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Distribution of Mast Cells in The Small Intestine in Different Periods of Pregnancy in Rats

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ABSTRACT

The gastrointestinal tract has different characteristics. The gastrointestinal tract is affected by the enlarging uterus as pregnancy progresses. The enlargement of the uterus changes the digestive organs, especially the metabolism of the stomach and intestines. Anatomical changes produce acute abdominal changes. In addition to the mechanical effects caused by the growing uterus, high progesterone levels cause delayed gastric emptying and increased gastrointestinal transit time. Stomach and intestinal motility are also affected, resulting in lower transit times. Mast cells are cells that are activated and show degranulation under all kinds of stress conditions. These cells are considered to be cells that play an important role in the physiological and pathological responses of the immune system by showing both proinflammatory and immunosuppressive functions. Maternal malnutrition adversely induces fetal bowel development and function through the maternal intestinal barrier, intestinal content, structure, and peripheral circulation. In this study, samples were taken from the small intestine in 3 different periods of pregnancy. Tissue samples taken from duodenum, jejunum and ileum were fixed in Carnoy solution for 12 hours. Paraffin blocks were prepared after tissue follow-up without washing. Paraffin blocks were formed from the samples and 5-6 µm thick sections were taken and stained with 0.5% Toluidine Blue to determine the distribution of mast cell number. After staining, the distribution of mast cells in the lamina propria + submucosa and tunica serosa layers was examined. It was determined that mast cells changed numerically in the duodenum, jejunum and ileum in the small intestine according to the layers and according to the gestational periods. It is thought that this study will contribute to the literature on this subject.

Keywords: Mast cell, Pregnancy, Rat, Small intestine

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Introduction

As pregnancy progresses, the gastrointestinal tract is affected by the enlarging uterus. Dilatation of the uterus; changes the metabolism of the digestive organs, especially the stomach and intestines. The resulting uterine dilatation causes anatomical and acute abdominal changes, as a result of which the peritoneum is stretched and may cause pain. In addition to the mechanical effects caused by uterine dilation, it causes a delay in gastric emptying and also an increase in gastrointestinal transit times and progesterone levels (Karaca and Yörük 2005). Mast cells are activated and show degranulation under all kinds of stress conditions. Mast cells originate from multipotent CD34+ precursor cells in the bone marrow and complete their differentiation in peripheral tissues. Under normal conditions, mature mast cells do not circulate in the bloodstream. However, mast cell progenitors migrate into tissues and differentiate into mast cells under the influence of stem cell factors and various cytokines. Mast cells are present throughout the body and play essential roles in maintaining many physiological functions (Gilfillan and Beayen, 2011; Dahlin and Hallgren, 2015; Krystel-Whittemore et al., 2016). These cells mature by reaching a unique phenotype by being affected by the tissue type and environmental conditions in which they are placed. Mast cells are found in large numbers in the skin, mucous membranes of the upper and lower respiratory tract, gastrointestinal tract, and mucous membranes lining the cavities of the body. Mast cells express the high-affinity IgE receptor (Brown et al., 2008; Tekeli, 2008; Wasserman, 1990). Many factors stimulate mediator release in mast cells. The physiological response of mast cells to stimuli is mostly through IgE receptors. (Halova et al., 2018). In the granules of mast cells, substances such as heparin, histamine, prostaglandin, neutral protease, β-glucuronidase, aryl sulfatase, tryptase, anaphylaxis, eosinophilic chemotactic factor (ECFA), slow reaction substance of anaphylaxis (SRS-A). Two main types of mast cells have been defined, considering the origins of mast cells, their localization, the response to the fixative solution used, their differences, functional criteria, and morphological characteristics of the cells (Bayramgürler and Demirsoy 2013; Demirbag et al., 2012). Mucosal mast cells (MMC) are mainly found in the mucosa of the gastrointestinal tract and in the lamina propria of the respiratory tract. Connective tissue mast cells (CTMC) are found in the gastrointestinal tract submucosa, skin, peritoneum, near vessels, and organ serosa (Maurice et al., 2005; Marshall, 2004). The aim of this study; is to examine the distribution of mast cells, which play an important role in the physiological and pathological responses of the immune system during pregnancy, in the small intestines.

