Original Article

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Evaluation of Parathyroid allo-transplantation with the Presence of Auto-CASR Antibody

Paratiroit Allo-Nakli ile Oto-CaSR Antikor Varlığının Değerlendirilmesi

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ABSTRACT

Objective: Parathyroid tissue is responsible for the regulation of calcium and vitamin D metabolism. It regulates parathyroid hormone release by the calcium-sensing receptor (CaSR) which is found on the parathyroid cell surface. Auto-antibody formation against this receptor has been reported in the literature. In this study; the probability of parathyroid transplantation triggering an auto-immune response against this receptor was evaluated. Individuals were screened for the presence of auto-CaSR antibodies after transplantation.

Methods: The nine individuals who underwent parathyroid transplantation were evaluated for the survival rate and were screened for the possible presence of auto-CaSR antibodies. Data were determined by ELISA from peripheral blood samples. A peripheral blood sample from one healthy volunteer was included as a negative control.

Results: Survival rate assessment of nine recipients was as follows; less than one month in three individuals and more than one year in six individuals. Any trace of possible auto-CaSR antibodies was not detected in any individual.

Conclusion: Auto-CaSR antibody formation was not observed after parathyroid transplantation. Thus, it has been shown for the first time in the literature that the recipients did not show an autoimmune-related response after parathyroid transplantation even with having heterogeneous survival rates. Moreover, it has

ÖZ

Amaç: Paratiroit dokusu kalsiyum ve D vitamini metabolizmasının düzenlenmesinde hayati öneme sahiptir. Parathormon salınımını taşıdığı kalsiyum-algılayıcı reseptör (CaSR) ile düzenler. Bu reseptöre karşı vücutta oto-antikor oluşumu literatürde bildirilmiştir. Bu çalışma da; paratiroit nakli uygulanmış bireylerde oto-CaSR antikor varlığı taranarak, paratiroit naklinin oto-immün bir yanıtı tetikleme ihtimali değerlendirilmiştir.

Yöntemler: Paratiroit nakli uygulanmış dokuz birey, nakil sağkalım verileri ile beraber periferik kan örneklerinde oto-CaSR antikor varlığı ELISA ile belirlenmiştir. Negatif kontrol grubu olarak bir sağlıklı gönüllüden alınan periferik kan örneği değerlendirmeye dahil edilmiştir.

Bulgular: Değerlendirmeye alınan dokuz bireyde sağkalım oranları; üç bireyde bir aydan daha az, altı bireyde ise bir yıldan fazladır. Oto-CaSR antikor varlığı ise tüm bireylerde negatif olduğu belirlenmiştir.

Sonuç: Paratiroit nakli sonrasında otoimmün ilişkili olarak oto-CaSR antikor oluşumu gözlenmemiştir. Böylelikle heterojen sağkalım farklılıklarına sahip alıcıların, paratiroit nakli sonrasında otoimmün bir yanıtı göstermemesi literatürde ilk defa bildirilmiştir. Ayrıca, paratiroit naklinin başarısını öngörmede alıcıların semptomatik olarak gösterdikleri iyileşmelerin sağkalım oranını belirlemede önem arz ettiği gösterilmiştir. Paratiroit transplantasyonundan sonra oto-CaSR hakkında güçlü sonuçlar çıkarılmadan önce daha geniş kohortlarla çalışmalara ihtiyaç vardır.

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been shown that the symptomatic improvements of the recipients in predicting the success of parathyroid transplantation are important in determining survival rate. Larger cohort studies are required before strong conclusions can be drawn about the auto-CaSR after parathyroid transplantation.

Keywords: Parathyroid transplantation, parathyroid hormone, survival rate, auto-CaSR antibodies

Anahtar Sözcükler: Paratiroit nakli, parathormon, sağkalım, oto-CaSR antikorları

Introduction

The causes of hypoparathyroidism are autoimmune, congenital, and most often iatrogenic. After thyroid surgery and neck dissection, excision of the parathyroid glands or disruption of their vitality causes a decrease in parathormone (PTH) secretion secondary to hypocalcemia (1, 2). When we look at the literature, the incidence of hypoparathyroidism after total thyroidectomy varies between 7% and 37% (3). This hypoparathyroidism is mostly temporary and resolves within a few weeks to a month (4). During this period, patients receive symptomatic treatment in the form of calcium and vitamin D replacement. However, post-operative hypoparathyroidism exceeding six months is considered permanent hypoparathyroidism (PH) (3,5).

