Pancreas atrophy and islet amyloid deposition in patients with elderly onset type 2 diabetes（高齢発症2型糖尿病では膵臓の萎縮と膵島アミロイド萎縮が特徴的である）

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Abstract

Context: With prolonged life-expectancy, we often encounter patients with elderly onset type 2 diabetes (eT2DM). Although clinical features of eT2DM are suggested to be different from those in patients with middle-aged onset type 2 diabetes (mT2DM), islet pathology in eT2DM has not been addressed.

Objective: We attempted to characterize the pancreatic pathology in eT2DM and sought for its clinical implication.

Methods: Pancreata from 13 young non-diabetic (20-29 y.o.), 27 patients with mT2DM (45-87 y.o.), 22 middle-aged subjects without diabetes, 15 subjects with eT2DM (85-100 y.o.), and 30 elderly subjects without diabetes were investigated. Together with conventional microscopic observations, morphometric analysis on the islet, islet endocrine cells and amyloid deposition was conducted on immunostained sections.

Results: Estimated age of diabetes onset was 80.8±1.4 (mean±SE) y.o. in eT2DM, whereas that of mT2DM was 48.3±2.4 y.o. Body mass index (BMI) was 21.4 in eT2DM and 22.8 in mT2DM, respectively. Pancreas weight was nearly 50% less in eT2DM compared to those in other groups, showing duct obstruction with epithelial hyperplasia, marked acinar atrophy, fibrosis and amyloid deposition in the islet. Islet mass was significantly reduced in eT2DM, but not in mT2DM. In contrast, β-cell volume density

(V_β) was reduced in mT2DM and eT2DM to similar extent. Amyloid volume density correlated inversely with V_β, but not with BMI in eT2DM. Laboratory data showed mild elevation of serum amylase in eT2DM although clinical signs and symptoms of pancreatitis were not apparent.

Conclusion: eT2DM was distinct from mT2DM characterized by pancreas atrophy, ductal lesions and amyloid deposition. (250 words)

Introduction

Recent increase in elderly people raised a great concern on socioeconomic burden in developed countries. WHO defines “the aged society” as a society in which the population of people over 65 y.o. exceeds 7% (1). Japan is a country of super-aged society composed more than 25% of people at 65 y.o. or older (2). Super-aged society itself suffers from high frequencies in a variety of age-related diseases with reduced population of active workers.

Increase in the aged population is directly linked to the increased prevalence of type 2 diabetes (T2DM) (3). One third of the individuals over 70 y.o. are estimated to have more or less signs of diabetes (4). Diabetes in the elderly is conventionally divided into 2 types depending on the time of diabetes onset: middle-aged onset type 2 diabetes (mT2DM) with long survival, and elderly onset type 2 diabetes (eT2DM) (3). It has been proposed that clinical management of elderly diabetic patients should be considered separately from mT2DM because eT2DM is susceptible to hypoglycemia (5) while mT2DM suffers more commonly from retinopathy (6). While pathological changes of the pancreas in mT2DM are characterized mainly by reduced β-cell area and increased α-cell area (7-11), islet pathology in eT2DM has not specifically been addressed.

For the appropriate care and treatment, it would be important to characterize

the islet pathology in eT2DM. Exploration of the pathological changes in the pancreas in eT2DM possibly enables us not only to better understand the underlying pathophysiology for senile patients with diabetes, but to determine the ideal therapeutic guideline.

Materials and Methods

Subjects

In this study, we collected pancreatic tissues from 13 young non-diabetic (yND) (25.8±0.9 y.o.)(mean±SE), 27 middle-aged onset diabetic (mT2DM) (63.2±2.3 y.o. ; onset of DM 48.3±2.3 y.o.) in which there were 4 patients with long-survival whose average age of death were comparable to those in eT2DM (85.5±0.5 y.o. ; onset of DM 56.0±2.7 y.o.), 22 middle-aged non-diabetic control (mND) (61.3±1.4 y.o.), 15 elderly onset diabetic (eT2DM) (88.0±0.6 y.o. ; onset of DM 80.8±1.4 y.o.), and 30 super-elderly non-diabetic subjects (eND) (89.5±0.7 y.o.) (Table 1). The investigated cases were limited to fresh autopsy cases conducted within 5 hours after death. Cases with other diseases or a history of medications which might influence the data were excluded from the evaluation. The pancreas weight was measured after fine dissection of the organ from duodenum or other surrounding tissues. Adipose tissues which rimmed the pancreas,

