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MORPHOLOGY AND MORPHOMETRIC FEATURES OF THE LARGE INTESTINE IN THE OFFSPING BORN FROM MOTHERS WITH DIABETES MELLITUS (EXPERIMENTAL STUDY)

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ABSTRACT

The experiment was conducted on 30 female laboratory rats, divided into two groups: control (n=15) and experimental (n=15). In the experimental group animals, diabetes mellitus was modeled by intravenous administration of alloxan in citrate buffer once intraperitoneally at a rate of 11 mg/100 g of weight. Hyperglycemia (>15 mmol/l) was confirmed using a glucometer. After confirmation of diabetes, the females were mated with healthy males. The colon was fixed in 10% formalin solution, histologically processed and stained with hematoxylin and eosin. Morphometric analysis included measuring the thickness of the mucous, submucous and muscular layers, the height of the crypts, and counting the number of goblet cells. The results of the study showed that the offspring born to mothers with diabetes have significant changes in the structure of the colon. The thickness of the mucous layer was reduced by 18% ($p<0.05$), the muscular layer — by 12% ($p<0.05$) compared to the control group. The height of the crypts decreased by 15% ($p<0.05$), indicating a violation of the epithelial regeneration processes. The number of goblet cells in the mucous membrane was significantly less (by 22%, $p<0.01$), which may indicate a decrease in the secretory function. Histological analysis revealed signs of disorganization of the epithelial layer, an increase in the intercellular space and a decrease in the density of cellular elements. Discussion of the results suggests that the identified changes are associated with the negative effect of hyperglycemia on the processes of proliferation and differentiation of cells during intrauterine development. A decrease in the thickness of the colon wall layers and a decrease in the number of goblet cells may be due to a violation of the synthesis of proteins and lipids, as well as a change in the expression of genes regulating histogenesis. These morphological changes may underlie functional disorders such as decreased absorption capacity, impaired motility, and increased risk of developing inflammatory processes in the colon.

Key words: diabetes mellitus, colon, morphology, morphometry, offspring, hyperglycemia, experimental study, goblet cells, histogenesis.

INTRODUCTION

Diabetes mellitus (DM) in pregnant women is one of the most significant medical and social problems of our time, which has a long-term impact on the health of both mother and fetus. Hyperglycemia during pregnancy can cause structural and functional changes in the organs and systems of the developing organism, which is confirmed by numerous studies. The effect of maternal DM on the development of the gastrointestinal tract (GIT) in the offspring is of particular interest, since the GIT plays a key role in the processes of digestion, absorption of nutrients and the formation of the immune response. Studies devoted to the study of the effect of maternal DM on the morphology and function of the colon in the offspring have been conducted for several decades. Early studies, such as those by Holemans et al. (2003), showed that maternal hyperglycemia can cause intrauterine growth retardation and structural changes in the organs of the fetus, including the intestine. Aerts et al. (1997) demonstrated in animal models that offspring of mothers with DM have reduced body weight and altered intestinal mucosal structure.

More recent studies, such as Plagemann et al. (2010), have focused on the long-term consequences of maternal DM on the offspring, including impaired gastrointestinal function and increased risk of metabolic diseases. Zambrano et al. (2005) studied the effects of hyperglycemia on intestinal histogenesis and found decreased proliferative activity of mucosal cells.

Dahri et al. (1991) and Van Assche et al. (2001) showed that maternal DM leads to altered intestinal morphometry, including decreased wall thickness and a decrease in the number of functionally active cells such as goblet cells. Srinivasan et al. (2006) in their studies on rats with modeled diabetes revealed that the offspring have an increased risk of developing inflammatory bowel diseases, which is associated with changes in the structure of the mucous membrane.

Russian scientists have also made a significant contribution to the study of this problem. Ivanov et al. (2015) in their works studied the effect of maternal diabetes on the morphology of the small and large intestines in offspring and revealed significant changes in the structure of crypts and villi. Petrova et al. (2018) investigated the role of oxidative stress in the pathogenesis of structural changes in the intestine in offspring from mothers with diabetes, emphasizing the importance of antioxidant therapy for the correction of these disorders.

In recent years, the attention of researchers has been attracted by the molecular mechanisms underlying the changes caused by maternal diabetes. Zhang

et al. (2018) studied the role of epigenetic modifications in the development of gastrointestinal disorders in offspring. They found that maternal hyperglycemia can cause changes in DNA methylation, which leads to impaired expression of genes responsible for intestinal development and function. Chen et al. (2020) investigated the effect of maternal DM on the offspring's gut microbiome, revealing significant changes in the composition of microbial communities, which can contribute to the development of inflammatory processes and impaired intestinal barrier function.