Although calcium and vitamin D replacement and, whenever possible, PTH injections are used in PH, the only curative treatment is parathyroid transplantation (10). Bezmialem Vakıf University Parathyroid Transplantation Unit is the first unit in Turkey to receive a parathyroid transplant permission from the Ministry of Health. Parathyroid transplantation in patients with PH shows survival of 1 to 5 years in some patients, but a survival of 1 to 6 months is observed in some patients (6-8).

Parathyroid tissue has an important role in the regulation of calcium and vitamin D metabolism. Parathyroid tissue provides cell functions with its surface receptor, the calcium-sensing receptor (CaSR) (9,10). CaSR senses the level of calcium in the blood, regulates PTH secretion, and plays an important role in calcium metabolism through urinary calcium excretion. The gene encoding the CaSR protein is located on the 3rd chromosome in humans (11). The parathyroid glands are a rare target for autoimmunity. It has been shown that antibodies to CaSR found on the surface of the parathyroid cells are present in the serum of patients with autoimmune hypoparathyroidism (12). In some individuals, auto-CaSR antibodies are thought to play a direct pathogenic role in hypocalcemia (12,13).

In this context, the relationship of parathyroid cell/tissue transplantation with the possibility of triggering the development of antibodies against CaSR (auto-CaSR antibody) during survival was investigated. In this sense, the presence/absence of auto-CaSR antibodies in blood serum samples of transplanted recipients with a transplant survival of less than 1 month and more than 1 year was evaluated in this study.

Methods

Informed consent forms were obtained from the recipients and one healthy individual for their peripheral blood samples to be used in this study, for which Clinical Researches Local Ethics Committee Approval was obtained in accordance with the World Medical Association Declaration of Helsinki (Document date and number of the approval of the ethics committee: 24/05/2018-8283).

Recipients

The ABO compatible parathyroid allotransplant was performed for the first time in 9 individuals diagnosed as having PH between 2013 and 2018, and individuals who had only one parathyroid transplant were included. There was no autoimmune disease in these individuals and no immunosuppressive drug use was reported during the collection of peripheral blood samples to be used in this study. Of the 9 individuals who voluntarily agreed to participate in the study, the survival of three was less than 1 month and the survival of six of them was more than 1 year. All transplant operations were carried out with a single application. Tissue transplantation (Recipients 1, 3, 4) of the three volunteers in the study was provided by applying the Cleaveland protocol (14), and the other five recipients (Recipients 2, 5, 6, 7, 8, 9) were transplanted parathyroid tissue with cell isolation (15) applied by mechanical method.

Peripheral blood samples were subjected to 10 min centrifugation at 4000 rpm. Serum samples were stored at -80°C until ELISA experiments were performed. As the negative control group, a peripheral blood sample from a healthy volunteer was included in the evaluation.

ELISA

A commercially available ELISA kit for Auto-CaSR antibodies was used (EDITM Human Anti-CaSR Autoantibody ELISA Kit, Epitope Diagnostics, Inc., San Diego, CA, USA). All samples were studied in pairs, in accordance with the kit's protocol. The absorbance values at 450 nm were evaluated relative to the positive and negative control.

Results

In Table 1, the type of transplantation, the region of administration, intact parathormone (iPTH) levels, serum calcium levels and current clinical status of the 9 parathyroid allotransplant recipients participating in the study are presented. Current clinical status of recipients were as follows: K0: no change was observed, P1: The doses of the drugs for the PH treatment were not decreased, but clinical improvement was observed, K2: the use of drugs due to PH treatment was reduced, and K3: drug use was discontinued (Table 1). In Figure 1, the

Table 1. Recipients of parathyroid allo transplant (n=9); type of transplant. place of administration. pre- and post-op intact PTH and serum calcium values. Post-op follow-up data are given for each recipient based on current survival times. clinical situation; It was scored according to the response (change) during the survival and the transplant follow-up status at the end of 2020 (K0: no change was observed. K1: The drugs in the CH treatment were not reduced. but clinical improvement was observed. P2: The drug use due to CH treatment was reduced. and K3: The drug use was discontinued)). PTH: Parathormone. CH: Permanent Hypoparathyroidism. iPTH: pg/mL. Serum calcium (Ca): mg/dL.

