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Immunohistochemical and morphometric study of the effect of maternal diabetes on rat foetal pancreatic islets

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The effect of maternal diabetes on the foetal endocrine pancreas has been the subject of extensive studies, but a detailed quantitative immunohistochemical investigation is not available. This study was therefore undertaken to investigate the effect of gestational diabetes on the morphology of foetal rat islets. Sections were stained with anti-insulin (B cells) antibodies and were used for morphometric analysis. The B cell volume density was significantly lower in the diabetic group, while islet volume density, islet diameter, islet volume and absolute islet cell numbers were significantly greater in the diabetic group. The B cell nuclear diameter and volume were not significantly different in the diabetic group. The results obtained from this investigation indicate that maternal diabetes induces foetal islet hypertrophy and causes an increase in the total islet cell number.

key words: quantitative, B cells, immunohistochemical

INTRODUCTION

It is believed that hyperglycaemia induces B cell proliferation and causes an increase in the total B cell mass. Glucose has a stimulatory effect on B cells *in vitro* and *in vivo*, including the ability to induce proliferation and insulin secretion of islet B cells [2, 3, 9, 10, 14, 18]. In diabetic pregnant rat the foetal blood glucose level correlates with maternal level and insulin synthesis and secretion of foetal pancreatic B cells are stimulated by gestational diabetes [12, 16, 17]. Infants of diabetic mothers also show hyperplasia of islet B cells [10, 14].

Severe diabetes in pregnant rats was reported to induce hypoinsulinaemia and hyperglycaemia in their foetuses [11, 20], while mild diabetes was found to produce intrauterine foetal B cell hyperplasia, hyperinsulinaemia and increased foetal body weight [8, 15, 20]. It seems that foetal exposure to maternal diabetes has a long-lasting effect. Mild diabetes during pregnancy in rats was reported to induce a decrease in insulin secretion in later life [21].

Despite the extensive work on the effect of maternal diabetes on foetal endocrine pancreas, a detailed quantitative immunohistochemical study has not been reported. The application of immunohistochemical techniques has allowed sensitive and clear identification of islet B cells, which contain insulin granules. The accurate identification of B cell granules by immunohistochemical stain in this study would allow the performance of accurate measurement of the various morphometric parameters. This study was therefore designed to examine the effect of maternal diabetes on foetal islet volume density, diameter, cell number and B cell volume density. The effect of maternal diabetes on foetal nuclear diameter and volume was also assessed.

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MATERIAL AND METHODS

Animal and tissue preparation

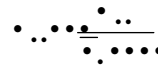
Adult male and female Lewis albino rats aged 60 d and weighing 180–250 g were used. The rats were allowed free access to food and water. They were kept at a constant temperature of 24°C and a light cycle of 12 h on/12 h off. Virgin female Lewis rats were made diabetic by intravenous injection of streptozotocin (Sigma, St Louis, Mo., USA). The dose of streptozotocin was 50 mg/kg. Non-fasting blood glucose concentrations were measured to confirm the presence of diabetes. Diabetic female rats were mated with males and pregnancy was confirmed. Rats of 19 and 21 days foetal age were used. Pregnant rats were anaesthetised by ether inhalation and foetuses were extracted from their uteruses. Blood glucose concentrations of the foetuses were measured. The pancreases were removed, fixed in buffered neutral formalin, dehydrated, embedded in paraplast (Sherwood Medical Co., St Louis, Mo., USA) and sectioned serially at 5 μm. Pancreases of 19- and 21-day-old foetal rats of non-diabetic mothers were obtained and used as controls. Six pancreases were examined per group, with a total of 24 pancreases.

Immunohistochemical staining

Four sections, 20 sections apart, were obtained from each specimen and stained by indirect immunoperoxidase method [19]. This method was used to localise the insulin-producing B cells. The primary antibody used was guinea pig anti-swine insulin serum (optimal dilution 1:500). The secondary antibody used was rabbit anti-guinea pig immunoglobulin conjugated with peroxidase (dilution 1:200). All sera and antisera were obtained from Dako Corporation, Carpinteria, CA, USA. The chromogen substrate used was 3, 3-Diaminobenzidine tetrahydrochloride (Sigma, St Louis, Mo., USA). The sections were counterstained with Harris' haematoxylin to facilitate nuclear identification.

