Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta Morphologica et Anthropologica, 31 (1-2)
Sofia ● 2024

Gastric Mucosal Mast Cell Activity in Children on Longitudinal Enteral Feeding with Percutaneous Endoscopic Gastrostomy, PEG

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Longitudinal enteral feeding via percutaneous endoscopic gastrostomy (PEG) challenges the gastric mucosa. The aim of our study is to make a morphological analysis of the gastric mucosa in PEG-children with an emphasis on the inflammatory component. The biopsy specimens from stomach of 37 children without GIT disease, age 0-18 years (20 with oral feeding – controls; 17 – with PEG; duration 24–48 month) are examined with histological and morphometric methods. In gastric mucosa of PEG children we find scanty amount of loose connective tissue, glands next to each other and hyperemic capillaries. In cases with cerebral palsy is noticeable an increased number of mast cells. Longitudinal enteral feeding via PEG doesn‘t cause significant morphological disorders in the structure of the stomach. Regarding increased mast cell activity further studies are needed to clarify whether this finding is related to the PEG or to the child‘s neurological disorder.

Key words: percutaneous endoscopic gastrostomy, cerebral palsy, gut, parietal cells, mast cells

Introduction

Enteral nutrition is a process of substrate satisfaction of the body’s needs for nutrients and energy, using the gastrointestinal tract (GIT) through a specially introduced probe of specially prepared medicinal foods. It is a type of nutritional therapy administered by gavage or stoma directly into the GIT, distal to the oral cavity. Enteral feeding probes can be placed through the mouth - orogastric, nasal, ie. naso-gastral, naso-

jejunal or naso-postpyloric feeding tube, or through an endoscopically placed stoma, percutaneous endoscopic gastrostomy or with an extension in the jejunum or in the jejunum.

Longitudinal enteral feeding via percutaneous endoscopic gastrostomy (PEG) challenges the gastric mucosa. It is carried out in conditions of disturbed physiology - all stages of the digestive process until the food enters the stomach are absent. There is a risk of infection of the GIT, allergens.

Mast cells have been found to interact with neurons from autonomic ganglia in the gut wall. They secrete tryptase, histamine and serotonin, which leads to increased organ sensitization and interference with their functions [26]. In both variety- and volume-limited patients, antral and duodenal mast cell densities were increased [13]. Increased mast cell density is associated with slower gastric emptying and increased gastric dysrhythmia in children with functional dyspepsia [25].

The aim of our study is to make a morphological analysis of the gastric mucosa in children with longitudinal enteral feeding through PEG with an emphasis on the inflammatory component.

Materials and Methods

The biopsy specimens from stomach of 37 children without GIT disease from the Clinic of Pediatrics and Medical Genetics MU - Plovdiv at age 0-18 years (20 of them are on oral feeding - controls and 17 on enteral feeding by precutaneous endoscopic gastrostomy (PEG) duration of 24–48 month were examined with light-microscopy and morphometric analysis. Written informed consent was obtained from the children's parents for the histological examination and the publication of the obtained data. The biopsy specimens were fixed in 10% neutral formalin and incorporated into formalin. Hematoxylin-eosin and Toluidine blue staining was performed on paraffin sections with a thickness of 5 μm. The observation and photo documentation of the microscope slides are performed with a digital photomicroscopic camera of a light microscope "Olympus BX51".

Using morphometric image analysis software ImageJ 1.8.0 we counted the number of parietal cells in the biopsy specimens (ImageJ, 2021). The statistical analysis of the results were carried out to the conventional standard statistical procedures using computer statistical analysis by SPSS, version 26.0 for Microsoft Windows XP.

Results

The morphological characteristics of gastric mucosa in children on longitudinal enteral feeding with PEG and that of the controls did not show significant differences in the morphological structure.

In the mucosa of children with oral feeding we observe areas with preserved covering columnar epithelium and transversely or longitudinally cut fundus glands. The parietal cells that secrete the hydrochloric acid of the gastric juice are rounded, eosinophilic, some of them binucleate. The chief cells synthesizing the enzyme pepsinogen have basophilic cytoplasm. In the controls, the parietal cells are mainly located in the body, and the chief cells occupy the base of the glands (**Fig. 1**).

In the mucosa of children with PEG we establish a small amount of loose connective tissue in the lamina propria. The glands are densely located next to each other, well supplied with blood with slightly hyperemic capillaries between them. Parietal cells are evenly distributed in the neck, body and bottom of the glands. The chief cells retain their localization at the bottom of the glands (**Fig. 2**).

No pronounced inflammatory lymphocytic infiltration was found in both groups, but in some cases with PEG with neurological disease (cerebral palsy) we established the significant presence of mast cells. The produced images in **Fig. 3** depict that the mast cells were localized in the connective tissue under the covering epithelium and near the fundic glands of the mucosa. Some of the mast cells were in a state of degranulation.

