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Histological study of donor's duodenal mucosa biopsy specimens in the diagnosis of pancreatoduodenal complex rejection: the experience of N.V. Sklifosovsky Research Institute for Emergency Medicine

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Background. *The verification and identification of the transplanted pancreas rejection type requires a morphological examination of the graft tissue. The pancreas graft transcutaneous biopsy procedure is associated with a high risk of surgical and infectious complications, and with the risk of the graft loss. Relatively safe is the biopsy of the donor duodenum mucosa.*

Objective: *to evaluate the efficacy of morphological examination of the donor duodenum mucosa in early diagnosis of an acute rejection crisis of pancreas graft.*

Material and methods. *The study presents a retrospective analysis of 35 donor duodenum mucosa biopsies performed in 19 recipients. In order to assess the correlation between clinical and morphological signs of rejection, the patients were divided into two groups. The first group included 6 patients with clinical signs of graft dysfunction; the second group included 7 patients without signs of rejection. Statistical processing of the study results was made using the descriptive statistics methods.*

Results. *The signs of immunological complications were identified in 18 donor duodenum mucosa biopsies (51.4%) in 12 recipients (63.2%). In most cases (n = 13; 72.2%), the histological signs of mild rejection were found, the signs of moderate and severe rejection were less frequent (n = 3; 16.6% and n = 2; 11.1%, respectively). Morphological signs of acute rejection were found in all the patients of the 1st group (n = 6), including the signs of mild rejection in 4 cases (66.6%), of medium and severe rejection in 1 case each (16.7%). In the 2nd group, morphological signs of mild rejection were found in 3 patients (42.9%). Differences between the groups in the incidence of immunological complications were statistically significant (p <0.05).*

Conclusions: *the biopsy of donor duodenum mucosa is an important criterion in the diagnosis of an acute rejection crisis of the pancreatoduodenal complex, and also remains a safe method, even in the earliest postoperative period.*

Keywords: pancreas transplantation, diagnosis of acute rejection crisis

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ARC, acute rejection crisis

DD, duodenum

DDM, duodenal mucosa

EGDS, esophagogastroduodenoscopy

IDA, interduodenal anastomosis

MRI, magnetic resonance imaging

PAS, periodic acid-Schiff

PDC, pancreatoduodenal complex

PG, pancreas graft

US, ultrasonography/ultrasound

Introduction

Pancreas transplantation (PT) is one of the treatment methods for patients with type 1 diabetes mellitus suffering from end-stage chronic renal disease. If the glomerular filtration rate is reduced <20 ml/min/1.73 m² or if the patient is already receiving renal replacement therapy, a combined kidney and pancreatoduodenal complex (PDC) transplantation or DD transplantation after previous kidney transplantation is indicated [1]. According to different authors, a 1-year pancreas graft (PG) survival rate reaches 95% [2], a 5-year graft survival ranges from 69% to 80% [3]. It was noted that the causes of PDC function loss differ in different time periods after transplantation. So, the main etiological factors of PDC dysfunction during the first week after surgery include the surgical complications (vascular thromboses), and the immunological ones (acute graft rejection). The most significant factors of the functional loss a week later after transplantation include the infectious complications, and a PDC rejection a month later posttransplant [4].

In PDC transplantation, the rejection is diagnosed based on the criteria reflecting the PG functional status, such as the blood levels of amylase and lipase, glycemia, C-peptide, etc. To diagnose an acute rejection, some instrumental methods may also be useful: radionuclide scintigraphy, angiography, Doppler ultrasonography (US), magnetic resonance imaging (MRI), and computed tomography. But none of these methods is reliably

specific. Verification and evaluation of the rejection type requires a morphological examination of the graft tissue. A transcutaneous biopsy of PG is associated with a high risk of surgical complications that may be accompanied by infectious complications and the risk of graft loss. Much safer is the PDC duodenal mucosa (DDM) biopsy. Earlier it was possible only by using the method of bladder drainage: the pancreas transplantation with draining the exocrine secretions into the urinary bladder. In literature, one can find the works of American scientists where they used a large number of such biopsies to show a correlation of changes in the PDC DDM with the development of the PG rejection [5]. According to the international registry, from 10% to 28% of patients who were operated on earlier by using that technique subsequently had to be subjected to relaparotomy and reconstructive surgery to divert the exocrine secretion into the bowel due to the development of urological and metabolic complications.

To date, most transplantation centers conducting pancreas transplantations prefer a more physiological, intraintestinal method of draining exocrine secretions; and for that purpose various intestinal anastomoses are used. For PG intestinal drainage, some surgeons use a Roux-excluded loop formed of a jejunum or even ileum, with the pancreas head being directed cranially, caudally, or laterally [6].