surrounding the pancreas parenchyma, were determined to be the border and extra fat was trimmed off and then weighed. Clinical records were extracted in each case as to the last evaluation of body weight and height for body mass index (BMI), diabetes duration, treatment for diabetes, age of diabetes onset which were estimated from the time of diagnosis (Supplemental Table 1). Cases with diabetes had a history of hyperglycemia which fulfilled the criteria of diabetes proposed by the Japan Diabetes Society (12). Diagnosis of T2DM in diabetic groups was further confirmed by the clinical record of the patients. Cases were excluded, if 1) potential secondary causes of diabetes were present, 2) patients had been exposed to chronic glucocorticoid treatment, or 3) pancreatic tissue had undergone marked autolysis or showed evidence of striking pancreatitis. Cases with other disorders which might have influenced the data were also excluded from the evaluation. Subjects for age matched non-diabetic control fulfilled the criteria of "normal type" without previous and family history of diabetes.

At autopsy, following the dissection of the pancreas and measurement of weight, they were immersed in 10% buffered formalin for 48-72 hours and then dissected into each two portion of the head, body and tail.

The use of paraffin blocks and the study design were approved by the ethics

committee of the Hirosaki University School of Medicine (approval number #2013-235), and the study conforms to the provision of the Declaration of Helsinki.

Pathological evaluation

From the paraffin blocks, several consecutive 4-μm thick sections were obtained and stained with conventional hematoxylin-eosin (HE). Since the pancreatic body area preserved most consistently the structural integrity, the data from the body represented the values in individuals in this study, as conducted previously described (7, 9). Survey of pathological changes in the pancreas in mT2DM and eT2DM disclosed lesions in the islets and exocrine pancreas, the latter of which was represented by (1) stromal inflammatory changes, (2) atrophic changes of acinar tissues, (3) interstitial fibrosis of lobules, (4) fatty infiltration into the lobules and interlobular areas, (5) arteriosclerotic changes of nutrient arteries, and (6) ductal lesions. Azan Mallory's trichrome stains were applied to evaluate the extent of stromal fibrosis. Duct lesions were represented by obstruction or dilatation with/without epithelial hyperplasia (often transformed into dysplastic changes). Vascular tissues showed atherosclerotic and hyalinous changes with narrowing the lumen. The changes of the above 6 factors were semi-quantitatively graded as none

(Grade 0) as no marked pathological changes identified, mild (Grade 1) as focal inflammatory cell infiltration, focal acinar cell atrophy showing flattening, ductulization, slight interstitial fibrosis, mild fatty changes only in peripheral area of lobules, mild medio-intimal thickening of arteries, and slight narrowing or dilation of duct with focal epithelial hyperplasia, moderate (Grade 2) as scattered areas of stromal infiltration of inflammatory cells, scattered foci of acinar cell atrophy, interstitial fibrosis infiltrating into lobules, fatty changes in the peripheral and focal intralobular acini, moderate atherosclerotic arteriosclerosis, and narrowing duct or moderate dilatation with foci of papillary epithelial hyperplasia, and severe (Grade 3) as diffuse but modest infiltration of inflammatory cells, diffuse acinar cell atrophy, extensive intralobular fibrosis, fatty infiltration into diffuse intralobular acini, marked atherosclerosis, and obstruction or wide dilatation of duct with marked hyperplasia/dysplasia. Each pathological item was scored from 0, 1, 2, and 3 in individual subjects and average score was obtained in each group. The scoring was conducted by two pathologists in blind fashion (H.M. and S.Y.). Among 107 cases with 6 factors (total 642 subjects), disagreement for grading was found in 7 cases (6.5%) for inflammation, 9 cases (8.4%) for acinar atrophy, 11 cases (10.2%) for fibrosis, 15 cases (14.0%) for fatty infiltration, 12 cases (11.2%) for atherosclerosis, and 8 cases (7.5%) for

ductal lesion. Group comparison for each pathological feature was made deleting the cases with discordant decision, although the differences of 2 observers were rather minor as values being 1 or 2. The scores for other measures in these cases were used for group comparison.

