Graft survival effect of HLA-A allele matching parathyroid allotransplantation

Beyza Goncu ,[1,2] Harika Salepcioglu Kaya,[3] Emrah Yucesan,[4] Yeliz Emine Ersoy,[3,2] Adem Akcakaya[3,5]

[1]Experimental Research Center, Bezmialem Vakif University, Istanbul, Turkey
[2]Parathyroid Transplantation Laboratory, Bezmialem Vakif University, Istanbul, Turkey
[3]Faculty of Medicine, Department of General Surgery, Bezmialem Vakif University, Istanbul, Turkey
[4]Faculty of Medicine, Department of Medical Biology, Bezmialem Vakif University, Istanbul, Turkey
[5]Organ Transplantation Unit, Bezmialem Vakif University, Istanbul, Turkey

Correspondence to
Dr Beyza Goncu, Experimental Research Center, Bezmialem Vakif University, 34093 Istanbul, Turkey; bsgoncu@gmail.com

Accepted 9 December 2020
Published Online First 22 December 2020

To cite: Goncu B, Salepcioglu Kaya H, Yucesan E, *et al*. *J Investig Med* 2021;**69**:785–788.

ABSTRACT

Permanent hypoparathyroidism is an endocrine disease that is mostly associated with the disruption of the parathyroid glands during surgery. Allotransplantation is the most promising approach for treatment particularly for its cost-effective and exact curative potential. Herein our aim was to evaluate human leukocyte antigen (HLA)-A allele matching effect on clinical improvement and graft survival after parathyroid transplantation. We performed parathyroid transplantation between ABO/Rh compatible recipient and an unrelated donor who has chronic kidney disease. Preoperative immunological tests include panel reactive antibody, T-flow cytometry crossmatch, B-flow cytometry crossmatch, autoflow cytometry crossmatch, and complement-dependent cytotoxicity crossmatch tests were performed. After histopathological evaluation, half of the resected parathyroid gland cells were isolated and transplanted to the omentum surface by laparoscopy. The transplantation outcome was followed up throughout 382 days. The recipient discharged 2 days after transplantation without any complication. During follow-up, calcium and vitamin D supplementation reduced to a one-third dose; even the intact PTH levels remained low. However, clinical improvement was observed by serum calcium levels. The recipient still continues with low-dose supplementation after 382 days of post-transplantation. Parathyroid cell transplantation to the omental tissue is the most promising option even with only one allele matching for patients with using lifelong high-dose supplementation. Clinical improvements and long-term effect of HLA-A allele matching should be evaluated with more studies and in larger cohorts as well.

INTRODUCTION

Permanent hypoparathyroidism (PHP) is an endocrine disorder that leads to hypocalcemia, hyperphosphatemia, and hypercalciuria.[1 2] Symptomatic treatment requires lifelong medication, such as oral calcium, active vitamin D/analogues, and even intravenous calcium administration. As side effects, gastritis, urolithiasis, and nephrocalcinosis occur in most patients.[3 4] There are two main treatment options for the absent/non-functioning parathyroid gland: parathormone (PTH) replacement therapy and parathyroid allotransplantation (PA). Continued PTH replacement therapy provides a remarkable advance; however, it is expensive and long-term effects are still controversial.[1 5 6] On the other hand, PA is a promising approach and targets the true aspects of PHP by eliminating supplementation requirements. Currently, preoperative immunological criteria were upgraded by recent studies.[7 8] Herein, we have performed PA between human leukocyte antigen (HLA)-A allele matching and ABO/Rh compatible recipient with only minimized immunosuppression.

METHODS

Donor

The donor has a secondary hyperparathyroidism due to chronic kidney disease (CKD), which is one of the accompanying conditions of CKD. The donor was first diagnosed with renal insufficiency and commenced hemodialysis in 2010. The donor was treated with 6000 mg/day oral calcium and 4800 IU/day cholecalciferol (Calcimax-D3; Basel Ilac Co, Istanbul, Turkey). Donor could not use calcimimetics because of its severe side effects. In addition, clinical symptoms include bone and severe joint pain, and laboratory examination revealed hypercalcemia and extremely high PTH levels. The preoperative intact PTH level was 1715.1 pg/mL. The imaging examination showed that parathyroid glands were enlarged. The donor was screened for viral markers (anti-HIV, antihepatitis C virus, anti-hepatitis B core, anti-hepatitis B surface, anti-hepatitis B envelope antibodies, Epstein–Barr virus IgG and IgM, and venereal disease research laboratory), and subtotal parathyroidectomy was performed after all.

