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Age-related histochemical reactions of stomach in Indian domestic pigs (*Sus scrofa domestica*)

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Abstract

In this study, histochemical features revealed a negative reaction for PAS-AB in epithelium and lamina muscularis of non-glandular part but showed a positive reaction for PAS. In the cardiac region, there was mild to moderate PAS reaction in the lining epithelium of the apical surface, glands, and lamina propria. With PAS-AB, the apical and middle portion of cardiac glands showed a positive reaction in 6th day piglet, while in 21st day piglets reaction was in the upper half. In fundic region in G-I, PAS reaction occurred on the surface epithelial cells, gastric pits, and base of the fundic gland. The intensity of the PAS reaction was higher in the lower third of the fundic glands in G-II and III. With PAS-AB intensity of the reaction was higher in epithelial cells and the upper half of the gland in groups II and III. In the pyloric region, all surface cells in mucosa, glands, and pits showed a positive reaction to PAS and PAS-AB with higher intensity in G-III than in the other two groups.

Keywords: Nonglandular region, cardiac region, fundic region, pyloric region

Introduction

Domesticated Pigs or Swine are omnivorous and their stomach is a complex organ falling in between that of a carnivore's simple stomach and an herbivore's compound stomach. Swine species are being increasingly used in biomedical research both as a general large animal biological model in teaching and research and for the study of specific disease conditions due to their similar anatomical and physiological features. Pig has become a desirable experimental model for humans due to its similarity in size, nutrition, and digestive system. Even though domestic pigs are considered omnivorous, their gastric chambers have undergone considerable modifications, making them primarily herbivorous. For microbial digestion of ingesta, the stomach's top portion has a diverticulum or pouch.

Materials and Methods

The present study was carried out in the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad. Indian domestic Pig stomachs will be collected at regular weekly intervals from a minimum of eighteen (18) healthy Pigs, irrespective of their breed, sex, and nutritional status from local slaughterhouses in and around Hyderabad. The specimens will be divided into three groups *viz* Group I Piglets (birth to 4 weeks), Group II (weaners to 10 weeks), and Group III (Adults - above 10 weeks).

Soon after collection, the specimens were cleaned and washed thoroughly with normal saline to remove blood stains and were immediately packed neatly in polythene bags and kept in the ice box for transportation to the laboratory for necessary studies. For histochemical studies, the tissue pieces of the stomach were collected immediately after slaughter and fixed in 10% Neutral Buffered Formalin and Bouin's fluid. The fixed specimens were processed for routine paraffin embedding technique and sections of 4-5 μ m thickness were obtained and subjected to the following histochemical staining methods: Periodic acid-Schiff (PAS) technique for identifying neutral mucopolysaccharides and combined PAS-Alcian blue technique for identifying acid and neutral mucins.

Results and Discussion

In the present investigation, the stomachs of Indian domestic pigs in all post-natal groups were superficial layers of the epithelium and lamina muscularis of the non-glandular region (pars oesophagea) of pig stomach showed moderate to strong positive reaction for PAS. The surface lining cells of epithelium exhibited a negative reaction for PAS-AB and the rest of the epithelial cells reacted positively for PAS-AB. also observed that lining epithelium and lamina muscularis of the non-glandular region of pig stomach showed moderate to strong positive reaction for PAS and moderate reaction for PAS-AB.

The cardiac region of the stomach exhibited mild to moderate PAS reaction on the lining epithelium of the surface of cardiac glands and lamina propria which is similar to Imai *et al.* (1988) [2] who stated that the pig stomach showed a strong reaction for PAS at mucus neck cells in cardiac glands while cells of body and basal portions of glands showed moderate reaction. In group I, a PAS-positive reaction was observed at the lining epithelium of mucosal surface and gastric pits which is to the reports of Poonia *et al.* (2012) [3] who stated that columnar cells of surface epithelium in the cardiac gland region showed a strong PAS-positive reaction in sheep.