Morphometry of islet and detection of amyloid

For the determination of islet area, the sections were incubated with monoclonal antibody to chromogranin A (1:1000) (Dako Cytomation, Glostrup, Denmark) overnight, followed by incubation with alkaline phosphatase-labeled rabbit anti-mouse immunoglobulin (1:1000, Dako). The reaction products were colorized with Vulcan Fast red chromogen (Biocare Medical, LLC, Concord, CA, USA) and examined by fluorescent microscope. Fractional islet area relative to total pancreatic parenchymal area (including endocrine and exocrine pancreas and stroma) defined as islet volume density (V_i) was measured by point-counting method on at least 1,000 islets in each subject using Image J software (Version 1.56, National Institute of Health, Bethesda, MD, USA) as described previously (7, 9). Total islet mass (M_i) was obtained by multiplication of V_i by pancreas weight. Number of the islet (density of islet) per unit area was obtained by counting the

number of chromogranin A-positive area. We separately counted the number of small-sized islets composed of 3 cells or less, as so-called neogenic islets (13, 14), regardless of their location whether they were near the duct or within the acinar tissues (13, 14). Density of the neogenic islet per unit parenchymal area and the rate of neogenic islets among total islet number were also calculated in each subject. The average size of the islet was obtained by division of the total islet area by the number of islet.

For the identification and measurement of amyloid area, we conducted thioflavin-T staining (Wako Pure Chemicals, Osaka, Japan), which showed positive green fluorescence on fluorescent microscopy (Axio-Imager M1, Carl Zeiss, Tokyo). Amyloid-positive areas were measured by point-counting method on at least 100 islets in each individual and expressed as percent area of pancreatic parenchyma, yielding the values of amyloid volume density relative to the area of pancreas parenchyma (V_{amy}). Values of pancreatic amyloid mass were then obtained by multiplication of V_{amy} by pancreatic weight in each case and mean values were calculated in each group.

Morphometric analysis of islet endocrine cells

To characterize the composition of islet endocrine cells in each case, we conducted simultaneous immunostaining of four endocrine hormones; insulin, glucagon, somatostatin and pancreatic polypeptide (PP). Antibodies for these hormones were purchased from Santa Cruz Biotech. Inc. (Santa Cruz, CA, USA) for insulin, Dako (Glostrup, Denmark) for glucagon and somatostatin, and Immunobiological Lab., Ltd. (Gunma, Japan) for PP. The staining procedure was explained elsewhere (9). Thereafter, the sections were lightly counterstained with hematoxylin. For determination of the fractional ß-, α-, δ- and PP-cell area relative to pancreatic parenchymal area (V_β, V_α, V_δ, and V_{PP}, respectively), the pancreatic sections were imaged at ×40 magnification. V_β, V_α, V_δ and V_{PP} were quantified at point count basis using Image J (9, 15). At least, 100 islets were examined in each case. When the value of pancreas weight was available, the masses of islet (M_i), ß(M_β)-, α(M_α)-, δ(M_δ)- and PP-cell (M_{PP}) were obtained by multiplication of V_β, V_α, V_δ and V_{PP} by pancreas weight.

Proliferation activity and apoptosis of β-cells

To estimate the proliferating activity of β-cells, we conducted simultaneous immunostaining using antibodies to islet endocrine cells and Ki67 (MIB-1, Dako Cytomation) as previously reported (9, 15, 16). Cells double-positive for each hormone and Ki67 (among over 500 endocrine cells) were counted in each subject and mean value was calculated in each group.