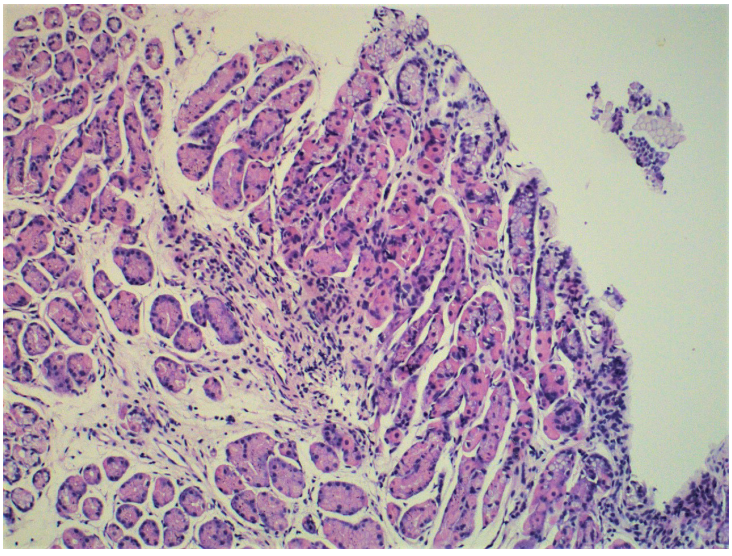

Fig. 1. Stomach controls. HE stain. x100.

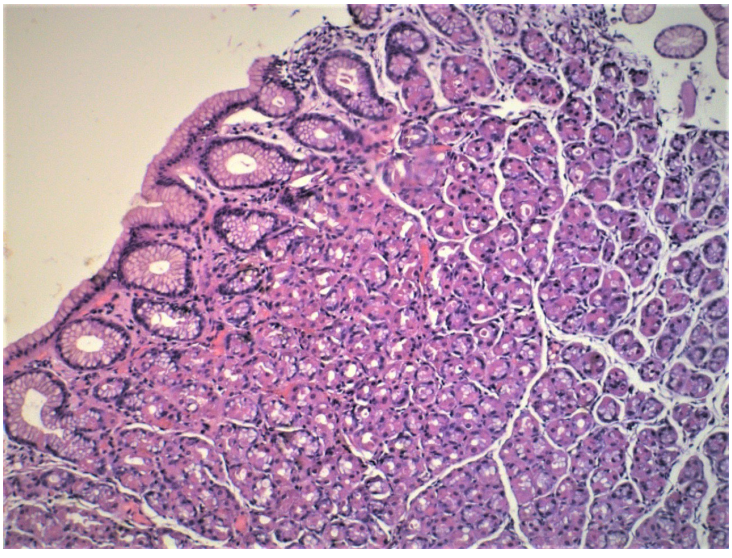

Fig. 2. Stomach PEG. HE stain. x100.

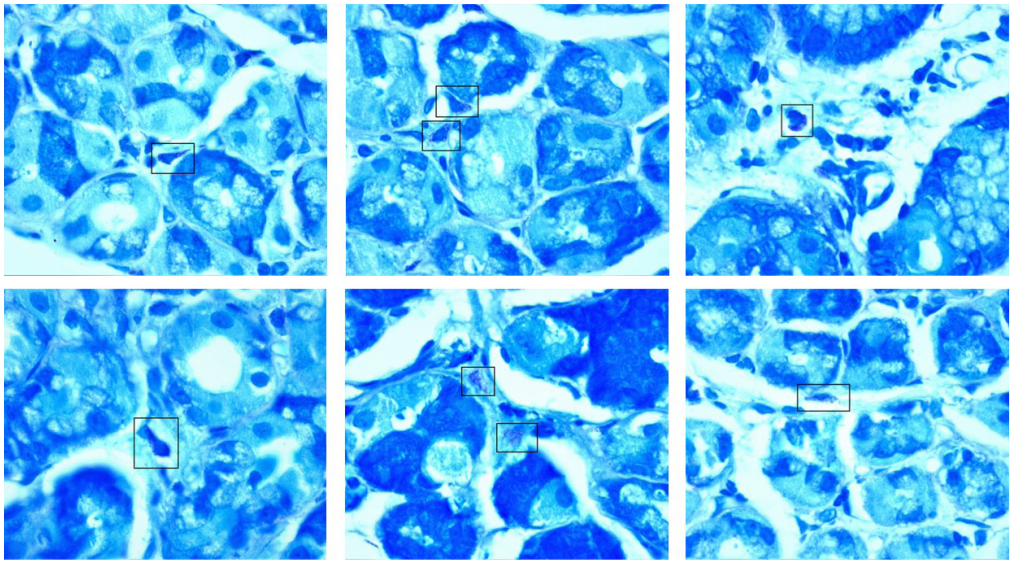

Fig. 3. Stomach PEG. Mast cells near the fundic glands. Toluidine blue stain. x 1000.

