

Original Article

Parathyroid autotransplantation in rats having hypoparathyroidism

Mehmet Erikoglu^{1*}, Bayram Colak^{2*}, Hatice Toy³, Mehmet Gurbilek⁴

Departments of ¹Surgery, ³Pathology, ⁴Biochemistry, Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey; ²Department of Surgery, Faculty of Medicine, Selcuk University, Konya, Turkey. *Equal contributors.

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Abstract: Re-implantation techniques of extracted parathyroid tissue were developed in order to prevent temporary hypocalcemia. During thyroid surgery; inadvertently removed or devascularized parathyroid gland is usually implanted in the sternocleidomastoid muscle. In this experimental study using rats with hypoparathyroidism, our aim was to investigate whether the excised parathyroid tissue could be seeded in the liver and in the peritoneum, instead of the SCM muscle. In our study, four different groups, each consisting of 10 Wistar albino rats were used (Control group, sternocleidomastoid muscle group, liver group, peritoneum group). Parathyroidectomy was performed and the parathyroid tissue was seeded into the sternocleidomastoid muscle, liver and peritoneum. After 14 days, the rats were sacrificed and levels of calcium, magnesium, phosphorus, alkaline phosphatase and parathyroid hormone were measured in rats' blood samples. The autotransplanted parathyroid tissue was then excised and examined. In all groups, parathyroid tissues were analyzed histopathologically according to calcification, necrosis, tissue loss, foreign body reaction, inflammation and fibrosis. Regarding Ca, Mg, PO₄, ALP; There were no difference between the groups. When we compared control group with the other groups; a difference was observed in the levels of PTH (P<0.05). In pathological examination; regarding tissue loss; there was a difference between liver and peritoneum groups (P<0.05). In our study, we expected better result in plantings inside liver and peritoneum compared to SCM. However, there were no difference between the groups.

Keywords: Parathyroid, autotransplantation, hypocalcemia

Introduction

Blood build-up of parathyroid tissue can be deteriorated or accidentally extracted in thyroid and head-neck region surgeries [1]. Re-implantation techniques of extracted parathyroid tissue were developed in order to prevent temporary hypocalcemia. Extracted parathyroid tissue can be planted in trapezius muscle [2], sternocleidomastoid muscle [3], fore arm subcutaneous tissue [4], fore arm brachioradial or flexor muscle group [5], subcutaneous tissue in front of sternum [6], stomach fat tissue [7].

Permanent hypoparathyroidism is a serious clinical situation after thyroid surgery. Parathyroid autotransplantation is an effective procedure to reduce the incidence of permanent hypoparathyroidism. During thyroid surgery; inadvertently removed or devascularized para-

thyroid gland is usually implanted in the SCM muscle [8].

In this experimental study using rats with hypoparathyroidism, our aim was to investigate whether the excised parathyroid tissue could be seeded in the liver and in the peritoneum, instead of the SCM muscle.

Material and methods

Study was carried out in Necmettin Erbakan University, Meram Medicine Faculty, Research and Application Center of Experimental Medicine. Ethics committee approval was taken prior to the study.

In our study, four different groups, each consisting of 10 Wistar albino rats were used.

Group 1 (Control group): Parathyroid exploration only was made.