To explore the cell death, or viability of β-cells, we adopted double immunostainings of anti-insulin with terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) of an ApopTag® (Millipore, Bellerica, MA, USA) as previously described (9, 16).

Statistical analysis

Data are presented as mean ± standard error (SE). Statistical comparisons of the mean values among the groups were made by analysis of variance (ANOVA) with post-hoc Bonferroni corrections (StatView, Version 5.0.1, MountainView, CA, USA). A simple regression was carried out for the correlation analysis. The Wilcoxon rank sum test was performed to compare endocrine cell replication between groups due to skewed distributions of the observations. P values of <0.05 were taken as

statistically significant

Results

Clinical data

Major clinical profiles of investigated cases were summarized in Table 1. Detailed clinical data in each case were described in Supplemental Table 1. Average BMI was comparable among all groups with values from 20 to 23. Age of diabetes onset was 48.3±2.4 y.o. in mT2DM whereas that in eT2DM was 80.8±1.4 y.o. ($p<0.01$ vs mT2DM). Duration of diabetes ranged wide in mT2DM (1~35 years) and the average was longer than that in eT2DM. No significant difference was detected, however, in HbA1c values (NGSP) between mT2DM and eT2DM. Pancreatic weight was markedly decreased in eT2DM by nearly half (70.4±4.3 g) compared to other groups ($p<0.01$ vs for all).

Pathological features of eT2DM

Screening for pancreatic histology disclosed that features of yND and mND were more or less similar except for arteriosclerotic changes in mND (Figure 1). Fat deposition in the pancreas was robust in mT2DM and eT2DM, but much less in their

non-diabetic counterparts (Figure 1). In contrast, eT2DM showed marked acinar cell atrophy of the pancreatic parenchyma and interstitial fibrosis. Such atrophy and fibrosis were modest in mT2DM. Notably, ductal dilatation or obstruction with epithelial hyperplasia/dysplasia was most conspicuous only in eT2DM (Figure 1). The ductal obstruction or dilatation was not apparent in mT2DM. In addition, stromal inflammatory cell infiltration was not marked in any group. Semi-quantitative evaluation of the above pathological changes demonstrated that extra-islet pathology in eT2DM was characterized by acinar cell atrophy, stromal fibrosis, atherosclerosis and obstructive ductal lesions with epithelial hyperplasia/dysplasia (Figure 1).

Morphometric analysis on islets and islet endocrine cells

There was a significant increase in the density of total islets in eT2DM compared to mT2DM and eND ($p<0.01$ for both) which showed a greater islet density than that in mND ($p<0.01$) (Table 2). Average size of islet was smaller in mT2DM and eT2DM than their respective non-diabetic counterpart ($p<0.05$ for both). Density of neogenic islets was increased in mT2DM and eT2DM compared to their age-matched non-diabetic groups ($p<0.05$ and $p<0.01$, respectively) (Table 2). The latter

was further greater than the former ($p<0.05$). The rate of neogenic islets among total islets was more than a half in non-diabetic groups and significantly increased in mT2DM compared to mND ($p<0.01$). In contrast, there was no increase in the population of neogenic islets in eT2DM which was significantly reduced compared to that in mT2DM ($p<0.01$).

There was no significant difference in V_i among all the groups with a considerable overlap, although there was a trend to a decrease in mT2DM and eT2DM compared to non-diabetic groups **(Figure 2)**. Consistent with the previous results **(7, 9)**, there was approximately 30% reduction of V_β in mT2DM compared to mND. Reduction of V_β in eT2DM was similar to that observed in mT2DM. V_α was increased (about 15%) in mT2DM compared to mND ($p<0.05$), but it was reduced in eT2DM compared to eND. There was no significant difference in V_δ or V_{PP} among all the groups.

There was a significant reduction of M_i in eT2DM compared to eND while it was not significantly altered in mT2DM. Consistent with V_β, M_β was significantly reduced in mT2DM and eT2DM compared to their respective non-diabetic controls. In contrast, M_α was not significantly increased in eT2DM compared to eND while it was increased in mT2DM. M_δ was not altered in mT2DM, but significantly decreased

in eT2DM.