Morphometry

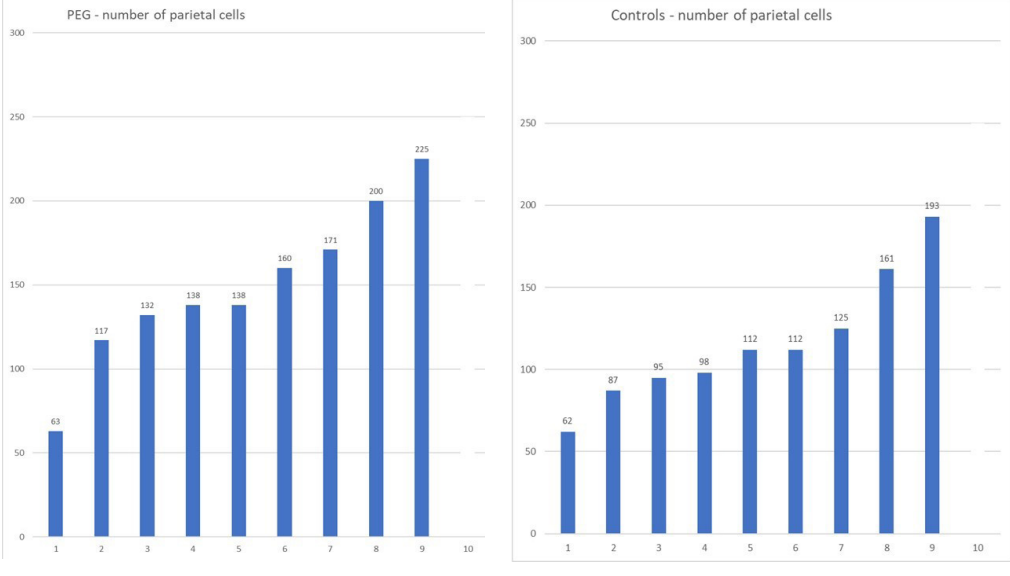

Fig. 4. Number of parietal cells.

The results in **Fig. 4** show that the number of parietal cells in the gasric glands of PEG patients was greater than in control patients. In each counting segment, more parietal cells were found in the PEG children than in the oral fed children.

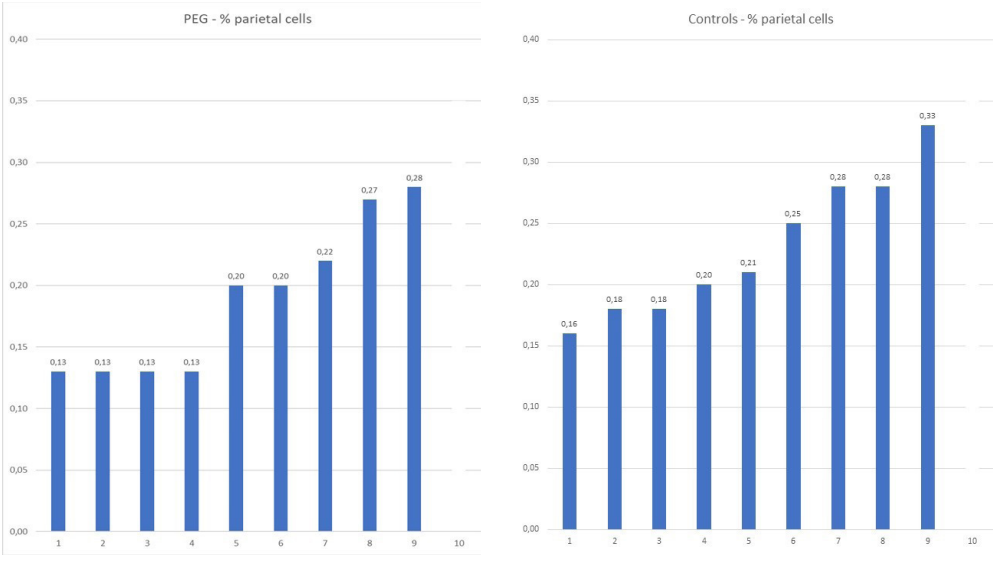

Fig. 5. Parietal cells %.

The results in **Fig. 5** show that the percentage of parietal cells in the gastric glands of patients with PEG is approximately equal to that of control patients, but in all segments we can see that it is decreased.

The greater number of parietal cells in the corpus mucosa in patients with percutaneous gastrostomy is associated with the greater number of closely spaced fundic glands, with tiny layer of connective tissue between them.