Morphometric analysis

Eight to twelve islets were examined per section with a total of 32–48 islets per foetus. The volume density of the islets per pancreatic tissue (V_{vi}) and the volume density of B cells per islet (V_{vb}) were calculated by the point counting method of Weibel [23]. The sections were examined at a magnification of × 400 to estimate V_{vi} and at a magnification of × 1000 to estimate V_{vb}. The number of cellular profiles per test area (N_{Ac}, no./μm^2) was calculated by dividing the number of cell nuclei profiles per islet profile by the islet profile's area. The nuclei were counted by direct counting method at a magnification of × 1000. A variant of DeHoff and Rhines formula [24] was used to calculate the numerical density of the cells per unit volume of islet (N_{vc}, no./μm^3).



where N_{Ac} represents the number of nuclear profile per unit area estimated in sections of thickness t and $\bar{D}$ represents the mean corrected nuclear diameter. The absolute number of total cells per islet was estimated by multiplying N_{vc} by islet volume.

A graticule of a calibrated linear scale was used to measure the major (a) and minor (b), at a right angle to (a), axes of the islet. The islet profile diameter was calculated from the equation $d_i = \sqrt[2]{ab}$ [24]. The mean axial ratio of the islets was calculated. On the assumption that the islets are spheroid structures, the formula of Fullman [24] was used to calculate the mean islet diameter $\bar{D}$.

where N represents the total profiles measured.

The B cell nuclear profile diameter (d_n) was measured by similar steps. Fifty nuclei were measured per slide at a magnification of × 1000. The Abercrombic method [24] was used to calculate the corrected mean nuclear diameter $\bar{D}$.

The mean islet volume and the mean B cell nuclear volume were calculated from the mean corrected islet diameter $\bar{D}$ and mean corrected nuclear diameter $\bar{D}$ [24].

Statistical analysis

The data were analysed statistically by Student's t-test. All the statistical computations were made using the statistical packages SPSS and Excel. The difference was considered as significant when $p < 0.05$.

RESULTS

Maternal blood glucose level was 357 ± 50 mg/dl and foetal blood glucose level was 414 ± 5 mg/dl. The mean islet axial ratios of 19- and 21-day-old foetuses were 1.24 ± 0.03 and 1.17 ± 0.04 respectively. The mean nuclear axial ratios of 19- and 21-day-old foetuses were 1.11 ± 0.03 and 1.10 ± 0.03 respectively.

Table 1. Volume density of the islets per pancreatic tissue, volume density of B cells per islet, islet diameter, islet volume and absolute number of total cells per islet of 19-day-old control and diabetic foetuses (n = 6)

	Control group	Diabetic group
Volume density of the islets per pancreatic tissue (V_{vi})	0.0381 ± 0.004	0.0588 ± 0.003*
Volume density of B cells per islet (V_{vb})	0.376 ± 0.03	0.233 ± 0.01*
Islet diameter [μm]	41.1 ± 1.4	58.3 ± 3.1*
Islet volume [μm^3]	39261 ± 2771	108954 ± 16712*
Number of total cells/islet	119 ± 20.2	195 ± 21.5*

Values are presented as mean ± SEM; *p < 0.05, diabetic group v. control group

Table 2. Volume density of the islets per pancreatic tissue, volume density of B cells per islet, islet diameter, islet volume and absolute number of total cells per islet of 21-day-old control and diabetic foetuses (n = 6)

	Control group	Diabetic group
Volume density of the islets per pancreatic tissue (V_{vi})	0.0488 ± 0.006	0.0696 ± 0.003*
Volume density of stained B cells per islet (V_{vb})	0.525 ± 0.03	0.332 ± 0.01*
Islet diameter [μm]	64.9 ± 5.7	93.7 ± 2.3*
Islet volume [μm^3]	171240 ± 43313	437075 ± 31763*
Number of total cells/islet	281 ± 42.4	463 ± 51.5*

Values are presented as mean ± SEM; *p < 0.05, diabetic group v. control group

The mean volume density of the islets per pancreatic tissue (V_{vi}), the mean volume density of B cells per islet (V_{vb}), the mean islet diameter, the mean islet volume and the mean absolute number of total cells per islet of 19-day-old control and diabetic groups are shown in Table 1. It was observed that the mean volume density of the islets per pancreatic tissue ($V_{vi,}$), the mean islet diameter, the mean islet volume and the mean absolute number of total cells per islet of the diabetic group were significantly greater than the corresponding values of the control group ($p < 0.05$). The volume density of B cells per islet (V_{vb}) of the diabetic group was significantly lower than that of the control group ($p < 0.05$).

At day 21 of gestation, the mean volume density of the islets per pancreatic tissue ($V_{vi,}$), the mean islet diameter, the mean islet volume and the mean absolute number of total cells per islet of the diabetic group were significantly greater than the corresponding values of the control group ($p < 0.05$). The volume density of B cells per islet (V_{vb}) of the diabetic group was significantly lower than that of the control group ($p < 0.05$). Table 2 shows these mean values.

The volume density of the islets (V_{vi}), the islet diameter, the islet volume, the volume density of B cells (V_{vb}) and the absolute number of total cells per islet increased from day 19 to day 21 of gestation in control and diabetic group (Tables 1 and 2).