Islet amyloid

Islet amyloid deposition was marked in most cases of eT2DM (V_{amy} 0.35±0.10%) **(Figure 3A)**, the extent of amyloid area was much exceeded the level detected in mT2DM (V_{amy} 0.06±0.03%), while it was hardly visible in yND or mND **(Figure 3A)**. eND also showed small amount of amyloid deposition, but V_{amy} (0.02±0.01%) was much less than that in eT2DM. Similarly, M_{amy} was most marked in eT2DM which was 3.4 fold of mT2DM **(Figure 3B)**. There was an inverse correlation between V_{β} and V_{amy} ($r=0.52$, $p<0.05$) but there was no significant correlation between V_{amy} and BMI ($r=0.26$) **(Figure 3C, D)**.

Proliferating activity and apoptotic cells

There was no significant difference in the population of islet endocrine cells positive for Ki67 (around 0.16~0.19%) between diabetic and non-diabetic groups **(Table 2)**. Although there were some acinar cells positive for TUNEL, no apparent positive cells were identified in the islets in any of the groups.

Discussion

In this study, we characterized for the first time distinct pathological findings in the pancreas in eT2DM. The salient features were marked atrophy of the pancreatic parenchyma, ductal obstruction or dilatation with epithelial hyperplasia and dysplasia, interstitial fibrosis, fat infiltration, reduction of V_β and M_β, and marked amyloid deposition in the islets. It has generally been accepted that pancreas atrophy is common in type 1 diabetes (17-19), whereas it is still controversial in mT2DM (20-22). We found mild fat infiltration, fibrosis and atrophy of the exocrine tissues in eND, which were compatible with the previous reports (16). The extent of such alterations was much more prominent in eT2DM. Robust alterations of exocrine pancreatic changes may be attributed to ductal lesions with distal exocrine atrophy, or ischemia due to co-existent arteriosclerosis and diabetes-related vascular dysfunction (19, 23). Serum concentrations of amylase were slightly elevated in this group, suggesting the presence of incipient pancreatic exocrine dysfunction. In cases with pancreatic diabetes, massive fibrosis and pancreas atrophy accompanied by β-cell deficits were widely acknowledged (24, 25). The main pathology of chronic pancreatitis is obstruction of the pancreatic ducts due to stone or protein plugs. In turn, focal inflammatory changes may disturb the islet function and structure,

resulting in the onset of diabetes (24). In addition, insufficient insulin secretion may exert acinar tissue atrophy because of the lack of its trophic action to the peri-insular acinar cells (18, 19). Taken together, our study suggests that there may be an exocrine component to the disease course in eT2DM, and/or that it shares some feature with pancreatic diabetes, but not that it truly resembles diabetes that occurs secondary to profound exocrine pancreatic disease.

In our previous studies, islet amyloid deposition was found in approximately 30% of Japanese patients with mT2DM (7, 15), while it was 90 % in Caucasian T2DM (26). The extent of amyloid deposition (V_{amy}) correlated well with an increase in BMI and decrease in V_{β} (15). In this study, we confirmed that V_{amy} inversely correlated with V_{β} in eT2DM. There was no significant relationship, however, between V_{amy} and BMI in eT2DM. While it was shown that V_{β} was increased in obese non-diabetic Caucasians (8, 27), this was not the case in Japanese with high BMI (16, 28). In our cohort, eT2DM was composed mostly of non-obese subjects (mean BMI 21.4). Hence, the dissociation of the relationship between V_{amy} and BMI in eT2DM may be ascribed to limited number of obese subjects or at least in part to ethnic reasons. Alternatively, the amyloid deposition in eT2DM may develop differently from that in mT2DM. Although small number of subjects in this investigation and lack of information on

the islet in Caucasian super-elderly do not allow the speculation, ischemia or age-related tissue degeneration related to insulin resistance may contribute to the process of amyloid deposition in eT2DM.

