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PECULIARITIES OF THE MORPHOLOGY OF LIVER BIOPSY SAMPLES OF DONORS ABOVE 60 YEARS OF AGE

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Objective: to study the differences in the frequency of pathological processes in liver biopsy samples of donors older than 60 years (group 1) and donors currently recognized as "standard" by age – 60 years and younger (group 2). **Material and methods.** Of the total pool of 300 consecutive donors with brain death, there were 28 (9.3%) donors over 60 years old (61 to 73 years old; 19 men and 9 women). **Results.** The frequency of pathology is independent of gender in both groups ($p > 0.05$). In elderly donors, compared with "standard" donors, mild ($p < 0.05$) and significantly more often severe ($p < 0.05$) albuminous degeneration are significantly less frequent, and there is only a tendency ($p > 0.05$) to more frequent mild hepatic steatosis. Dystrophic processes are the result of more severe ischemic injury to the liver of elderly donors. Ischemic liver injury determines the risk of more frequent biliary complications, which require careful monitoring and maintenance at an optimal level of hemodynamics for donors in the intensive care unit. Based on other morphological parameters, the liver of donors above 60 years of age does not significantly differ ($p > 0.05$) from the liver of donors 60 years and younger. **Conclusion.** To expand the donor pool, age restrictions should be removed when selecting a liver for transplantation, thereby maximizing the use of donor potential.

Keywords: elderly liver donors, "standard" liver donors, albuminous degeneration of hepatocytes, fatty hepatosis, liver fibrosis.

Liver transplantation (LT) is the only effective treatment for end-stage acute or chronic liver disease. However, there is worldwide shortage of organs to perform these surgical procedures. Many waitlisted patients do not live up to LT. That is why medical researchers are now searching for additional ways of increasing the pool of donors. Transplantation of a part of the liver (transplantation of a part of the liver of a living donor and transplantation of a split liver) is helping to overcome donor organ shortage to a certain extent [1]. Besides, using organs from expanded criteria donors is one of the ways of increasing the number of LT. An expanded criteria donor is any deceased donor over the age of 60, a donor with hepatitis C virus, a donor whose cold ischemia has lasted for over 12 hours, a non-heart-beating donor, or a donor with hypernatremia, macrovesicular steatosis >30%. In recent years, the most discussed issues include the wisdom of using elderly donors for liver transplantation [2].

Pre-transplant histopathological evaluation is known to be an effective, accurate and reliable tool for assessing the quality of liver retrieved from deceased donors. Pre-transplant biopsies are critical in selecting donor livers for transplantation, especially in cases of expanded criteria donor. The biopsies should be performed more often to avoid unnecessary loss of organs suitable for transplantation and prevent transplantation of inappropriate organs [3].

The objective of this study is to examine differences in the incidence of pathological processes in liver biopsy specimens from donors over the age of 60 (group 1) and among donors currently recognized as "standard" in age – 60 years and below (group 2).

MATERIALS AND METHODS

Donor maps were analyzed. Histological evaluation of incision liver biopsy samples of 300 consecutive donors with brain death was performed. Biopsies were carried out prior to cold storage of liver.

Biopsy specimens of donor liver were fixed in 10% neutral-buffered formalin at pH 6.8–7. They were dehydrated in alcohols of an ascending strength and poured into paraffin blocks. Sections (4–5 μm thick) were prepared on a Leica RM 2145 microtome. After dewaxing, the histological sections were stained with hematoxylin and eosin, as well as with Masson's trichrome. Histological preparations were studied using a Leica DM6000 B microscope. Level of albuminous degeneration of hepatocytes, fatty hepatosis, liver fibrosis, and liver inflammation were evaluated.

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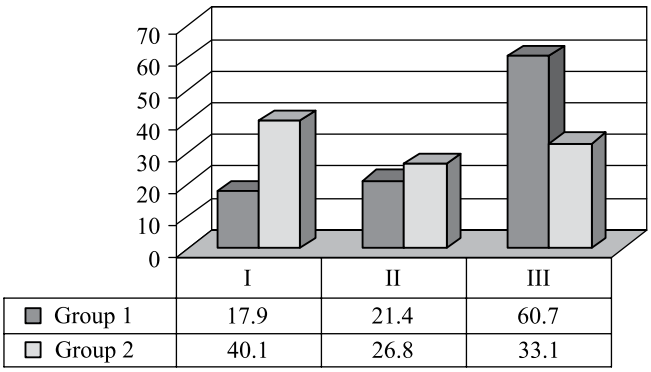
Results were statistically processed using software package Statistica 7.0 and Excel spreadsheets. The results were processed using student's t-test and chi-square test. Significance of differences was taken at $p \leq 0.05$.

RESULTS

In our study, out of the total pool of consecutive 300 brain dead donors, there were 28 (9.3%) donors over 60 years old (19 men and 9 women), aged 61 to 73 years (group 1). The control group (group 2) included 272 donors (196 men and 76 women) aged 18 to 60 years. In both groups, no relationship between incidence of pathological changes and gender was found ($p > 0.05$).