At 19 and 21 days of gestation, the mean B cell nuclear diameter and volume of the diabetic group did not vary significantly from the corresponding values of the control group ($p > 0.05$). These mean values are shown in Tables 3 and 4.

Table 3. B cell nuclear diameter and volume of 19-day-old control and diabetic foetuses (n = 6)

	Control group	Diabetic group
Nuclear diameter [μm]	6.76 ± 0.14	6.66 ± 0.4^{NS}
Nuclear volume [μm^3]	162 ± 10	160 ± 28.7^{NS}

Values are presented as mean ± SEM; NS: p > 0.05, diabetic group v. control group

Table 4. B cell nuclear diameter and volume of 21-day-old control and diabetic foetuses (n = 6)

	Control group	Diabetic group
Nuclear diameter [μm]	6.62 ± 0.34	7.14 ± 0.08^{NS}
Nuclear volume [μm^3]	156 ± 22.6	191 ± 6.9^{NS}

Values are presented as mean ± SEM; NS: p > 0.05, diabetic group v. control group

Numerous scattered small islets were found in the exocrine pancreas of 19- and 21-day-old diabetic foetuses (Fig. 1). At 19 and 21 days of gestation, the islets of the diabetic and control groups were nearly rounded, well defined and the B cells occupied the central part of the islet (Fig. 2–5).

DISCUSSION

The results reported here show that islet diameter and islet volume of the diabetic group at 19 and 21 days of gestation were significantly greater than the corresponding values of the control group. This may be due to hypertrophy of pancreatic islets and

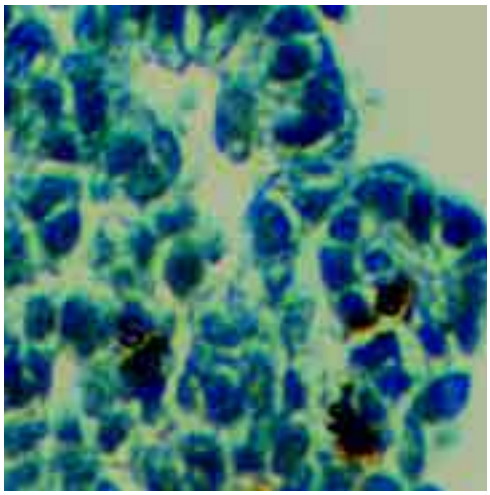


Figure 1. Light micrograph of small pancreatic islets (S) of 21-day-old diabetic foetus stained with indirect immunoperoxidase method to demonstrate insulin producing B cells. × 400.

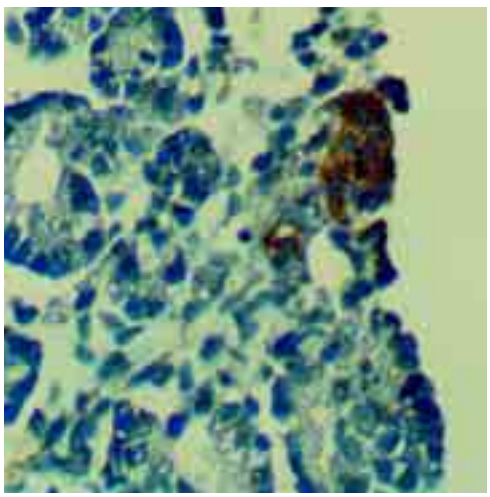


Figure 2. Light micgrograph of pancreatic islet (I) of 19-day-old control foetus stained with indirect immunoperoxidase method to demonstrate insulin producing B cells. × 200.

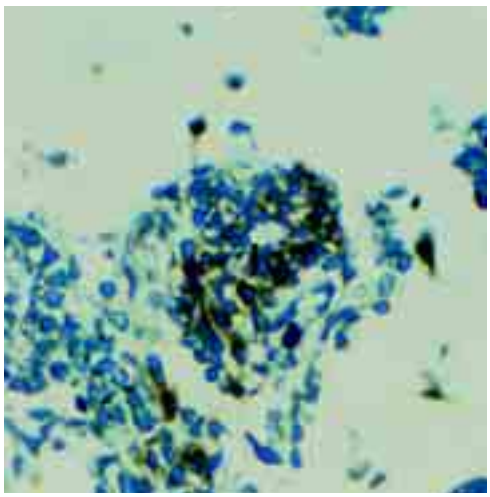


Figure 3. Light micrograph of pancreatic islet (I) of 19-day-old diabetic foetus stained with indirect immunoperoxidase method to demonstrate insulin producing B cells. × 200.