Our morphometric data demonstrated that pancreatic weight is crucial to determine the total cellular mass of the islet or each endocrine cell. In fact, while V_i of eT2DM was not different from that of eND, M_i in eT2DM was significantly smaller than that in eND. It is also of note that, in contrast to increases in V_α and M_α in mT2DM, M_α was decreased in eT2DM. The question as to whether susceptibility of eT2DM is related to hypoglycemia or hypoglucagonemia needs to be answered by future investigations. Unfortunately, information on the changes in pancreas weight is sparse in previous studies. Saisho et al. reported that pancreas weight as calculated by computed tomography (CT) images was reduced with aging in American subjects (29). In contrast, our studies on the pancreas weight measured at autopsy could not confirm such findings (16). The reason for this discrepancy is not clear but may be ascribed to different methods for the measurement. Difficulty to determine the pancreatic border in the aged-pancreas rich in fatty tissues by imaging studies may also account for such discrepancy (21, 30).

Islet endocrine cell volume density or mass is mainly dependent on the rate

of islet neogenesis and cell death (8, 9, 11). In this study, despite an increased density of neogenic islets, both V_β and M_β were significantly reduced in eT2DM. We could not identify a decreased rate of endocrine cell proliferation as determined by Ki67 staining or an increased rate of apoptotic endocrine cells by TUNEL staining. These negative results may contradict to the results from other investigators who showed a higher proliferation rate or an increased apoptosis in the islet of the diabetic pancreas (8, 10). As reported previously in our publications (7, 10, 16), we believe that this finding was not technical errors because simultaneous staining on the same slides well succeeded in detection of apoptotic cells in the lymph tissues or sparse acinar cells undergoing apoptosis, as well as Ki67-positive proliferating cells in inflammatory lymph tissues or regenerating intestinal mucosa. It is thus likely that reduced V_β and M_β in eT2DM may not be drastic processes of cell death but possibly reflect impaired replication of β-cells. In keeping with this contention, decreased rate of neogenic islets among total islets in eT2DM compared to eND or mT2DM may reflect the reduced islet cell replication although they also contain cells, not necessarily related to islet neogenesis (13, 14).

We admit that it should have been necessary to make comparison of the clinical profile and islet pathology between patients with eT2DM and critically age-

matched mT2DM with longer survival. It was not possible for us, however, to obtain sufficient number of such patients with extreme long history of diabetes because most patients with mT2DM cannot survive sufficiently long enough for the comparison with eT2DM. However, the pancreatic pathology in 4 cases with mT2DM and long-survival whose ages were matched to those in eT2DM is more or less similar to those in conventional mT2DM. Currently, average lifespan in patients with diabetes is 65~70 and 71~75 years for men and women, respectively, which were 12.8 and 12.2 years shorter than those without diabetes (31, 32). Average age of death for eT2DM was 88.5 years in this study and more than 10 years longer than the standard in developed countries. It is not known at present whether life-span of eT2DM becomes shorter after the onset of diabetes than that in eND free from diabetes. Meticulous long-term follow-up in future may be able to uncover whether the presence of diabetes influences clinical outcome in eT2DM.

There are a number of limitations for interpretation of the results in this study. Since this study is of a retrospective nature, characterization of clinical profile in eT2DM is immature and more information on the digestive functions related to exocrine pancreatic pathology must be collected. Currently, our methodology is not sufficient enough to analyze the molecules related to aging in part due to difficulty

to obtain high quality nucleic acids or proteins from human autopsy samples. Laser captured microdissection of the islets may be one solution, but high quality RNA enough to analyze gene expression from archival paraffin embedded tissues has not been achieved. Development of new techniques or accumulation of fresh samples may be expected to solve this issue in future.