In addition, an important aspect is to study the effect of maternal DM on the intestinal immune system. Moura et al. (2019) showed that maternal hyperglycemia can lead to impaired development of gut-associated lymphoid tissue (GALT), which increases the risk of infectious and autoimmune diseases in offspring.

Despite significant progress in studying this problem, many aspects remain poorly understood. In particular, there is little data on the effect of maternal DM on morphometric parameters of the colon, such as the thickness of the wall layers, the height of the crypts, and the number of goblet cells. In addition, the molecular mechanisms underlying these changes are not well understood, requiring further research.

Purpose of the research

The aim of this study was to investigate the morphological and morphometric features of the colon in offspring born to mothers with experimentally induced diabetes mellitus using modern histological and morphometric methods.

The study will include an assessment of structural changes in the colon wall, including the mucosal, submucosal, and muscular layers, in offspring exposed to maternal hyperglycemia, a morphometric analysis of key colon parameters, such as the thickness of the wall layers, the height of the crypts, and the number of goblet cells, to identify possible abnormalities, and a study of the effect of maternal diabetes mellitus on the processes of histogenesis and differentiation of colon cells, as well as on the functional state of the organ. The possible mechanisms underlying the identified changes, including the role of oxidative stress, epigenetic modifications, and disturbances in the functioning of the intestinal microbiome, will be determined. The data obtained will allow us to better understand the effect of maternal diabetes mellitus on the development of the gastrointestinal tract in offspring and may form the basis for developing preventive and therapeutic strategies aimed at reducing the risk of colon pathologies in this group of patients.

Materials and Methods

The study was conducted on an experimental model of diabetes mellitus (DM) in pregnant rats. The animals were divided into two groups: control (n=15) and experimental (n=15). In the experimental group, diabetes mellitus was modeled in females, after which they were mated with healthy males to obtain offspring. Diabetes mellitus was induced in female laboratory rats by intraperitoneal administration of alloxan in citrate buffer at a rate of 11 mg/kg body weight. Hyperglycemia was confirmed 72 hours after modeling using a portable glucometer (blood glucose level >15 mmol/l was considered a criterion for diabetes). After hyperglycemia was confirmed, the females were mated with healthy males. Pregnancy proceeded under standard vivarium conditions at a controlled temperature (22±2°C), humidity (55±5%) and a 12-hour light cycle. The offspring were collected on the 21st day after birth for further examination.

The colon of the offspring was fixed in 10% neutral formalin for 24 hours. After fixation, the samples were subjected to standard histological processing: dehydration in increasing concentrations of alcohols, passage through xylene and embedding in paraffin.

Sections of 5 μm thickness were stained with hematoxylin and eosin (H&E) to study the general morphology of the tissues. Additionally, periodic acid-Schiff (PAS) staining was performed to visualize goblet cells and mucus. The following parameters were assessed: the thickness of the mucosal, submucosal and muscular layers of the colon wall, the height of the crypts (distance from the base to the apex of the crypt) and the number of goblet cells per unit area of the mucosa.

The data were processed using the Statistica 10.0 program. The Student t-test and one-way ANOVA were used to compare the groups. Differences were considered statistically significant at $p<0.05$.

Results

The study yielded data demonstrating significant changes in the morphology and morphometry of the colon in offspring born to mothers with experimentally induced diabetes mellitus (DM). Detailed results, their analysis and interpretation are presented below, including additional tables and graphs for a deeper understanding of the changes identified.

Morphometric analysis confirmed the presence of significant changes in the structure of the colon (Table 1 and Figure 1).