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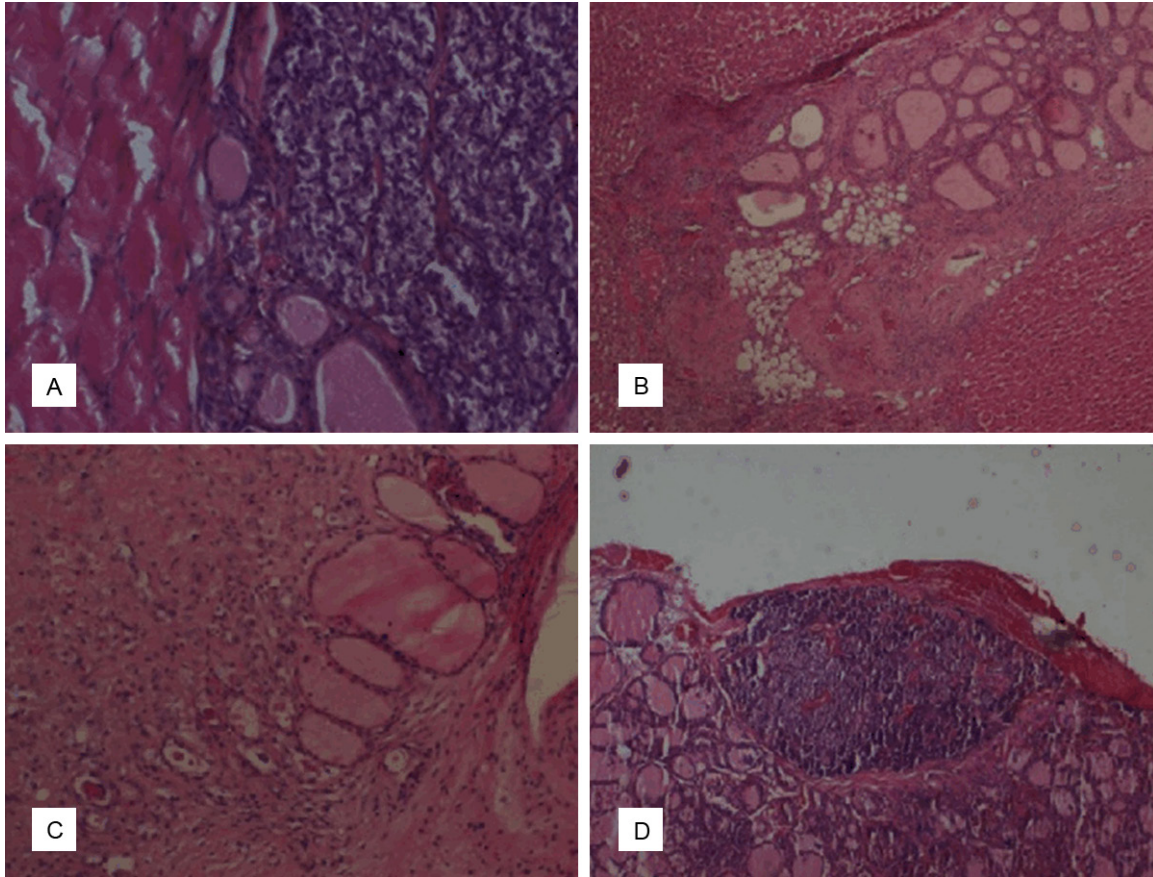


Figure 1. Histopathological image (10 ×) of seeded parathyroid tissue. A: Group 2; B: Group 3; C: Group 4; D: Group 1.

Group-2 (SCM group): Parathyroidectomy was performed and the parathyroid tissue was seeded into the sternocleidomastoid muscle.

Group-3 (Liver group): After parathyroidectomy, laparotomy was performed and the parathyroid tissue was seeded in the liver.

Group-4 (Peritoneum group): After parathyroidectomy, laparotomy was performed and the parathyroid tissue was seeded in the peritoneum.

Before the experiment, feathers on the necks of the rats were shaved. Betadine® solution and antiseptics were applied on their neck skin. Operative proceedings were carried out under sterilized conditions. Cuts were applied on the necks of the rats in middle line in all groups in the length of approximately 1-2 cm. Thyroid and parathyroid tissues were found.

Group 1: Parathyroid exploration was carried out. No parathyroidectomy was applied.

Group 2: Parathyroidectomy was applied. Parathyroid tissue was kept in normal saline of 0.9% and divided into pieces. From the same incision, plantation was carried out inside sternocleidomastoid muscle and it was sutured.

Group 3: Parathyroidectomy was applied. All the tissues were kept in normal saline of 0.9% and divided into pieces. Laparotomy was carried out under sterilized conditions. From the incision of 0.5 cm on the liver, it was planted inside liver parenchyma and it was sutured.

Group 4: Parathyroidectomy was applied. All the tissues were kept in normal saline of 0.9% and divided into pieces. Laparotomy was carried out under sterilized conditions. It was planted between peritoneal leaves in fore abdominal wall.

2 rats from Group 1 and 3, 1 rat from Group 2 and 4 died during the study and dead rats were substituted by the new ones and the study continued.