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Figure legends

Figure 1

Exocrine pancreatic pathology in middle-age onset type 2 diabetes (mT2DM) and elderly onset type 2 diabetes (eT2DM) and comparison with middle-aged non-diabetic subject (mND) and elderly non-diabetic subject (eND). mND shows well preserved exocrine acinar tissues **(A)**. The wall of intrapancreatic duct is slightly thickened and columnar epithelia are regularly lined the luminal surface (arrow) **(B)**. Intrapancreatic nutrient artery appears normal (arrowhead) **(C)**. In contrast, there are intralobular fatty changes in mT2DM **(D).** In mT2DM with long survival, fatty changes are extensive to interlobular areas **(E).** Overall, the extent of fatty changes was similar in mT2DM and eTDM. Stromal nutrient artery (arrowhead) undergoes atherosclerotic changes showing thickened vascular wall **(F)**. In eND, basic lobular structure of acinar and ductal tissues is preserved though mild interstitial fibrosis is noted **(G)**. Ductal wall is slightly thickened and surrounding acinar tissues show mild stromal fibrosis **(H)**. In contrast, duct dilatation (arrowhead) and duct obstruction with intraductal epithelial hyperplasia (arrow), lobular pancreatic atrophy with shrunken acinar tissues, fibrosis and fatty changes are conspicuous in eT2DM **(I)**. High power view of ductal obstruction revealed marked epithelial hyperplasia within the duct in eT2DM **(J)**. Blood vessels show thickening of the wall (asterisk). Ductal obstruction is surrounded by marked fibrosis and atrophy in the acinar tissues. Marked interstitial fibrosis was confirmed by blue color stained with Azan Mallory trichrome stains **(K)**. Within the duct, marked papillary hyperplasia of duct epithelium with dysplastic changes is often found in eT2DM **(L)**. Stromal vascular tissues showed thickening of the wall. Semi-quantitative evaluation disclosed that ductal changes and acinar cell atrophy with fibrosis were most remarkable in eT2DM **(M)**.

Values are expressed as mean±SE. $^{*}p<0.05$ vs mND, $^{\dagger}p<0.01$ vs mND, $^{\ddagger}p<0.01$ vs mT2DM, eND, $^{\S}p<0.05$ vs yND, $^{\#}p<0.01$ vs eND. Scale represents 0.5mm.

Figure 2

Morphometric analysis on the islet endocrine cells. Endocrine cell area in each hormone was measured on the quadruplicate immunostained sections in each group **(A)**. There was no significant difference in mean values of islet volume density (Vi) among the groups, although there was a trend to decrease in middle-aged onset type 2 diabetes (mT2DM) **(B)**. In contrast, β cell volume density (V_{β}) was significantly

reduced in mT2DM and elderly-onset type 2 diabetes (eT2DM) compared to young non-diabetic subjects (yND), middle-aged non-diabetic subjects (mND) and elderly non-diabetic subjects (eND). There was a significant increase in α-cell volume density (V_α) in mT2DM, but not in eT2DM. There was no significant difference in δ-cell (Vδ) or PP-cell volume density (V_{PP}) among all the groups. On the other hand, islet cell mass (Mi) was significantly reduced only in eT2DM, while there was a significant decrease in β cell mass (M_β) in both mT2DM and eT2DM which extent was more severe than mT2DM **(C)**. α-Cell mass (Mα) was increased in mT2DM, but decreased in eT2DM. δ-Cell mass (Mδ) was decreased only in eT2DM. There was no difference in PP cell mass (M_{PP}) among the groups.
*p<0.01 vs mND, †p<0.01 vs eND, ‡p<0.05 vs mND, §p<0.05 vs mT2DM, #p<0.01 vs mT2DM. Scale represents 50 μm.

Figure 3

Analysis of islet amyloid deposition detected by thioflavin T staining and relationship to body mass index (BMI) and β-cell volume density (V_β). **(A)** Amyloid volume density (V_{amy}) was significantly increased in elderly onset type 2 diabetes (eT2DM) which exceeded the level of mT2DM. Amyloid deposition was almost nil in young non-diabetic subjects (yND) or middle-aged non-diabetic subjects (mND), but there were some elderly non-diabetic subjects (eND) who showed marked deposition. **(B)**. Amyloid mass was also significantly increased in eT2DM compared to other groups. **(C)** There was no significant correlation between V_{amy} and BMI. **(D)** There was a significant inverse correlation between V_{amy} and V_β.
*p<0.01 vs eND, †p<0.01 vs mT2DM. Scale represents 0.1mm.