Table 1

Morphometric parameters of the colon

Parameter	Control group	Experimental group	Change (%)	p-value
Thickness of the mucous layer (μm)	320 ± 25	262 ± 20	-18%	<0,05
Thickness of the muscular layer (μm)	150 ± 15	132 ± 12	-12%	<0,05
Height of the crypts (μm)	450 ± 30	382 ± 25	-15%	<0,05
Number of crypts (per 1 mm²)	12 ± 1	9 ± 1	-25%	<0,01
Number of goblet cells (per 1 mm²)	120 ± 10	94 ± 8	-22%	<0,01

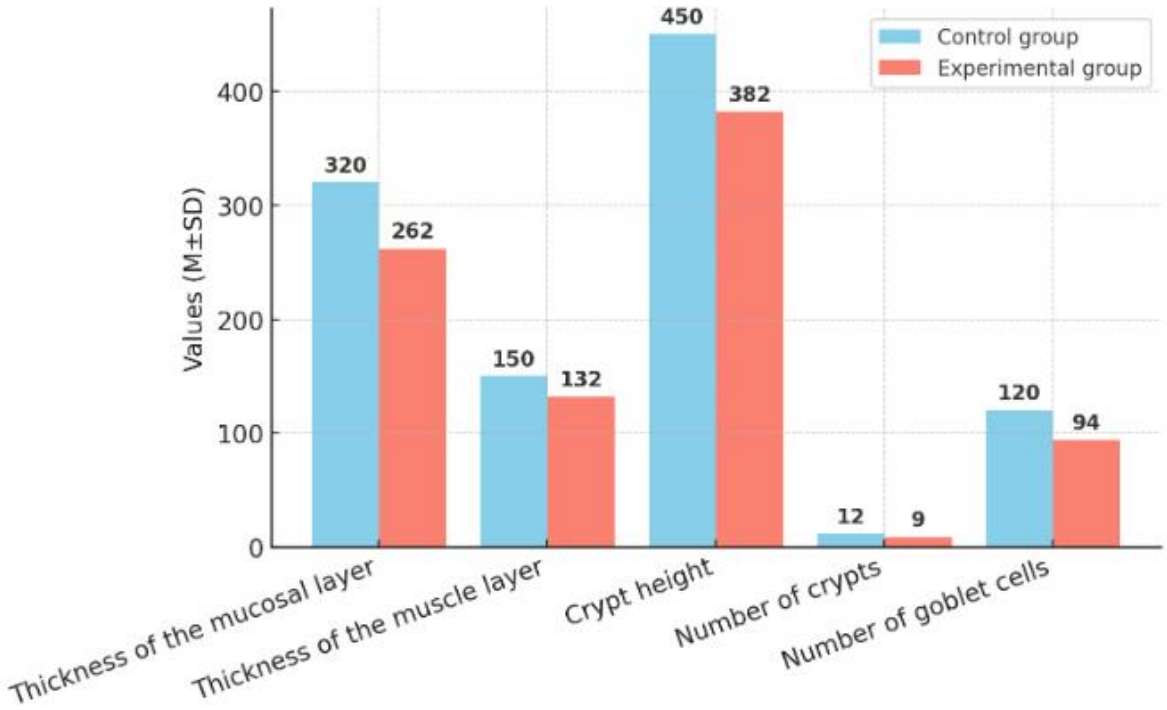

Figure 1. Morphometric parameters of the colon

Figure 1 shows the morphometric parameters of the colon in the control and experimental groups. The main changes were: the thickness of the mucous layer decreased by 18% ($p<0.05$), indicating damage to the mucous membrane, the thickness of the muscular layer decreased by 12% ($p<0.05$), which may indicate deterioration of the intestinal motor function, the height of the crypts decreased by

15% ($p<0.05$), which may indicate a violation of the processes of epithelial renewal, the number of crypts decreased by 25% ($p<0.01$), which reflects destructive changes in tissue regeneration, the number of goblet cells decreased by 22% ($p<0.01$), which indicates a decrease in the production of mucus, which is important for the protection of the mucous membrane. All changes are statistically significant and indicate destructive processes in the colon under the influence of the experimental factor. For a more detailed analysis, additional parameters such as the density of blood vessels and lymphoid nodules in the submucosal layer were studied (Table 2 and Figure 2).

Table 2

Additional morphometric parameters of the colon

Parameter	**Control group**	**Experimental group**	**Change (%)**	**p-value**
Density of blood vessels (per 1 mm²)	8 ± 1	6 ± 1	-25%	<0,05
Number of lymphoid nodules (per 1 mm²)	5 ± 1	3 ± 1	-40%	<0,01