Three enlarged parathyroid glands were located during surgery, and each of the dissected tissue was fragmented into the half with careful examination. Each part of each gland was divided; one part was sent to the pathology department; and remaining parts were snap-frozen with liquid nitrogen, directly transported to the parathyroid cell culture laboratory and stored at −80°C until histopathological confirmation of hyperplasia for transplantation. The donor was discharged after surgery from the hospital without any complications.

Recipient

The recipient underwent total thyroidectomy in 2009. PHP symptoms occurred in the early

Table 1 Matching/mismatching HLA alleles between donor and recipient

	HLA genotyping				
	Class I			Class II	
	HLA-A	HLA-B	HLA-C	Hla-dr	Hla-dqb1
Recipient	**A*24** **A*02**	B*35 B*44	Cw*04 Cw*16	DRB1*04 DRB1*11	***DQB1*03*** *DQB1*03*
Donor	**A*24** **A*02**	B*55 B*52	Cw*01 Cw*12	DRB1*01 DRB1*14	***DQB1*05*** *DQB1*05*

Bold and italic indicate the matching and homozygous HLAs, respectively.
HLA, human leukocyte antigen.

stage after surgery and the recipient was using 4000 mg/day oral calcium and 3200 IU/day cholecalciferol (Calcimax-D3, Basel Ilac Co), 1 µg/day calcitriol (Rocaltrol; Deva Ilac Co, Istanbul, Turkey) to relieve cramps and numbness in the neck, hands, and legs. The dosage was increased to 6000 mg/day and 4800 IU/day for calcium and cholecalciferol, respectively, to ease the increasing symptoms. Additionally, the recipient was hospitalized at least three times in a month for intravenous calcium administration because of severe muscle cramps, while the treatment continues with the reported doses. The preoperative serum calcium level was 12.4 mg/dL; the intact PTH level was below one pg/mL; and phosphorus level was 3.9 mg/dL. Also, the recipient is taking 125 µg/day levothyroxine sodium (Levotiron; Abdi Ibrahim Ilac Co, Istanbul, Turkey). The recipient was in our transplantation waiting lists for more than 2 years.

Preoperative immunological assessment

The donor and the recipient was ABO/Rh-compatible. HLA genotyping was carried out by sequence-specific oligonucleotide-primed PCR method as described previously,[7] and the results are given in table 1. In addition, T-flow cytometry crossmatch (T-FCXM), B-flow cytometry crossmatch (B-FCXM) and autoflow cytometry crossmatch (auto-FCXM), respectively, and complement-dependent cytotoxicity crossmatch (CDCXM) tests were performed between the donor and the recipient. Panel reactive antibody (PRA) test was also performed only for the recipient.[2 7]

HLA homozygosity was observed for different loci of HLA-DQB1 in donor and recipient alleles. The recipient was found PRA negative, and all FCXM and CDCXM test results between donor and recipient were negative.

Transplantation

Transplantation was carried out with the permission of the National Scientific Board for Transplantation, and our parathyroid transplantation unit is the first officially permitted center to conduct PA since 2013.

After immunological assessment and histopathological approval of the donor tissue, we decided to perform PA. The recipient received only 250 mg methylprednisolone 1 day before transplantation and 125 mg at 1 hour before PA to minimize host reaction.

Parathyroid tissue was removed from cryopreservation and cell isolation was performed by the method we developed (patent pending, application number: 2018/15244). Viability results of the 24 hours of cultivation are presented in figure 1. The PTH level of parathyroid cells was 1313 pg/mL after 24 hours of cultivation. Collected suspension parathyroid cells were washed by centrifugation at 300 *g* for 7 min with isotonic saline solution two times for the removal of cultivation media. Then, 115×10^6 parathyroid cells were resuspended and sent to the operating room in a 10 mL isotonic saline solution.

Under general anesthesia, transplantation of cells was performed by the laparoscopic approach as described previously.[9] The cells were transferred directly to the omentum surface. The abdomen was closed with two stitches after transplantation. The recipient was discharged after 2 days without any complication.

RESULTS

The graft survival was followed up for 382 days, and calcium and active vitamin D supplementations were elevated while the recipient reported having reduced numbness and muscle cramps. The most important outcome of this transplantation is that the recipient does not require intravenous calcium administration still. Interestingly, at day 90, the recipient reported that muscle pain, cramps, and numbness occur rarely and after that, supplementation reduced to a one-third dose (1000 mg/day oral calcium and 800 IU/day cholecalciferol and 0.5 µg/day calcitriol/day) even with low PTH levels. Between 90 and 270 days'