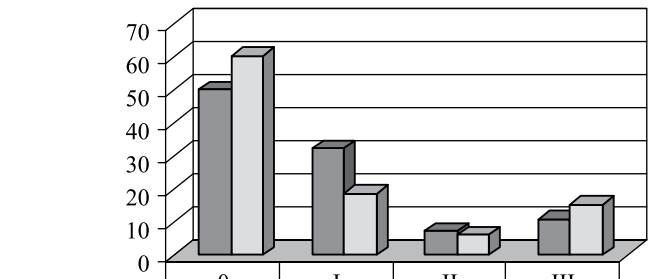
Severity of ischemic liver injury was evaluated by the level of albuminous degeneration of hepatocytes in the elderly (group 1, n = 28) and in the "standard" (group 2, n = 272) donors (Fig. 1).

In mild degree (1), predominantly granular dystrophy was observed in hepatocytes. Only small areas of hydropic degeneration were found in the liver lobules. This degree of potentially reversible albuminous degeneration was found in five (17.9%) biopsy specimens of elderly donors and 109 (40.1%) biopsy specimens of "standard" donors. That is, slight degree of liver injury was significantly more common ($p < 0.05$) in group 2 donors. At a moderate degree (2), hepatocytes had predominantly hydropic degeneration, but there were also small ballooning degeneration foci (group 1: n = 6; 21.4% and group 2: n = 73; 26.8%). The frequency of moderate degree (group 2) of albuminous degeneration in both groups did not significantly differ ($p > 0.05$). In severe degree (3), hydropic and ballooning degeneration were common in equal measure in all hepatocytes (group 1: n = 17; 60.7% and group 2: n = 90; 33.1%). Such severe degree of albuminous degeneration was significantly more likely ($p < 0.05$) observed in group 1 donors. Thus, in comparison with "standard" donors, age-related donors are reliably less likely to have mild degree (1), but more likely to have severe degree (3) of albuminous degeneration in the liver.



	I	II	III
Group 1	17.9	21.4	60.7
Group 2	40.1	26.8	33.1

Fig. 1. Comparative frequency of various degrees of albuminous degeneration in liver biopsy specimens of elderly (group 1) and "standard" (group 2) donors



	0	I	II	III
Group 1	50	32.1	7.1	10.7
Group 2	60.3	18.5	5.9	15.1

Fig. 2. Comparative frequency of various degrees of fatty hepatosis in liver biopsy specimens of elderly (group 1) and "standard" (group 2) donors

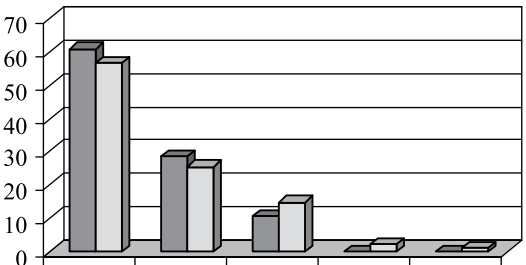
Based on the generally accepted classification, we divided fatty hepatosis into four degrees. Zero (0) degree – fatty hepatosis is absent in the absence or presence of fatty degeneration of up to 5% of hepatocytes; mild (1) degree of fatty hepatosis – fatty degeneration of more than 5% but not more than 33% of hepatocytes with fatty degeneration; moderate (2) degree of fatty hepatosis – fatty degeneration of more than 33% but not more than 66% of hepatocytes with fatty degeneration. Severe (3) degree of fatty hepatosis – more than 66% of hepatocytes with fatty degeneration.

Figure 2 shows the comparative incidence of various degrees of fatty hepatosis in liver biopsy specimens of age-related (group 1) and "standard" (group 2) donors.

Fatty hepatosis was less common in group 1 donors (n = 14; 50.0%) than in group 2 (n = 164; 60.3%). In contrast, mild and moderate fatty hepatosis were more common in age-related donors (degree 1: n = 9; 32.1%; degree 2: n = 2; 7.1%) and not in "standard" donors (degree 1: n = 51; 18.5%. degree 2: n = 16; 5.9%). Severe fatty hepatosis was more common in the young (n = 41; 15.1%) and not in the elderly (n = 3; 10.7%) donor group. However, all these differences were not significant ($p > 0.05$).

Extent of fibrosis was staged according to the METAVIR scoring system: F0 (no fibrosis), F1 (portal fibrosis without septa), F2 (portal fibrosis with few septa), F3 (numerous septa without cirrhosis), and F4 (cirrhosis).

Figure 3 shows the comparative frequency of various degrees of fibrosis in liver biopsies in age-related (group 1) and "standard" (group 2) donors. Liver fibrosis was absent in more than half of the observations, both in the age-related (n = 17; 60.7%) and in the "standard" (n = 154; 56.6%) donors. F1 fibrosis was found in 8 (28.6%) and 69 (25.4%) biopsies in group 1 and group 2, respectively. F2 fibrosis was detected in 3 (10.7%) and 40 (14.7%) biopsies, respectively. Severe fibrosis (F3 and F4) were absent in elderly donors and were rare in "standard" donors (F3: n = 6; 2.2%. F4: n = 3; 1.1%).