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Table 1. Measured values of calcium, magnesium, phosphorus, PTH and LDH in rats' blood products within 14 days (Calcium (8.4-10.2 mg/dL), phosphor (2.3-4.7 mg/dL), magnesium (1-4.5 mg/dL), ALP (0-500 u/L), PTH (12-88 pg/ml) (normal value)

Groups	N	Calcium	Magnesium	Phosphorus	ALP	PTH
Control (1)	10	7.87 ± 1.82 ^a	2,22 ± 0.30 ^a	9.30 ± 2.55 ^a	225.00 ± 83.19 ^a	68.04 ± 59.41 ^a
SKM (2)	10	8.51 ± 1.39 ^a	2,12 ± 0.13 ^a	8.90 ± 1.49 ^a	234.50 ± 102.47 ^a	5.92 ± 2.57 ^b
Liver (3)	10	9.30 ± 0.61 ^a	2,25 ± 0.17 ^a	7.77 ± 1.13 ^a	215.40 ± 32.01 ^a	9.77 ± 5.29 ^b
Peritoneum (4)	10	8.55 ± 1.11 ^a	2,19 ± 0.24 ^a	7.94 ± 1.18 ^a	213.90 ± 105.83 ^a	29.28 ± 20.28 ^b

PTH P values: 1-2: 0.000; 1-3: 0.001; 1-4: 0.021; 2-3: 0.812; 2-4: 0.156; 3-4: 0.234. a, b: The differences between the averages in the same column with different letters are important (P<0.05).

Similarly, parathyroid tissues were divided into pieces and kept in normal saline of 0.9% [9]. parathyroid tissues in pieces were planted inside SKM, Liver and peritoneum. In some of the studies; adequate period for the determination of serum calcium level in rats, on which tiroparathyroidectomy was applied, was determined as 14 days [9]. Therefore; rats were sacrificed for 14 days in our study.

After 14 days, the rats were sacrificed and levels of calcium, magnesium, phosphorus, alkaline phosphatase and parathyroid hormone were measured in rats' blood samples. The autotransplanted parathyroid tissue was then excised and examined. Calcification, necrosis, tissue loss, foreign body reaction, inflammation and fibrosis were examined in the parathyroid tissue.

Rats were sacrificed by high dose anesthetic material and the experiment were ended. parathyroid tissues explored from Group 1 were extracted together with the parathyroid tissues planted in Group 2, 3, 4.

Pathological examination

Tissues taken from the rats for pathological examination were fixed in formalin of 10%. Appropriate tissues were cut on the sutured regions and embedded in paraffin blocks after routine follow up transaction. Through Microtome, sections of 4 µ were taken. They were dyed with hematoxylin eosin and analyzed in light microscope, the brand of which is OlympusBX51 (Figure 1A-D).

These tissue were analyzed in terms of calcification, necrosis, tissue loss, reaction of foreign body, inflammation or fibrosis. Findings were scored as (-), +, ++, +++, +++++. Scoring was made as the following: If not available: -, if avail-

able 25%: +, if available 50%: ++, if available 75%: +++, if available 100%: +++++.

Statistical analysis

SPSS 16.0 computer programme was used. The arithmetical average and standard deviations of all parameters was calculated. "One way Analysis of Variance" (ANOVA) test was used for the detection of intergroup differences. "Bonferroni" test was used for determining are differences caused by the which groups. P value of less than 0.05 was regarded as significant.

Results

Prior to the experiment, serum calcium, phosphorous, magnesium, alkaline phosphatase and parathormone levels of all the rats were normal.

There were no differences between the groups (P>0.05) regarding serum calcium, phosphorous, magnesium, alkaline phosphatase levels (Table 1). When we compare group 1 and other groups (2, 3, 4), a difference was observed in the levels of PTH (P<0.05) (Table 1). The second best value about PTH was in the peritoneum group (Table 1).

In all groups, there were parathyroid tissues in tissues taken from the groups. There were no available calcification, necrosis, tissue loss, reaction of foreign body, inflammation or fibrosis in Group 1 (Figure 1D). Calcification occurred significantly only in group 4 (peritoneum). In the other groups the formation of calcification was not detected. 1st, 2nd and 3th groups were similar (P>0.05). The 4th group was significantly different from the others (P<0.05). Necrosis was only seen in groups 2 and 3 but it is not statistically significant (P>0.05). Tissue loss