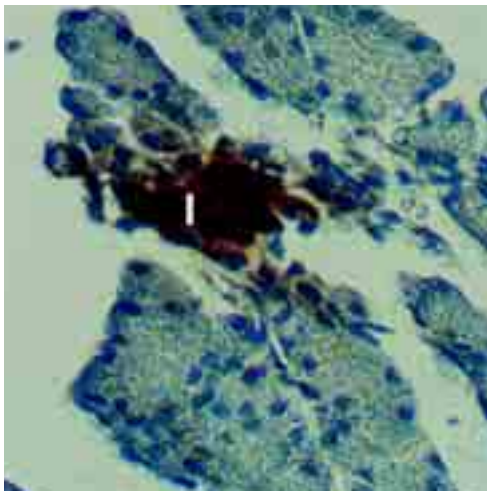


Figure 4. Light micgrograph of pancreatic islet (I) of 21-day-old control foetus stained with indirect immunoperoxidase method to demonstrate insulin producing B cells. × 200.

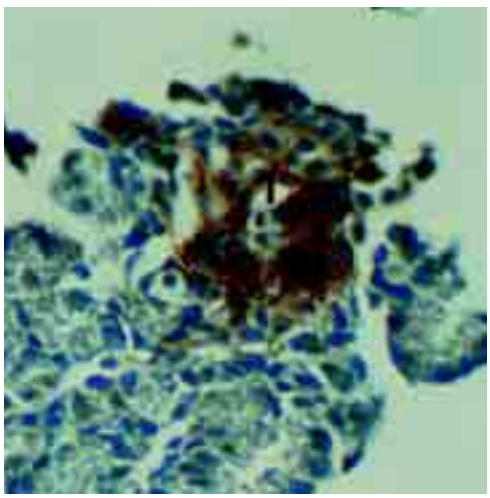


Figure 5. Light micrograph of pancreatic islet (I) of 21-day-old diabetic foetus stained with indirect immunoperoxidase method to demonstrate insulin producing B cells. × 200.

a rise in the total number of islet cells, which are caused by the stimulatory effect of hyperglycaemia present in the diabetic foetal group. This increase in B cell number may be a mechanism to increase insulin secretion in response to glucose stimulation. Previous studies reported similar findings. Aerts and Van Assche [2] reported that the endocrine pancreas of foetuses and newborn rats of diabetic mothers shows morphological and ultrastructural changes of hypertrophy and hyperplasia. Milner et al. [13] demonstrated that the addition of glucose and amino acids to the organic culture of foetal rat pancreas causes an increase in insulin granule diameter. Milner et al. [14] reported that foetal islet B, A and PP cells of diabetic mothers show hyperplasia. They suggested that foetal hyperglycaemia in early pregnancy has a trophic effect on the stem cells, which develop into B, A or PP cells. Scharfmann et al. [18] and Kaung [10] reported that glucose stimulates B cell proliferation and causes an increase in B cell population size of cultured foetal and neonatal rat pancreases. Bernard et al. [3] and Hue-Lee [9] demonstrated that glucose stimulates beta cell proliferation and causes an increase in beta cell volume in foetal culture. In the present investigation most of the parameters of the diabetic and control groups increased from day 19 to day 21 of gestation. This can be due to growth of foetal endocrine pancreas. To avoid any effect of streptozotocin on foetal pancreas the drug was injected one week before mating.

The amount of foetal endocrine tissue per total pancreatic tissue of the diabetic group, which is represented by the volume density of the islets, was significantly greater than that of the control group at 19 and 21 days of gestation. This result may suggest that hyperglycaemia stimulates the proliferation of the cells in the endocrine pancreas. It may also suggest that the endocrine pancreas of diabetic foetuses appears earlier than that of the control group. This confirms the data of previous publications [2, 5, 6]. On the other hand, the volume density of islet B cells of diabetic group at 19 and 21 days of gestation was significantly lower than that of the control group. This finding may be due to the rise of the islet volume with the unchanged B cell number. It may also be due to the degranulation of islet B cells caused by severe foetal hyperglycaemia. These findings are in agreement with those of Eriksson and Swenne [4], Verhaeghe et al. [22], Abdel-Rahman et al. [1] and Fu et al. [5].

The absolute number of total cells per islet of diabetic group was significantly greater than that of the control group. This is in agreement with the increase in islet diameter and volume found in the diabetic group.

The mean B cell nuclear diameter and volume were not significantly different in the diabetic group. It seems that maternal diabetes does not have any effect on B cell nuclear diameter or volume. Scattered small islets formed of few cells were observed in the exocrine part of diabetic foetal pancreases (Fig. 1). The presence of these islets may be due to the effect of marginal (tangential) section of the large islets. A similar finding was reported by Grasso et al. [7].

In conclusion, the results reported in this study suggest that maternal diabetes induces foetal islet hypertrophy and causes an increase in total islet cell number. They further show that gestational diabetes does not seem to affect B cell nuclear diameter or volume.

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