Material and Methods

A total of 18 Wistar Albino rats were used in the study, six animals in each group, in three different periods of pregnancy, on the 5th day of pregnancy, on the 12th day of pregnancy, and the 19th day of pregnancy. All animals were housed in rooms with adjustable 12-hour light/dark cycles at appropriate humidity and temperature. Standard rat feeding was practised throughout the study, and water was given ad libitum. All the experimental protocols were carried out and approved by the Department of the Experimental Animal Ethics Committee, Mehmet Akif Ersoy University (17.03.2021 date). Samples taken from the duodenum, jejunum, and ileum of each animal were fixed in Carnoy for 12 hours, and the tissues were directly kept in 70% alcohol for 12 hours without washing, and after routine tissue follow-up, they were blocked with paraplasts. Serial sections of 5 µm thickness were taken from the prepared blocks and stained with 0.5% concentration toluidine-blue (pH 0.5, 10 minutes) (Uslu and Yörük 2015). The stained slides were evaluated under the research microscope (Zeiss Primostar) with an integrated digital camera for mast cell distribution. We acquired images from sections by using a digital camera. To determine the distribution of mast cells in the slides stained with toluidine blue, 100 square ocular micrometres were used for cell counting. Mast cells were counted in 100 square units of the ocular micrometre at 40X objective magnification. The arithmetic average of these numbers was calculated by measuring the mast cells in the serial sections. Then, the average mast cell in the area covered by 100 square ocular micrometres was determined. For 40X objective magnification, the area of 100 square ocular micrometres was calculated with the help of a micrometric slide. These data were then converted to the number of mast cells per unit area of 1 mm^2. Mast cell counts SAS v. Variance analyzes were performed using the 12.0 package program. Differences were determined by Duncan's Test (SAS, 1998).

Results

Lamina propria-submucosa and Tunica muscularis-Tunica serosa layers were examined in duodenum, jejunum, ileum tissues in each experimental group. It was observed that mast cells were located in the duodenum, jejunum and ileum, between the crypts and inside the crypts, around the villi, close to the blood vessels, in the tunica mucosa and tunica serosa (Figure 1). Localization of mast cells was found to be similar in three different pregnancy periods. In the examination performed to determine the quantitative distribution of the mast cell, the lamina propria and submucosa layers were evaluated together. On the other hand, the number of mast cells in the tunica serosa layer was lower than in the propria and submucosa. It was determined that the number of mast cells changed from the duodenum to the ileum. It was determined that the number of mast cells increased in the 2nd and 3rd periods compared to the 1st period. However, these differences are not statistically significant (Table 1).

Table 1. Distribution of mast cells.

		Lamina p + Submucosa		T. mucosa +T. serosa	
		2nd Period	3rd Period	2nd Period	3rd Period
Duodenum	1st period	0.74648	0.18703	0.61961	0.63960
	2nd Period		0.77899		0.08592
Jejenum	1st Period	0.61879	0.15921	0.36838	0.28203
	2nd Period		0.01608*		0.89352
Ileum	1st Period	0.20749	0.27478	0.60655	0.66302
	2nd Period		0.19298		0.44843