In group II cardiac glands showed a PAS-positive reaction at their apex, although the staining was much weaker than that in epithelial cells of gastric pits and luminal surface. Similarly, also mentioned that the apex of cardiac gland cells properly showed a PAS-positive reaction but the staining was much weaker than the epithelial cells of gastric pits and luminal surface. In group III, the PAS reaction showed more intensity in the cardiac glands when compared to the surface epithelial cells and gastric pits. The intensity of the PAS reaction increased as the age advanced from group I to III. The above findings are similar to Shibata *et al.* (1990) [6] in cats, Trang *et al.* (2012) [7] in pigs, and Raja *et al.* (2022) [4] in guinea pigs. All of them stated that the cardiac region showed mild to moderate PAS reaction at the lining epithelium of the apical surface, around the cardiac glands and lamina propria. The intensity of the PAS reaction was more around the cardiac glands than on the apical surface. These findings are contrary to the observations made by Chande *et al.* (2020) [1], who reported that neither the cells in fundic glands showed a positive Alcian blue reaction indicating that acidic mucins are not synthesized in fundic region in guinea pig stomach.

With PAS-AB stain six-day-old piglet surface epithelial cells and gastric pits showed a strong reaction but in a twenty-one-day piglet of the same age group I, a positive reaction was observed at the apical and middle portion of the cardiac gland. These findings are in agreement with the reports of Sujana *et al.* (2017) [8] in pigs who stated that a positive PAS reaction was seen at the apical and middle portions of cardiac glands. In groups II and III the lining epithelium of the mucosal surface and around the upper half of the glands showed a positive reaction while the middle and base of the gland were in accordance with Imai *et al.* (1988) [2], who reported that a strong alcian blue reaction was seen in neck and upper portions of the body of cardiac glands. In the present study cardiac gland cells remained completely unstained by AB which is similar to that of Shibata *et al.* (1990) [6] in cat stomachs and Leus *et al.* (1999) [9] in pigs who noted that with PAS-AB, only PAS-positive reaction was seen in cardiac gland cells.

Moderately positive PAS and PAS-AB reactions were observed in fundic gland region in this study. Similarly, Roy

(1974) [5] stated that surface epithelial cells of porcine fundic stomach were suggestive sites for neutral polysaccharides and acidic mucin. In group I, both six-day and twenty-one-day piglets showed PAS reaction at surface epithelial cells, gastric pits, and at the base of fundic gland. PAS reaction was more in the lower third of fundic glands than the upper third in groups II and III. The intensity of the PAS reaction was higher in fundic region and the level of intensity increased as age advanced from group I to III. This is in concurrence with Raja *et al.* (2022) [4] who stated that PAS reaction was more in the fundic region and the intensity of PAS reaction was greater in the lower third of fundic glands than the upper third. The level of intensity for PAS increased as age advanced from 0-2 weeks to 16-32 weeks in guinea pigs.

With PAS + AB stain only a few epithelial cells of the gastric pit and luminal surface reacted positively in group I. While in groups II and III the intensity of the reaction was higher in the epithelial cells and the upper half of the gland. The lower half of the gland remained unstained with AB. These findings are similar to Chande *et al.* (2020) [1] who reported that the cells in fundic glands in a guinea pig's stomach were all negative for AB reaction, which shows that they do not synthesize mucins from the category of acidic ones even in small quantities. These findings are contrary to reports of Shibata *et al.* (1990) [6] who revealed that glandular cells in the basal portion of gastric glands in cats reacted moderate to strong state for PAS AB stain.

In a six-day-old piglet, all surface cells in gastric mucosa, gastric pits, and pyloric glands showed a positive reaction towards PAS. Similar observations were made by Roy (1974) [5] in pigs, Poonia *et al.* (2012) [3], Sujana *et al.* (2017) [8], and Chande *et al.* (2020) [1] who stated that mucous neck cells, body, and base of pyloric glands reacted positively for PAS demonstrating that these cells synthesize neutral mucins. Intensity of PAS reaction increased with age in groups which is in accordance with Raja *et al.* (2022) [4] in postnatal age groups of guinea pigs.

In the present study surface epithelial cells, gastric pits, and the deep half of the glands in pyloric region showed a positive reaction for PAS-AB stain which was more in group III than in groups I and II. Our observations are related to the findings of Poonia *et al.* (2012) [3] in sheep, Sujana *et al.* (2017) [8] in pigs, and Chande *et al.* (2020) [1] in guinea pigs who reported that gland apices exhibited PAS-AB positive reaction similar to epithelial cells which showed strong alciano-philic reaction since they synthesis acidic mucins.