In 2007, a new pancreas transplantation technique was proposed implying its retroperitoneal location and the formation of interduodenal anastomosis (IDA) for draining the exocrine secretions. Since 2011, this modification of the operation has been implemented in the practice of the Sklifosovsky Research Institute for Emergency Medicine. Such surgical technique has a number of obvious advantages. First of all, this is the most physiological way of drawing away pancreatic juice, since DDM is resistant

to the effect of aggressive enzymes contained in the juice. Also, the IDA formation is surgically simple compared to other interintestinal anastomoses. The retroperitoneal PG position contributes to circumscribing pathological foci in case of surgical complications and does not lead to peritonitis development. Another important advantage is the possibility of frequent and safe esophagogastroduodenoscopy (EGDS) to be performed to assess the interintestinal anastomosis condition and DDM biopsy aimed at early and reliable diagnosis of the rejection reaction.

The purpose of the study was to evaluate the efficacy of the morphological study of DDM for early diagnosis of PG acute rejection crisis (ARC).

Material and methods

The study presents a retrospective analysis of 35 DDM biopsies made in 19 recipients. The number of biopsies performed at different time points after PDC transplantation is presented in Table. 1.

Table 1. The number of duodenal mucosa biopsies performed at different time points after pancreatoduodenal complex transplantation

0-1 month	1-6 months	6 months - 1 year	1-2 years	2-3 years
13	7	2	8	5

EGDS is included in the list of mandatory studies in patients who underwent pancreas transplantation with duodenal-duodenal anastomosis (DDA) formation. Starting from 2013, the DDM biopsy for further histological examination in the early postoperative period has been

performed to 13 patients in the Sklifosovsky Research Institute for Emergency Medicine. Among them, there were 7 men (53.8%) and 6 women (46.2%). The age of patients ranged from 25 to 51 years and averaged 35.3 (± 6.3) years. EGDS was performed at 15.5 ± 4.56 postoperative days (Fig. 1).

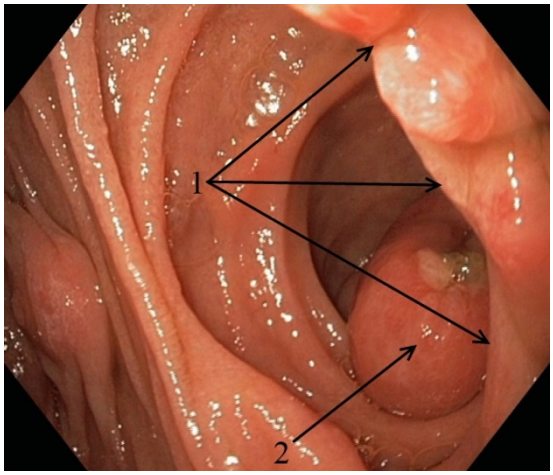

Fig. 1. Endoscopic view of intestinal anastomosis in esophagogastroduodenoscopy: 1, interduodenal anastomosis line; 2, invaginated stump of donor duodenum

The indications for EGDS with DDM biopsy in the long term after surgery were clinical (dyspepsia events, pain syndrome), instrumental (edema and the increased graft size, deteriorated blood flow parameters obtained at Doppler US examination, MRI, spiral computed tomography), and laboratory ones (increased levels of amylase, lipase, blood sugar), the signs of the transplanted pancreas dysfunction.

Six patients who had indirect signs of PDC dysfunction at the time of the study were included in the main group, and 7 patients who had no clinical or laboratory abnormalities at the time of the study made up the comparison group.

We should note that in 9 (47.4%) of 19 patients, the biopsy was made 2 or more times to assess the treatment efficacy or in routine examinations.

To diagnose the rejection and to assess its severity, the histological examination included the evaluation of a number of parameters: intestinal villi condition, the presence of goblet cells, the brush border state, the presence and extent of stroma infiltration, lamina muscularis mucosa, the presence of ulcer defects, vessel condition, and the presence of apoptotic bodies.

For a morphological study, the biopsy material was fixed in a 10% formalin solution, embedded in paraffin; the sections were stained with hematoxylin and eosin. A histochemical PAS reaction was used to evaluate the basal lamina, and the MSB reaction was used to detect vascular abnormalities.

Statistical processing of the study results was performed using descriptive statistics methods. The groups were compared using Fisher's exact test. The significance level threshold was assumed equal to 0.05.