	F0	F1	F2	F3	F4
Group 1	60.7	28.6	10.7	0	0
Group 2	56.6	25.4	14.7	2.2	1.1

Fig. 3. Comparative frequency of varying degrees of fibrosis in liver biopsy specimens of elderly (group 1) and "standard" donors (group 2)

Differences in the absence or presence of fibrosis in the groups are not significant ($p > 0.05$).

DISCUSSION

A retrospective study of biopsies of 300 consecutive donor livers showed that mild albuminous degeneration of hepatocytes (conditional "norm") in the liver of age-related donors is less common ($p < 0.05$), while severe albuminous degeneration of hepatocytes is significantly more common ($p < 0.05$) than in the liver of "standard" donors. The same trend was observed with respect to mild fatty hepatosis, although these differences were not significant ($p > 0.05$).

We believe that symptoms of both albuminous degeneration and fatty hepatosis are a manifestation of ischemic liver injury. They are associated with unstable hemodynamics in age-related donors, in whom cardiovascular pathology is typically more common and more severe than in young donors [4, 5]. A more severe ischemic injury to the liver of elderly donors is one of the main causes of more common biliary complications [6–8], although graft and patient survival rates do not differ significantly [6–10]. Therefore, the main task involved in improving outcomes of liver transplantation from elderly donors is to reduce ischemic injury to the donor liver. One may agree with the opinion expressed by Ghinolfi et al. [11] that *ex situ* normothermic machine perfusion (NMP) might perhaps be one of the future ways, which might minimize ischemia-reperfusion injury to liver grafts. The authors examined 10 primary liver transplantation recipients of older grafts (≥70 years) after NMP. Patients with similar characteristics served as control, but liver graft was used after cold storage. Electron microscopy showed decreased mitochondrial volume density and steatosis and increased volume density of autophagic vacuoles in the liver at the end of transplantation in NMP versus cold storage patients ($p < 0.001$). Thus, histological evidence showed that the use of *ex situ* NMP with older liver grafts (≥70 years) reduced ischemia-reperfusion injury. However, the clinical benefit of this method remains to be demonstrated.

In our study, there were no statistically significant differences in the incidence of various degrees of fatty hepatosis and fibrosis depending on the age of donors ($p > 0.05$). There are reports showing evidence that with liver graft fibrosis after transplantation, fibrosis does not progress, it even regresses. So, after LT (101 patients) with mild to moderate fibrosis (F1 and F2), the degree of the condition did not progress in 40% of patients; decreased fibrosis was observed in 30%. When observed for an average of 71 months, 63 patients (63%) maintained transplant function, six (6%) had liver transplantation, while 35 patients died. Graft survival was 82% and 69% after one year and five years, respectively. It was found that differences in graft survival are not statistically significant depending on the extent of liver fibrosis: five-year graft survival (73% for F1 and 62% for F2, $p = 0.24$). In addition, a group of recipients of liver graft with fibrosis was compared with control group of 208 consecutive patients who received liver graft without fibrosis. The 5-year graft survival rate did not significantly differ between the groups (69% in the liver fibrosis group versus 75% in the non-fibrosis group, $p = 0.19$). The 5-year survival rate of patients also did not statistically differ between the groups (73% survival in the liver fibrosis group versus 79% in the non-fibrosis group, $p = 0.2$). In patients with HCV, differences in 5-year graft survival rate were also not statistically significant: 60% in the fibrosis group versus 70% in the non-fibrosis group ($p = 0.22$). This study demonstrated that allografts with mild to moderate degrees of fibrosis achieve acceptable long-term survival after liver transplantation [12].

So, our study showed that, with exception of more severe ischemic liver injury in elderly donors, there are no significant differences in the histology of the liver of elderly and "standard" donors. In the absence of liver diseases, it retains its biological youthfulness, unlike the heart and kidney, regardless of the chronological age of the donor. In this regard, geriatrics data on heterogeneity of the aging process are also of great interest – the higher the individual's chronological age, the less he corresponds to his biological age. Geriatric-like measures can be used to select livers of chronologically old, but biologically young donors [13]. The use of chronologically old, but biologically young liver donors will expand the donor pool and reduce waitlist mortality [14].

CONCLUSION

When compared with "standard" (< 60 years) donors, older donors (≥60 years of age) are significantly less likely to have mild ($p < 0.05$) and significantly more likely to have severe ($p < 0.05$) albuminous degeneration. There is only a tendency ($p > 0.05$) towards more common mild fatty degeneration. Degenerative processes result from more severe ischemic injury to the liver of elderly donors

that are known to have more common and more severe cardiovascular pathology. Ischemic liver injury leads to more frequent biliary complications. In order to reduce the severity of ischemic liver injury in elderly donors and the risk of biliary complications, careful monitoring and maintenance of optimal hemodynamics in donors in the intensive care unit is required. Based on other histological parameters, the liver of donors >60 years of age, in the absence of liver congenital or acquired disorders, does not significantly differ ($p > 0.05$) from the liver of donors ≤60 years. To expand the donor pool, age restrictions should be removed when selecting a liver for transplantation. This would maximize the use of donor potential.

The authors declare no conflict of interest.

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