Discussion

An important characteristic of enteral feeding is the absence of physiological stimuli of the digestive process. Both visual, olfactory and gustatory stimuli, as well as complex neuro-humoral reflexes involving autonomic and sensory neuronal circuits, are involved. The synchrony in the hormonal activity of the enteroendocrine cells is also disturbed. All this affects the secretory functions of the GIT and in particular the secretion of hydrochloric acid from the parietal cells of the gastric glands.

Literature data on enteral nutrition and gastric acid secretion are mainly based on data from pH-metry of gastric contents. Clinical studies with pH-metry of gastric juice in enteral, parenteral and standard nutrition found that enteral nutrition stimulates acid secretion. Gastric content titration data, however, depend on the type of diet, as high protein administered in the stomach buffers hydrogen cations and gives a falsely elevated pH [4]. Gastric aspirate pH testing in infants on enteral nasogastric tube

feeding is used as a criterion for proper tube placement and is included in guidelines for gastric tube feeding [GUIDELINE. Gastric Tube Feeding in the NICU. Child and Adolescent Health Service. Government of Western Australia.]. A pH value ≤5.5 is considered predictive of proper gastric placement. The use of pH testing in infants, however, has limitations. It is unclear whether infants can produce adequate gastric acid to achieve a pH of 5, whether feeding frequency and medication use (proton pump inhibitor, histamine-2 receptor antagonist, etc.) affect reported pH results [14].

Percutaneous endoscopic gastrostomy has been a valuable tool in nutritional rehabilitation since its inception in 1980 [8]. The main and most common indication for PEG in children and adults are neurological disorders with inability to swallow or dysphagia [16, 19]. The majority of the children are suffering from cerebral palsy with inability to swallow. Some of these patients may also suffer from epilepsy, in which case they will need the tube for medication intake. Recurrent aspiration secondary to neurological disorder may result in failure to use the oral route safely and the insertion of a PEG tube should be undertaken [23]. Cerebral palsy (CP) is one of the main disabling disorders, characterized by permanent brain damage that affects motor and cognitive functions with different clinical symptoms in children [6]. The pathogenesis of CP is associated with increased systemic inflammatory response during intrauterine period or period before the age of 3.

The reason for PEG feeding in some of our patients is precisely CP. In the biopsy specimens of these patients we established the significant presence of mast cells under the covering epithelium and near the fundic glands of the mucosa.

Mast cells are the multifunctional immune cells that are one of the most important sources of proinflammatory cytokines. In a retrospective study of children with CP, the investigators detected higher levels of several perinatal circulating cytokines, including IL-9 [18]. It was found that in children with CP aged 3-18 years plasma levels of proinflammatory cytokines IL-1β, IL-6 and histamine levels were higher in individuals with CP compared to healthy controls [5]. The majority of mast cells are located in the gut wall. Here, they function as an important part of the immunological barrier between the internal milieu and luminal content [1]. Mast cells are present in all layers within the gastrointestinal tract. The close proximity of MCs and nerves is the emblem of the neuro-immune network and has indicated the existence of a bidirectional crosstalk between MCs and nerves acting in tandem with other neural and immune cells [2]. This dialog is crucial in the maintenance of intestinal homeostasis and it is responsible for diseases and in pain visceral perception [21].

The relationship between GIT and CNS disease is bidirectional. The role of the gut brain axis is to monitor and integrate gut functions, link affective and cognitive centers of the brain with peripheral intestinal functions and mechanisms such as immune activation, gut permeability, enteric reflex, and enteroendocrine signaling [7]. Neurological and neurodegenerative diseases are associated with complex pathologies including inflammation in both the central [3] and autonomic nervous system [27, 20, 10, 24]. These diseases are often characterized by poor gut health due to the loss of gut-barrier integrity [15, 17], which underscores the role of the brain-gut axis.

It has been established that mast cells play a role in multiple pediatric gastrointestinal disorders including eosinophilic esophagitis, functional dyspepsia, and irritable bowel syndrome [22]. A role for mast cells in feeding difficulties is biologically plausible and possibly related to increased gastric sensitivity. Mast cell infiltration and activation

has been shown to increase visceral sensitivity in both animal models and humans [12]. Mast cell infiltration and activation could account for hypersensitivity to stretch and possibly also for chemical sensitivity as has been demonstrated in adults with functional dyspepsia with enteral lipid infusion [9, 11].

Conclusion

Long term enteral nutrition via PEG does not cause significant morphological disorders in the structure of the stomach wall. The predominance of the glandular component over the connective tissue component in the gastric mucosa of children with PEG is probably an adaptation mechanism of the body for the secretion of a sufficient amount of gastric juice. Regarding increased mast cell activity in children with cerebral palsy further studies are needed to clarify whether this finding is related to the PEG or to the child‘s neurological disorder.

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