Results

The (ARC) signs were seen in 18 DDM biopsies (51.4%) of 12 recipients (63.2%). Mild or slightly pronounced ARC episodes were the most commonly detected (n = 13, 72.2%). Moderate ARC was seen in 3 biopsy specimens (16.6%), severe ARC was seen in 2 (11.1%). The timing of the ARC onset is presented in Table 2.

Table 2. The incidence of acute rejection crisis of pancreatoduodenal complex at different time points after surgery

ARC	Time after transplantation				
	0 -1 month (n = 13)	1 - 6 months (n = 7)	6 months - 1 year (n = 2)	1 - 2 years (n = 8)	2 - 3 years (n = 5)
Mild, n	7	3	1	1	-
Moderate, n	1	1	-	1	-
Severe, n	1	-	-	1	-

The histological examination of the biopsy material diagnosed a mild ARC in 13 of 19 cases. The morphological pattern was characterized as follows: alterations in intestinal villi (thinning, flattening, shortening) were noted in 84.6% of cases (n = 11), pronounced abnormalities with the damaged absorbing epithelium in 7.7% (n = 1), intact intestinal villi in 15.4% of cases (n = 2). Goblet cells in adequate numbers were detected in 9 examinations (69.2%), and their numbers were found decreased in 4 (30.8%) cases. Meanwhile, the brush border (PAS reaction) was kept safe in most of the obtained biopsy material (92.3%). The infiltration of donor DD lamina propria with mononuclear cells was seen as extreme in 5 cases (38.5%), moderate in 8 (72.5%). No vasculitis signs were seen in the obtained material. There were no ulcerative or cicatricial changes in any of the cases. Apoptotic bodies were identified in 2 biopsy specimens (15.4%) (Fig. 2).

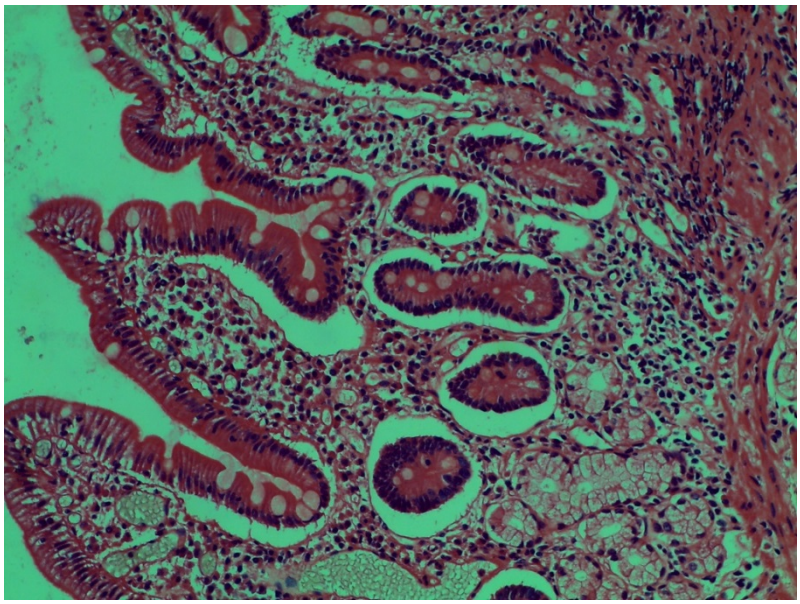

Fig. 2. A mild acute rejection crisis. Flattening, shortening of intestinal villi, lymphoplasmocytic infiltration of the mucosa lamina propria of donor DD (staining with hematoxylin and eosin, magnification x 200)

In moderate ARC, the intestinal villi lesions were more common, namely in 2 cases (66.6%); intestinal epithelium with a thinned brush border (PAS reaction) was present only in one case (33.4%). In all observations, there was a pronounced lymphocyte-plasmocyte infiltration; the vasculitis signs with perivascular infiltration of blast cells up to vascular wall necrosis were identified in 66.6%. Mucosa ulceration defects were seen in all cases (Fig. 3).