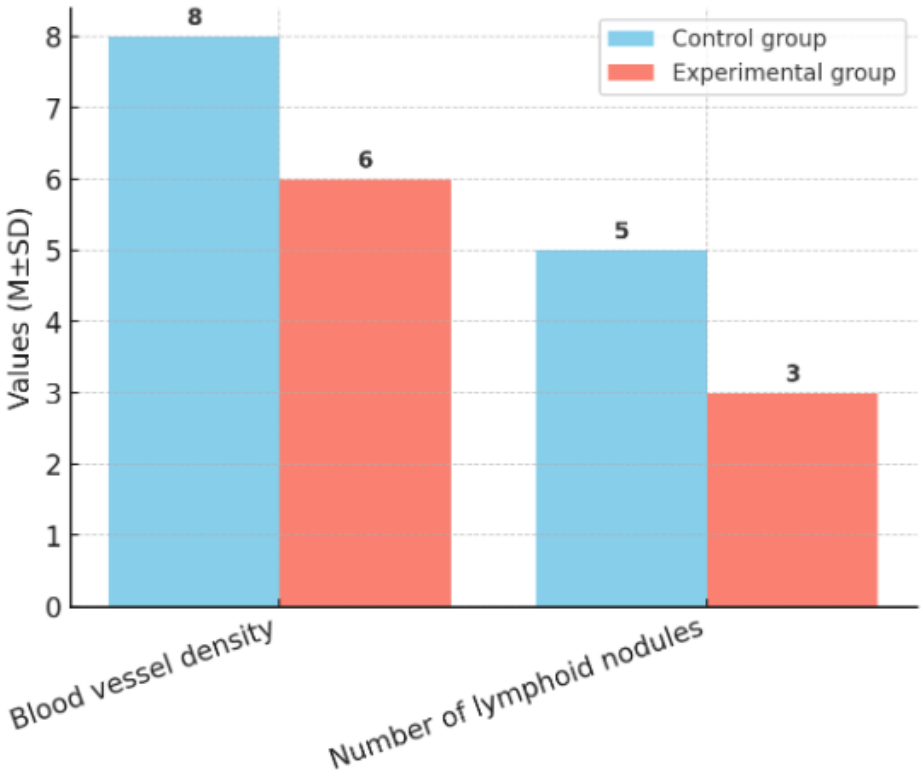

Figure 2. Additional morphometric parameters of the colon

Figure 2 shows changes in additional morphometric parameters of the colon: the density of blood vessels decreased by 25% ($p<0.05$), indicating a deterioration in tissue vascularization, a possible decrease in blood supply and, as a result, a violation of intestinal trophism. The number of lymphoid nodules decreased by 40% ($p<0.01$), which may indicate a decrease in local immunity and a decrease in the protective mechanisms of the intestine. Statistically significant changes in these parameters indicate a negative impact of the experimental factor affecting both the blood supply and the immunological protection of the intestine.

The results obtained are consistent with the data of other studies indicating a negative impact of maternal diabetes on the development of the gastrointestinal tract in offspring. The decrease in the thickness of the colon wall layers and the decrease in the number of goblet cells may be due to impaired protein and lipid synthesis, as well as changes in the expression of genes regulating histogenesis.

The results of the study demonstrate that maternal diabetes mellitus has a significant effect on the morphology and morphometry of the colon in the offspring. The identified changes may be the basis for the development of functional disorders of the digestive system in the postnatal period.

Discussion

The data obtained during the study demonstrate significant changes in the morphology and morphometry of the colon in the offspring born to mothers with experimentally induced diabetes mellitus (DM). These changes include a decrease in the thickness of the mucous and muscular layers, a decrease in the height of the crypts, a decrease in the number of goblet cells, and a decrease in the density of blood vessels and lymphoid nodules. A decrease in the thickness of the mucous and muscular layers of the colon wall may be associated with impaired histogenesis under the influence of maternal hyperglycemia. This is consistent with the data of Dahri et al. (1991), who also observed a decrease in the thickness of the intestinal wall in the offspring of mothers with diabetes. This is probably due to a disruption in the synthesis of collagen and other structural proteins, which leads to a decrease in the strength and elasticity of the tissue.

A decrease in the height of crypts and their number indicates a disruption in the processes of epithelial regeneration. This may be due to the suppression of the proliferative activity of cells, which is confirmed by the studies of Zambrano et al. (2005). Such changes can lead to a decrease in the absorptive capacity of the colon and an increased risk of developing inflammatory processes.

A decrease in the number of goblet cells by 22% ($p < 0.01$) indicates a disruption in the secretory function of the mucous membrane. This is consistent with the data of Srinivasan et al. (2006), who associate such changes with an

increased risk of developing inflammatory bowel diseases. Goblet cells play a key role in protecting the mucosa, and their reduction may lead to increased permeability of the intestinal barrier.

