant with our technique cost $130 only.

In addition, we could perform a transplant within 24 to 48 hours after a recipient is found. Graft function rate was 70% (7/10).

In our method, collected tissues were isolated from blood vessels, gland capsule, connective, and fatty tissues via a sterile filter, and were mechanically disintegrated in 1X phosphate buffered saline. But in Nawrot and associates' study, during the culture, apart from morphologic assessment, cell phenotype was established using immunohistochemical methods with the following monoclonal antibodies: anti- CD3, -CD4, -CD8, -CD22, -CD31, -CD68, -HLA type I and II. Also, our cultivation time is much shorter than the other cell cultivation methods. Because of that, our method is less expensive, easier, and faster than the others.

A 12-month follow-up seems insufficient to support our claim regarding the success of our hybrid technique, which is simple, fast, and cost-effective, and has minimal equipment and staff requirements. Long-term follow-up and larger patient groups are required for a more objective evaluation of our technique. One may consider PH as a serious health issue, and calcium/vitamin D replacement therapy carries with it high costs and produces either adverse effects or complications. Invention of new simple, fast, and cost-effective PA-t techniques, as we mentioned here, seems to be a significant step to solve this clinical pathology.

References

1. Al-Azem H, Khan AA. Hypoparathyroidism. B*est Pract Res Clin Endocrinol Metab.* 2012;26(4):517-522.
2. Glockzin G, Hornung M, Kienle K, et al. Completion thyroidectomy: effect of timing on clinical complications and oncologic outcome in patients with differentiated thyroid cancer. *World J Surg.* 2012;36(5):1168-1173.
3. Khan MI, Waguespack SG, Hu MI. Medical management of postsurgical hypoparathyroidism. *Endocr Pract*. 2011;17(suppl 1):18-25. Erratum in: *Endocr Pract*. 2011;17(6):967. dosage error in article text.
4. Richard CS, Nason RW, McLeod RS; CAGS Evidence Based Reviews in Surgery Group. Canadian Association of General Surgeons Evidence Based Reviews in surgery. Decision analysis of total thyroidectomy versus thyroid lobectomy in low-risk, differentiated thyroid cancer. *Can J Surg.* 2002;45(6):450-452.
5. Giordano D, Valcavi R, Thompson GB, et al. Complications of central neck dissection in patients with papillary thyroid carcinoma: results of a study on 1087 patients and review of the literature. *Thyroid.* 2012;22(9):911-917.
6. Prager G, Kalaschek A, Kaczirek K, et al. Parathyroidectomy improves concentration and retentiveness in patients with primary hyperparathyroidism. *Surgery.* 2002;132(6):930-935; discussion 935-936.
7. Bislev LS, Sikjaer T, Rejnmark L. Effects on calcium homeostasis of changing PTH replacement therapy of postoperative hypoparathyroidism from intact PTH to teriparatide: a case series. *Calcif Tissue Int.* 2014;95(4):374-381.
8. Kukreja SC, Johnson PA, Ayala G, Bowser EN, Williams GA. Allotransplantation of rat parathyroid glands: effects of organ culture and transplantation into the adrenal gland. *Experientia*. 1979;35(4):559-560.
9. Sollinger HW, Mack E, Cook K, Belzer FO. Allotransplantation of human parathyroid tissue without immunosuppression. *Transplantation.* 1983;36(6):599-602.
10. Decker GA, Stark JH, Botha JR, Margolius LP, Decker G. Allotransplantation of parathyroid cells. *Lancet*. 1995;345 (8942):124. No abstract available. Erratum in: *Lancet* 1995;345 (8947):464.
11. Tołłoczko T, Woźniewicz B, Sawicki A, Górski A. Allotransplantation of cultured human parathyroid cells: present status and perspectives. *Transplant Proc.* 1997;29(1-2):998-1000.
12. Chapelle T, Meuris K, Roeyen G, et al. Simultaneous kidney-parathyroid allotransplantation from a single donor after 20 years of tetany: a case report. *Transplant Proc*. 2009;41(2):599-600.
13. Woźniewicz B, Migaj M, Giera B, et al. Cell culture preparation of human parathyroid cells for allotransplantation without immunosuppression. *Transplant Proc.* 1996;28(6):3542-3544.
14. Hasse C, Klöck G, Schlosser A, Zimmermann U, Rothmund M. Parathyroid allotransplantation without immunosuppression. *Lancet*. 1997;350(9087):1296-1297.
15. Nawrot I, Woźniewicz B, Tołłoczko T, et al. Allotransplantation of cultured parathyroid progenitor cells without immunosuppression: clinical results. *Transplantation.* 2007;83(6):734-740.
16. Wells SA Jr, Burdick JF, Hattler BG, et al. The allografted parathyroid gland: evaluation of function in the immunosuppressed host. *Ann Surg*. 1974;180(6):805-813.
17. Tołłoczko T, Woźniewicz B, Górski A, et al. Cultured parathyroid cells allotransplantation without immunosuppression for treatment of intractable hypoparathyroidism. *Ann Transplant*. 1996;1(1):51-53.
18. Cabané P, Gac P, Amat J, et al. Allotransplant of microencapsulated parathyroid tissue in severe postsurgical hypoparathyroidism: a case report. *Transplant Proc.* 2009;41(9):3879-3883.
19. Hasse C, Zielke A, Klöck G, et al. First successful xenotransplantation of microencapsulated human parathyroid tissue in experimental hypoparathyroidism: long-term function without immunosuppression. *J Microencapsul.* 1997;14(5):617-626.
20. Moskalenko V, Ulrichs K, Kerscher A, et al. Preoperative evaluation of microencapsulated human parathyroid tissue aids selection of the optimal bioartificial graft for human parathyroid allotransplantation. *Transpl Int.* 2007;20(8):688-696.
21. Bjerneroth G, Juhlin C, Rastad J, Akerström G, Klareskog L. MHC class I and II antigen expression on parathyroid cells and prospects for their allogenic transplantation. *Transplantation.* 1993;56(3):717-721.
22. Flechner SM, Berber E, Askar M, Stephany B, Agarwal A, Milas M. Allotransplantation of cryopreserved parathyroid tissue for severe hypocalcemia in a renal transplant recipient. *Am J Transplant.* 2010;10(9):2061-2065.
23. Timm S, Otto C, Begrich D, et al. Short-term immunosuppression after rat parathyroid allotransplantation. *Microsurgery.* 2003;23(5): 503-507.
24. Aysan E, Kilic U, Gok O, et al. Parathyroid Allotransplant for Persistent Hypocalcaemia: A New Technique Involving Short-Term Culture. *Exp Clin Transplant.* 2016;14(2):238-241.
25. Aysan E, Kilic U, Gok O, et al. A novel non-surgical, minimally invasive technique for parathyroid autotransplantation: a case report. *Pediatr Transplant*. 2015;19(2):E37-E40.