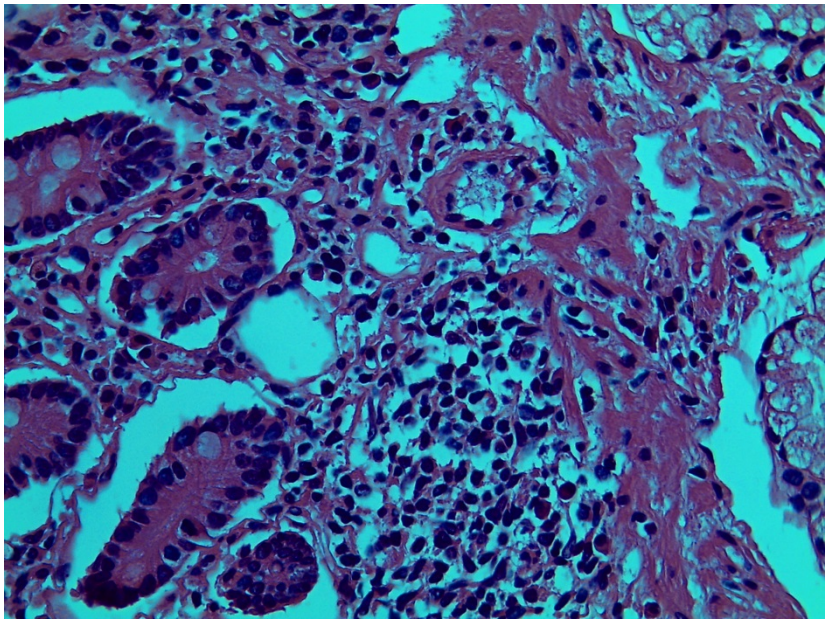

Fig. 3. Moderate cellular rejection. Large-focal infiltrate in the crypta sections of mucosal lamina propria of donor DD, hyperchromia of the arteriolar endotheliocyte nuclei (staining with hematoxylin and eosin, magnification x 400)

At identified severe ARC, the biopsy specimens displayed pronounced alterations of intestinal villi, absent goblet cells, widespread infiltration of the mucosal lamina propria with mononuclear cells, ulcerations of donor DD lamina muscularis mucosa with purulent necrotic detritus, the fibrinoid and hemorrhagic imbibition with arterial wall necrosis and venous thrombosis (Fig. 4).

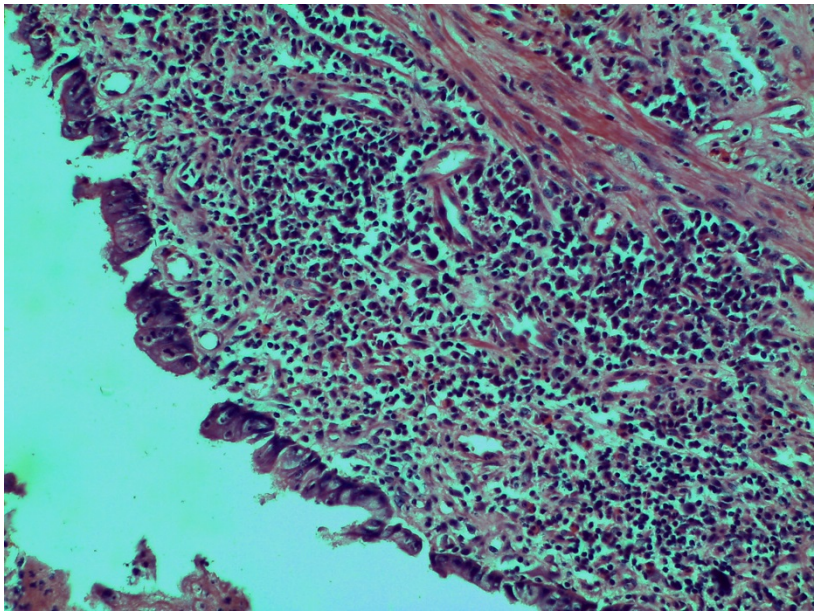

Fig. 4. Severe cellular rejection. Severe flattening and atrophy of villi, the lymphoplasmocytic infiltration that involves the donor DD lamina muscularis mucosa (staining with hematoxylin and eosin, magnification x 200)

To assess the clinical significance of DDM biopsy in the early postoperative period, we compared the results of histological examination between the main and control groups. In all patients of the main group (n = 6), the morphological signs of acute rejection were revealed alongside the clinical and laboratory signs of PDC graft dysfunction. Meanwhile, severe ARC was diagnosed in one case (16.7%), moderate ARC in one (16.7%), and mild ARC was diagnosed in 4 cases (66.6%) (Fig. 5). In the patients without clinical signs of graft dysfunction in the comparison group (n = 7), morphological studies revealed the development of only a mild rejection reaction in 3 cases (42.9%). Differences between groups were significant ($p < 0.05$).