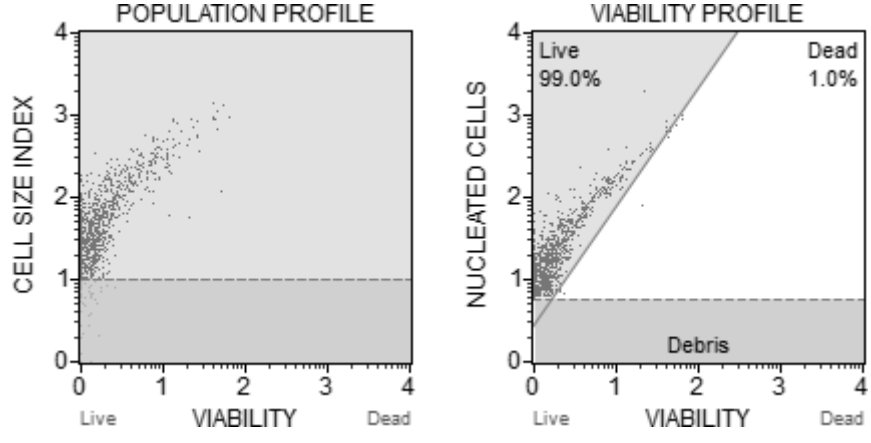

Figure 1 Viability results of parathyroid suspension cells before transplantation. The datum was analyzed on a MUSE cell analyzer and 2000 events were acquired.

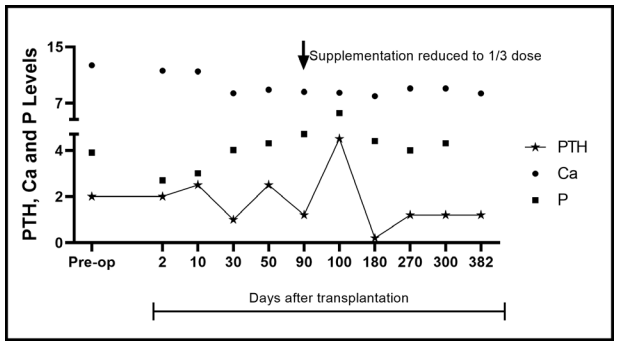

Figure 2 Post-transplantation follow-up of recipient PTH, Ca and P levels for 382 days. Preoperative supplementation reduced to one-third dose at day 90. Intact PTH values are indicated in picogram per millilitre; serum Ca and serum P values are indicated in milligram per decilitre. Reference ranges were PTH: 15–65 pg/mL, Ca: 8.6–10 mg/dL, P: 2.5–4.5 mg/dL. Ca, calcium; P, phosphorus; PTH, parathormone.

period, the physicians suggested if the cramps become more frequent, then the recipient may have double the dose of the supplementation of calcium. During the time period, intact PTH levels were elevated between 1 and 5 pg/mL; serum calcium and phosphorus levels remained at the reference ranges (Ca: 8.6–10 mg/dL, P: 2.5–4.5 mg/dL), except at day 180 serum phosphorus level was shown increased above the normal range (figure 2). The same day (at day 180) the PTH levels decreased to the lowest range and followed by decreased phosphorus levels into a normal range. After day 270, the recipient decided to improve the immune system by personal decision because of the COVID-19 pandemic, and currently, the recipient is taking 100 mg/day vitamin C and 10 mg/day glucan-coenzyme Q10 (Imuneks Q10; ImuneksFarma, Istanbul/Turkey) since. Currently, after 382 days of post-transplantation, the recipient is still using the reduced (1000 mg/day oral calcium and 800 IU/day cholecalciferol and 0.5 µg/day calcitriol/day) doses of calcium and active Vitamin D supplementation and have the option to double up the dose of calcium (not the active vitamin D) if the muscle cramps in the neck or hands occur.

DISCUSSION

PHP is mainly characterized by low or insufficient PTH levels. Postsurgical trauma such as thyroid or neck surgery, and/or removal/disruption of parathyroid glands is the main cause of the disease. Optional treatment requires either hormone replacement or replacing the removed/damaged tissue by transplantation, which is the only curative solution.

The parathyroid transplantation is an easy-to-handle and cost-effective indication for PHP. Therefore, the evaluation of compatibility between the donor and the recipient requires a definition. Major organ transplantations, for example, the heart and/or the kidney, already have a common consensus about the preoperative assessment and postoperative follow-up criteria.[10 11] Pre-evaluation of HLA typing has been reported to a decreased risk of rejection.[12] In 2019, Yucesan *et al* reported a preoperative evaluation of negative crossmatch (T-FCXM, B-FCXM, auto-FCXM, and CDCXM) and negative PRA tests could provide insight for graft survival for 4 years.[7] However, ABO compatibility is more frequent than T-FCXM, B-FCXM, auto-FCXM, and CDCXM for PA.[13] The lack of common immunological criteria for PA is the true obstacle in this field.