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Table 2. Histopathological examination (calcification, necrosis, tissue loss, reaction of foreign body, inflammation or fibrosis) of the groups evaluated with the ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Calcification	Between Groups	.792	3	.264	12.570	.000
	Within Groups	.756	36	.021		
	Total	1.548	39			
Necrosis	Between Groups	.169	3	.056	1.421	.253
	Within Groups	1.425	36	.040		
	Total	1.594	39			
Tissue loss	Between Groups	5.419	3	1.806	27.094	.000
	Within Groups	2.400	36	.067		
	Total	7.819	39			
Reaction of foreign body	Between Groups	3.219	3	1.073	33.587	.000
	Within Groups	1.150	36	.032		
	Total	4.369	39			
inflammation or fibrosis	Between Groups	3.488	3	1.163	54.885	.000
	Within Groups	.763	36	.021		
	Total	4.250	39			

has occurred in other groups except group 1. Control group was different from others in terms of foreign body reaction ($P < 0.05$). 2, 3 and 4 groups were similar ($P > 0.05$). Inflammation and fibrosis has occurred in other groups except group 1. Group 2 were similar with group 3 and 4 about inflammation and fibrosis ($P > 0.05$). Group 3 and 4 were different significantly from each ($P < 0.05$) (**Table 2**).

Discussion

Hypocalcemia which develops after thyroidectomy is either temporary or permanent. If treatment is stopped in 1 year at the latest after the surgery and no Hypocalcemia symptoms are observed, it is regarded as temporary. But if it is stopped after 1 year and Hypocalcemia still goes on, it is regarded as permanent [10]. Permanent hypoparathyroidism is a serious complication. If the patients having had thyroidectomy before are not adequately followed up after the surgery, it is impossible to make a diagnosis and treatment because there are generally no apparent symptoms and findings developed especially in patients having partial or latent hypoparathyroidism. There can be such serious complications as cataract, calcification of brain basal ganglion and cerebellum, papiledema which occur in the patients who are not treated [11]. No matter how careful surgeries are carried out in thyroid surgeries, there

are always risks of deterioration and extraction of blood built-up of parathyroid glands [12]. Parathyroid autotransplantation was firstly carried out by Lahey in 1926 [13]. Postoperative hypoparathyroidism gives illness is a frequently seen illness in patients having experienced retrosternal goitre and rethyroidectomy [14]. In various publications it is stated that hypocalcemia ratio increases between 2 and 10 times in secondary surgeries compared to primary surgeries. Marchesi and Torre [15] encountered temporary hypocalcemia in such high ratios as 57% and 1.5%, 21% and 24% in complementary thyroidectomies applied in recurrences of MNG, Aganval [16] thyroid cancer and Nicolosi [17] Graves illnesses.

Zedenius J et al. [18] stated in their studies that planting of at least one parathyroid gland inside stenokleidomastoid muscle during thyroidectomy as a routine can reduce the risk of permanent hypoparathyroidism to zero. Lo CY at al. [19] stated in their studies, in which they compared the patients to whom they applied selective and routine parathyroid plantings, that both methods can minimize the risk of hypoparathyroidism. However; they also stated that routine planting increased temporary hypocalcemia incidence.

Today, there is no method which enables a certain protection for the functions of parathyroid

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glands in thyroid surgery. Therefore, lots of surgeons accept parathyroid autotransplantation as an alternative method to protect parathyroid function when parathyroids cannot be protected adequately [20].

Although neck is appropriate for parathyroid autotransplantation in thyroid surgery, there are also different parts described for autotransplantation [21]. Fore arm is mostly recommended because re-exploration is easy there when necessary [22]; in addition to this, trapezoid muscle, pectoralis major muscle and quadriceps are also recommended [21].

Lots of methods have been applied so far in order to prevent permanent hypoparathyroidism. Parathyroids, whose feeding is deteriorated or accidentally extracted during thyroid and head and neck surgery, are traditionally planted inside SKM muscle. Liver is an organ which becomes bloodshot in the body while peritoneum has a good capacity of high absorption. Therefore, we planted inside liver and peritoneum.

Parathyroids extracted in our study were planted inside SKM, liver and peritoneum and the results were evaluated. Our expectations were such that hypocalcemia would be seen less in groups in which planting was made inside liver and peritoneum when compared to SKM group. However, in accordance with the results, we can say that there were no apparent differences observed between SKM, KC and peritoneum groups in terms of biochemical and also histological. In our study, we expected better results in plantings inside liver and peritoneum compared to SKM. However, there were no difference between the groups.

As a result, it was concluded that during thyroid surgery, the excised parathyroid tissue, in selected cases, may be planted in the liver and peritoneum.

Disclosure of conflict of interest

None.

Address correspondence to: Bayram Colak, Department of Surgery, Faculty of Medicine, Selcuk University, Selcuk Universitesi Tıp Fakültesi Alaeddin Keykubat Kampusu, Konya 42075, Turkey. Tel: +90 (530) 1164264; E-mail: bayro.99@gmail.com

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