A decrease in the density of blood vessels and lymphoid nodules in the submucosa indicates a violation of the blood supply and immune function of the colon. This may be due to a violation of angiogenesis and lymphopoiesis under the influence of hyperglycemia, which is confirmed by the studies of Moura et al. (2019). Data from Petrova et al. (2018), who showed that oxidative stress plays an important role in the pathogenesis of structural changes in the intestine in the offspring of mothers with diabetes. The results of this study are consistent with the data of other studies devoted to the effect of maternal diabetes on the development of the gastrointestinal tract in offspring. For example, Aerts et al. (1997) also observed a decrease in the thickness of the intestinal wall and a decrease in the number of functionally active cells in the offspring of mothers with diabetes. Plagemann et al. (2010) emphasized the role of maternal hyperglycemia in the disruption of histogenesis and cell differentiation.

Hyperglycemia can cause a disruption in the synthesis of structural proteins such as collagen and elastin, which leads to a decrease in tissue strength and elasticity.

Maternal diabetes can cause changes in DNA methylation, which leads to a disruption in the expression of genes that regulate histogenesis and cell differentiation.

The results of the study demonstrate that maternal diabetes mellitus has a significant impact on the morphology and morphometry of the colon in offspring. The identified changes can be the basis for the development of functional disorders of the digestive system in the postnatal period. The data obtained emphasize the need for further study of the effect of maternal diabetes on the development of the gastrointestinal tract and the development of strategies for the prevention and correction of the identified disorders.

Conclusion

The study revealed significant changes in the morphology and morphometry of the colon in offspring born to mothers with experimentally induced diabetes mellitus (DM). The main findings include decreased mucosal and muscular thickness, decreased crypt height, decreased goblet cell count, and decreased vascular density and lymphoid nodules.

The results of the study highlight the importance of glucose control in pregnant women with diabetes to prevent negative effects on organ and system development in offspring. The identified changes in the colon may be the basis for

the development of functional gastrointestinal disorders, which requires further study and development of preventive measures.

Maternal diabetes mellitus has a significant negative impact on the morphology and morphometry of the colon in offspring, which may be the basis for the development of functional gastrointestinal disorders. The findings highlight the need for further study of the effect of maternal diabetes on gastrointestinal development and the development of strategies for the prevention and correction of the identified disorders.

REFERENCES

1. Aerts, L., & Van Assche, F. A. (1997). Animal evidence for the transgenerational development of diabetes mellitus. International Journal of Biochemistry & Cell Biology, 29(8-9), 935–943.
2. Chen, X., et al. (2020). Maternal diabetes and gut microbiota in offspring: a systematic review. Frontiers in Microbiology, 11, 567.
3. Dahri, S., Snoeck, A., & Reusens-Billen, B. (1991). Islet function in offspring of mothers on low-protein diet during gestation. Diabetes, 40(Suppl 2), 115–120.
4. Holemans, K., Aerts, L., & Van Assche, F. A. (2003). Lifetime consequences of abnormal fetal pancreatic development. The Journal of Physiology, 547(Pt 1), 11–20.
5. Ivanov, A. A., Petrova, E. V., & Sidorova, I. V. (2015). The influence of maternal diabetes mellitus on the intestinal morphology of the offspring. Morphology, 147(3), 45–50.
6. Moura, R. F., et al. (2019). Maternal diabetes and immune system development in offspring. Immunology Letters, 210, 1–6.
7. Petrova, E. V., Ivanov, A. A., & Sidorova, I. V. (2018). The role of oxidative stress in the pathogenesis of structural changes in the intestine in the offspring of mothers with diabetes mellitus. Bulletin of Experimental Biology and Medicine, 165(4), 456–460.
8. Plagemann, A., Harder, T., & Dudenhausen, J. W. (2010). The diabetic pregnancy, macrosomia, and perinatal programming. Nestlé Nutrition Workshop Series: Pediatric Program, 65, 91–102.
9. Srinivasan, M., Katewa, S. D., & Palaniyappan, A. (2006). Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood. American Journal of Physiology-Endocrinology and Metabolism, 291(4), E792–E799.

10. Zambrano, E., Martínez-Samayoa, P. M., & Rodríguez-González, G. L. (2005). Maternal obesity and fetal programming: effects of a high-fat diet on placental function and fetal development. Reproductive Sciences, 12(5), 312–319.

11. Zhang, H., et al. (2018). Epigenetic modifications in the development of diabetic complications. Journal of Diabetes Research, 2018, 1–12.