* $p<0.05$

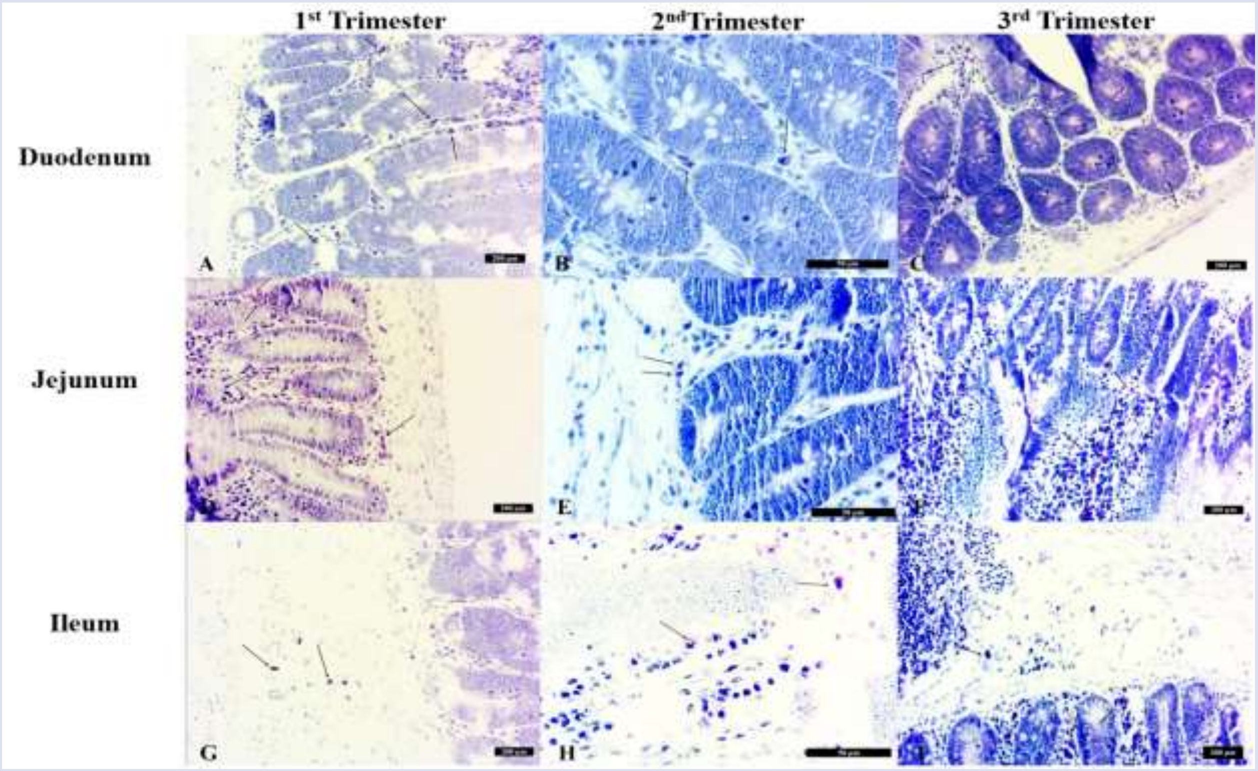

Figure 1. Distribution of mast cells in the intestines by gestational age. 1A: 1st Period, duodenum, Mast cells, Toluidine blue. 1B: 2nd Period, duodenum, Mast cells, Toluidine blue. 1C: 3rd Period, duodenum, Mast cells, Toluidine blue. 1D: 1st Period, jejunum, Mast cells, Toluidine blue. E: 2nd Period, jejunum, Mast cells, Toluidine blue. 1F: 3rd Period, jejunum, Mast cells, Toluidine blue. 1G: 1st Period, ileum, Mast cells, Toluidine blue. 1H: 2nd Period, ileum, Mast cells, Toluidine blue. 1I: 3rd Period, ileum, Mast cells, Toluidine blue.

Discussion

Due to the growing uterus and changing hormonal balance during pregnancy, the working and layout of many systems in the mother varies. In different periods of pregnancy, these changes affect other systems. In this study, the small intestines of the mother were examined in three different periods of pregnancy, and it was revealed that mast cells were affected by pregnancy metabolism in these processes. Mast cells are cells that are frequently affected by many stress-dependent metabolic events. They are cells that vary in number and location in many pathological conditions such as diabetes (Karaca et al., 2011) and colitis (Karaca et al., 2011). Similar to the changes in the number of mast cells in a similar study by Karaca et al., (2010, 2011) cell changes were also observed in our study. Since the small intestines (Peyer's patches) are structures related to the immune system, it is thought that this change in the number of mast cells in the small intestines may be effective in the immune response occurring in the intestines. Studies on multiple systems such as the cardiovascular system, the endocrine system, the respiratory system, and the digestive system during pregnancy have revealed that due to the location of the organs and physiological changes during pregnancy, both cellular structures and functional changes occur in the tissues (Tan and Tan, 2013). Our study determined mast cell increases in the small intestine mucosa were in the later stages of pregnancy, similar to the study by Tan and Tan (2013). Carlin and Alfirevic (2008) reported in their study that the physiological changes occurring in the first period of pregnancy and the changes occurring in the second and third periods are different. In addition, it has been reported that vascularization is different between the beginning of pregnancy and the following periods (Carlin and Alfirevic 2008). In our study, mast cell distribution was different in the first period of pregnancy compared to the second and third periods. Although mast cells are located similarly in three periods, their numerical distribution differs. Palladina et al. (2021) emphasized in their study that the mother's intestinal system and diet during pregnancy affect the nervous, endocrine, respiratory and digestive systems of the offspring and, therefore, the cellular distribution in the intestinal tract of the mother may be different. The mast cell numerical difference results obtained in our study support the findings of Palladina et al. (2021). Demirbag et al. (2012) stated that Carnoy detection is suitable for determining the metachromasia feature and numerical densities in mast cells in their study of the small intestines of rats. Our study revealed that Carnoy detection in the small intestines of rats at different periods during pregnancy is suitable for determining mast cell density and displaying their metachromatic features. Demirbag et al. (2012) found the highest number of mast cells in the duodenum in their study in the small intestines of rats, while in our study, more mast cells were found in the jejunum and ileum. However, this change was not statistically significant. A study of dogs' intestinal mucosa compared the lamina propria, and tunica muscularis layers of the jejunal mucosa showed that the number of mast cells was higher in the propria (Eren et al., 2000). In our study, we observed that mast cell density in the small intestines was higher in the tunica mucosa during pregnancy, and this situation was similar in different periods.

Conclusion

As a result, mast cells are cells that play an essential role in many diseases and physiological processes through the metabolic mediators they secrete. Due to the effect of intestinal physiology on fetal development, it has always been considered worth investigating during pregnancy. In our study, it was revealed that the distribution of Mast cells, which have both proinflammatory and immunosuppressive functions, changes in the duodenum, jejunum and ileum during pregnancy. It is thought that the obtained findings will lead to studies in which the effects on fetal development can be investigated and will contribute to the literature on this subject.

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