In our study, we have performed the first case on HLA-A matching PA with minimal immunosuppression and followed up the outcome for 382 days. Preoperatively, the donor and recipient were ABO/Rh compatible, crossmatch (T-FCXM, B-FCXM, auto-FCXM, and CDCXM) and PRA negative. As previously mentioned, the graft survival rate for PA is related to the intact PTH levels; however, in this case, we did not observe any significant increase in the intact PTH level. However, calcium response remained in the normal range while having decreased supplementation. In addition, the recipient showed a significant reduction in symptoms and did not require intravenous calcium administration after PA. Another important point is the recipient's CoQ10 supplementation usage. This may also explain the stability of the PTH level starting from day 270. As previously mentioned, CoQ10 acts as a modulatory agent for bone health, and as a result, serum calcium and PTH levels are affected indirectly.[14]

Clinically, serum calcium and relieved symptoms should be considered as contributing factors rather than intact PTH only when evaluating the graft survival for PA. Improved graft survival in PA may also be associated with HLA-A allele matching as well. The emergence of new case reports and larger cohorts may expand the applicability for parathyroid transplant patients.

Contributors BG participated in concetp/design, data analysis/interpretation, drafting and critical revision of the article. HSK and EY participated in data interpretation and collection. YEE and AA participated in critical revision and approval of the article.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Transplantation is carried out with the permission of the Turkish Ministry of Health, National Scientific Board for Transplantation. All of the protocols conformed to the ethical guidelines of the Helsinki Declaration, and written informed consent was obtained from all subjects. Our parathyroid transplantation unit is the first officially permitted center to conduct parathyroid allotransplantation since 2013.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iD

Beyza Goncu http://orcid.org/0000-0001-6026-8218

REFERENCES

1 Rubin MR. Recent advances in understanding and managing hypoparathyroidism. *F1000Res* 2020;9. doi:10.12688/f1000research.22717.1. [Epub ahead of print: 23 Jul 2020].
2 Yucesan E, Goncu B, Basoglu H, *et al*. Fresh tissue parathyroid allotransplantation with short-term immunosuppression: 1-year follow-up. *Clin Transplant* 2017;31. doi:10.1111/ctr.13086. [Epub ahead of print: 16 Oct 2017].
3 Al-Azem H, Khan AA. Hypoparathyroidism. *Best Pract Res Clin Endocrinol Metab* 2012;26:517–22.
4 Bilezikian JP, Khan A, Potts JT, *et al*. Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. *J Bone Miner Res* 2011;26:2317–37.
5 Bilezikian JP, Brandi ML, Cusano NE, *et al*. Management of hypoparathyroidism: present and future. *J Clin Endocrinol Metab* 2016;101:2313–24.
6 Cusano NE, Rubin MR, Bilezikian JP. PTH(1-84) replacement therapy for the treatment of hypoparathyroidism. *Expert Rev Endocrinol Metab* 2015;10:5–13.
7 Yucesan E, Goncu B, Ozdemir B, *et al*. Importance of HLA typing, PRA and DSA tests for successful parathyroid allotransplantation. *Immunobiology* 2019;224:485–9.

8 Aysan E, Yucesan E, Idiz UO, *et al*. Discharging a patient treated with parathyroid allotransplantation after having been hospitalized for 3.5 years with permanent hypoparathyroidism: a case report. *Transplant Proc* 2019;51:3186–8.
9 Yucesan E, Basoglu H, Goncu B, *et al*. Microencapsulated parathyroid allotransplantation in the omental tissue. *Artif Organs* 2019;43:1022–7. doi:10.1111/aor.13475
10 Ansari D, Bućin D, Höglund P, *et al*. Analysis of the influence of HLA-A matching relative to HLA-B and -DR matching on heart transplant outcomes. *Transplant Direct* 2015;1:e38.
11 Augustine J. Kidney transplant: new opportunities and challenges. *Cleve Clin J Med* 2018;85:138–44.
12 Montgomery RA, Tatapudi VS, Leffell MS, *et al*. HLA in transplantation. *Nat Rev Nephrol* 2018;14:558–70.
13 Barczyński M, Gołkowski F, Nawrot I. Parathyroid transplantation in thyroid surgery. *Gland Surg* 2017;6:530–6.
14 Quiles JL, Romero-Márquez JM, Navarro-Hortal MD, *et al*. Chapter 17 - The role of coenzyme Q10 in the protection of bone health during aging. In: Preedy VR, Patel VB, eds. *Aging*. 2 edn. Academic Press, 2020: 173–82.