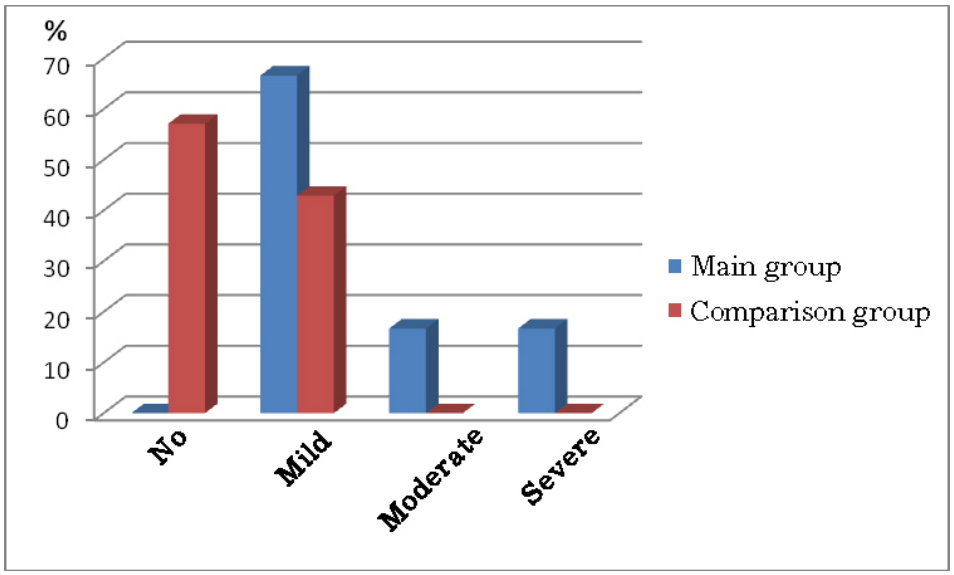

Fig. 5. Distribution of acute rejection crisis by its severity in pancreatoduodenal complex recipients in the early period after transplantation

The treatment of rejection in the recipients of the main group included the polyclonal antibodies administered in combination with plasmapheresis sessions in one case (16.7%), methylprednisolone (1500 mg) pulse therapy in 3 cases (50%), and the correction of immunosuppressive therapy in 2 cases (33.3%). Only in one case (16.7%) of severe ARC development, the anti-crisis therapy appeared ineffective, the transplanted organs were removed on the 5th day after transplant surgery. In the other cases, the PG function completely recovered.

In the comparison group, the correction of immunosuppressive therapy was required in one case (14.3%) due to failed attempts to achieve the target calcineurin concentration in blood (conversion from tacrolimus to cyclosporine). Two other recipients received anti-crisis therapy for the ARC of the renal graft that was diagnosed alongside the morphological signs of

the rejection reaction in DDM. In all the cases, the transplanted PDC function remained satisfactory.

In a retrospective data analysis, we found that the patients with detected PDC ARC had the following signs of PG dysfunction at the time of taking DDM biopsy:

- Two patients had clinical signs. In both cases, a severe ARC was noted. The crisis was manifested by a severe pain syndrome in the early postoperative period in one patient, by clinical signs of acute pancreatitis (abdominal pain, nausea, vomiting) in the other patient.

- The instrumental studies most commonly detected abnormal characteristics of intraorgan blood flow in PG with increased resistance indices in 4 cases (22.2%).

- Increased levels of the following laboratory parameters were observed: blood total amylase and pancreatic amylase in 7 patients (38.9%), blood lipase in 8 (44.4%), blood sugar in 5 (27.8%). In one case diagnosed with severe PDC ARC, significantly decreased levels of blood amylase and lipase were detected

Summary

PG rejection development can be suspected based on the data obtained in various ways. These can be clinical presentation data, laboratory results, or instrumental test results. However, the diagnosis can be confirmed or excluded only after obtaining the histology results of the PDC tissue biopsy. Given the high risk of PG puncture biopsy complications, the most appropriate and the least risky method is the donor duodenum biopsy at esophagogastroduodenoscopy. Performance of such donor DDM biopsy

became possible thanks to the practical implementation of PDC transplantation technique with creating interduodenal anastomosis.

This method of identifying immunologic complications might be associated with overestimated diagnosis of pancreatoduodenal complex acute rejection because the duodenum is rich in lymphoid tissue and therefore appears to be more immunogenic *than* the pancreas. Therefore, even if a pancreatoduodenal complex rejection is found to be mild, but without clinical manifestations, there is no need to prescribe anti-crisis therapy, and the correction of the baseline immunosuppression is quite sufficient.

Conclusions

Donor DDM biopsy serves as an important criterion in the diagnosis of an acute rejection crisis the pancreatoduodenal complex, while it is a safe invasive diagnostic method, even in the earliest postoperative period.

1. Donor DDM biopsy should be included in the routine examination of the patients who underwent pancreas transplantation with creation of duodeno-duodenoal anastomosis, both in the early and late postoperative periods.

Conflict of interests. Authors declare no conflict of interest.

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