	Transport type	Place of ıssue	Pre-op		Post-op		Overal survival ratio (month)	Clinical status (change/current status)
			iPTH	Ca	iPTH	Ca		
A1	Tissue	Deltoid muscle	0	8.8	0.3	8.5	<1	K0/K0
A2	Tissue	Deltoid muscle	9.9	8.9	27.3	7.5	≤20	K2/K1
A3	Tissue	Deltoid muscle	6	8.5	n/a	7.9	≤36	K2/K0
A4	Tissue	Deltoid muscle	3.1	8.5	2.9	9.2	<1	K0/K0
A5	Mechanical cell isolation	Deltoid muscle	0.1	12.5	15.5	10.4	60>	K2/K3
A6	Mechanical cell isolation	Deltoid muscle	10.4	7.3	10.6	7.8	<1	K0/K0
A7	Mechanical cell isolation	Omentum	10.7	8.4	18.6	6.6	<1	K1/K0
A8	Mechanical cell isolation	Omentum	2.2	8.2	8.4	3.5	<12	K1/K1
A9	Mechanical cell isolation	Omentum	9.7	8.4	12.7	8.8	24>	K2/K3

absorbance values at 450 nm obtained as a result of the ELISA experiment for auto-CaSR antibody levels, recipient samples (n=9), values of one healthy individual, and positive and negative controls are given.

Recipient #1 (A1); A 44-year-old woman underwent a parathyroid tissue transplant into the deltoid muscle three years after the diagnosis of PH and her survival time was <1 month. A1 continues on pretransplant medication regimen for PH symptoms. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 4th year after transplantation (Figure 1).

Recipient#2 (A2); A 28-year-old woman underwent parathyroid cell transplantation on the omentum with a laparoscopic approach, approximately 2 years after she was diagnosed as having PH. It was observed that the patient did not use medication until the 20th month after the transplant. Since the patient became pregnant 20 months after transplantation, it was reported that the drug doses used for the diagnosis of PH were reinitiated by half. It was observed that the transplant survival period lasted up to 20 months and her clinical status was still good in terms of drug doses. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 3rd year after transplantation (Figure 1).

Recipient #3 (A3); A 45-year-old woman underwent a parathyroid tissue transplant to the deltoid muscle 8 years after she was diagnosed as having PH and her survival time was 3 years. The recipient returned to the drug doses used for the symptoms of PH at 35 months after transplantation. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 2nd year after transplantation (Figure 1).

Recipient #4 (A4); A 45-year-old man underwent a parathyroid tissue transplant to the deltoid muscle ten years after the diagnosis of PH and his survival time was <1 month. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 2nd year after transplantation (Figure 1).

Recipient #5 (A5); A 56-year-old woman underwent a parathyroid cell transplant to the deltoid muscle two years after she was diagnosed as having PH. Her survival time is more than 5 years and she is still being followed up. The recipient stated that she did not show symptoms of PH and did not use any medication. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 5th year after transplantation (Figure 1).

Recipient #6 (A6); A 51-year-old woman underwent a parathyroid cell transplant into the deltoid muscle six years after being diagnosed as having PH. Survival time was less than 1 month. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 1st year after transplantation (Figure 1).

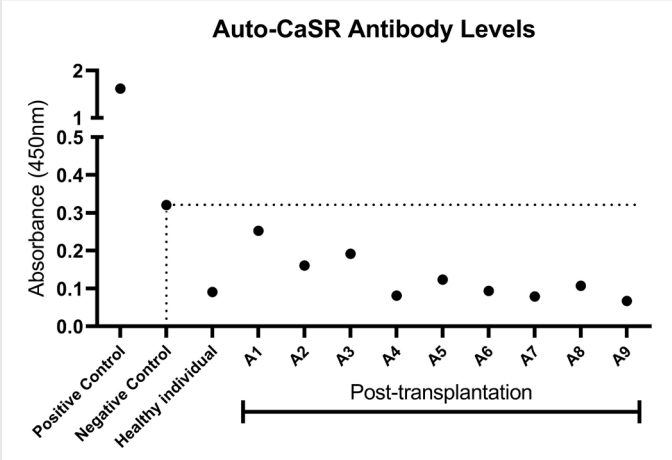

Figure 1. Comparison of auto-CaSR antibody levels by absorbance values at 450 nm

Recipient #7 (A7); A 39-year-old woman underwent parathyroid cell transplantation on the omentum using a laparoscopic approach two years after she was diagnosed as having PH. The transplant survival time was approximately 29 months. It was reported that the drug doses used for the treatment of PH symptoms were reduced to 1/3 of the initial doses, and then the doses were returned back to the initial drug doses. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 1st year after transplantation (Figure 1).

Recipient #8 (A8); A 49-year-old woman underwent a laparoscopic approach to parathyroid cell transplantation on the omentum 7 years after she was diagnosed as having PH, and the survival time of the transplantation was approximately one year. The drug doses used by the recipient for the treatment of PH symptoms were halved and she reported that she continued her medication as it was. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 9th month after transplantation (Figure 1).

Recipient #9 (A9); A 51-year-old woman underwent parathyroid tissue cell transplantation into the deltoid muscle two years after she was diagnosed as having PH. Transplant survival is >2 years and she is still being followed-up. The recipient stated that she did not show symptoms of PH and did not use any medication. In this study performed at the 10th month after transplantation, the presence of auto-CaSR antibodies was found to be negative (Figure 1).

Discussion

The patients with PH need lifelong, regular oral or parenteral calcium and vitamin D replacement, but in some patients, hypocalcemia and symptoms do not improve despite regular supplements. In addition to the morbidity due to hypocalcemia and hypoparathyroidism, the quality of life of the patients is seriously impaired and the cost of these lifelong preparations is high (16). Serum calcium level is primarily affected by PTH level in these patients, and also affected by the levels and functions of vitamin D, phosphate, renal system, calcium transporter/binding protein and CaSR (17-19).

The CaSR auto-antibodies were first found in patients with familial hypocalciuric hypercalcemia (FHH) (20,21). However, Kifor et al. stated that these auto-antibodies were not only found in patients with FHH, leading to a search for a basis for autoimmune symptoms (22). Autoimmune hypoparathyroidism may present as a clinical abnormality. Auto-CaSR antibodies can be seen in autoimmune polyendocrinopathy syndrome-1 (APS-1) or in APS-2 (23,24). APS-1 most commonly includes mucocutaneous, candidiasis, hypoparathyroidism, and Addison's disease. APS-2 includes two or more of the following: Addison's disease, Graves' disease, autoimmune thyroiditis, type 1 diabetes mellitus, primary hypogonadism, myasthenia gravis, or celiac extension (23,25,26). Studies have shown that auto-CaSR antibodies are present in approximately one third of these diseases (23,27). On the other hand, it has been reported that some patients with primary hypoparathyroidism may harbor auto-antibodies against human CaSR (28). Therefore, it is known that detecting this auto-antibody has clinical value to assess the autoimmune origin of the disease.

Study Limitations

In this study, according to the results of parathyroid allo-transplantation, tissue transplantation was applied to four individuals and parathyroid cell transplantation was applied to five individuals. In terms of administration site, six transplants were performed by injection into the deltoid muscle. The other three transplants were performed with the laparoscopic approach to the omental tissue. Survival rates were less than one month in four individuals and less than one year in one individual. Survival of up to two to three years was observed in two individuals. In the other two individuals, survival still continues. The presence of auto-CaSR antibody, on the other hand, was evaluated as negative despite differences in administration site, transplant type and survival times. It was observed that parathyroid allo-transplants did not pose any risk factor for auto-CaSR antibody formation. In addition, the criteria for clinical improvement were determined depending on the survival status in the examination made among the clinical conditions of the recipients. Based on our experience with parathyroid allo-transplants since 2013, it is thought that changes in PTH ratio alone are not sufficient to evaluate the outcome of transplantation treatment of PH. Contribution to clinical improvement should also be considered in cases such as the reduction of drug doses used for PH symptoms, long-term symptomatic relief at low doses, and/or complete disappearance of the need for intravenous calcium.

Conclusion

As it can be understood from the findings obtained as a result, genetic differences between individuals can lead to different course of the same disease with similar symptoms. This reveals the importance of personalized and translational medicine approaches. By expanding the patient cohort in future studies, it will be possible to minimize the individual differences resulting from genetic heterogeneity and to reconsider the transplantation processes taking into account the mentioned differences.

Ethics

Ethics Committee Approval: Clinical Researches Local Ethics Committee Approval was obtained in accordance with the World Medical Association Declaration of Helsinki (ethics committee consent document date and number 24/05/2018-8283).

Informed Consent: Informed consent forms were obtained from the recipients and a healthy individual for the peripheral blood samples to be used in this study.

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Authorship Contributions

Surgical and Medical Practices: H.S.K., S.Y., Y.E.E., A.A., Concept: H.S.K., S.Y.,

Design: H.S.K., B.G., Data Collection or Processing: B.G., Ö.F.D., Analysis or Interpretation: H.S.K., B.G., Ö.F.D., Y.E.E.,

Literature Search: H.S.K., B.G., Ö.F.D., Writing: H.S.K., B.G., Y.E.E., A.A.

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