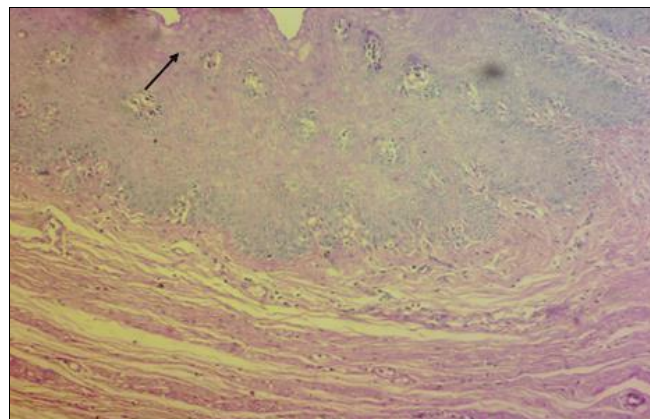


Fig 1: Photomicrograph of G-II pig stomach showing non glandular region with moderate PAS reaction in superficial cells of epithelium (arrow). PAS 4x

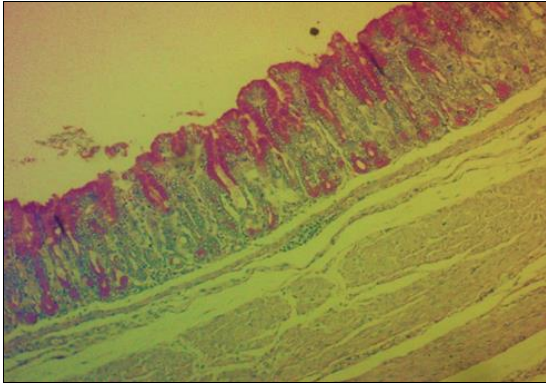


Fig 2: Photomicrograph of G-I (6 day) piglet stomach showing positive PAS reaction in lining epithelium, gastric pits and glands in cardiac region (arrow)., PAS 10x

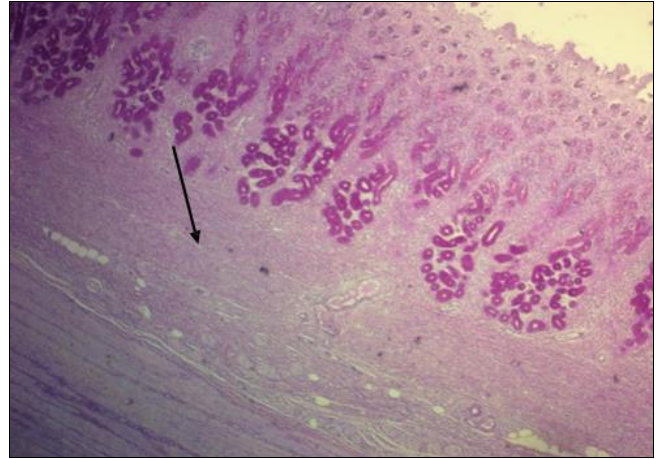


Fig 6: Photomicrograph of G-III pig stomach PAS reaction showing more intensity in cardiac glands(arrow) in cardiac

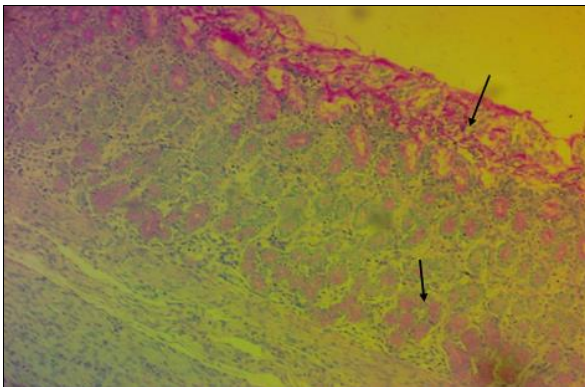


Fig 3: Photomicrograph of G-I (6 day) piglet stomach showing fundic gland region with moderate PAS reaction in epithelial cells, gastric pits and base of fundic glands (arrow). PAS 10x

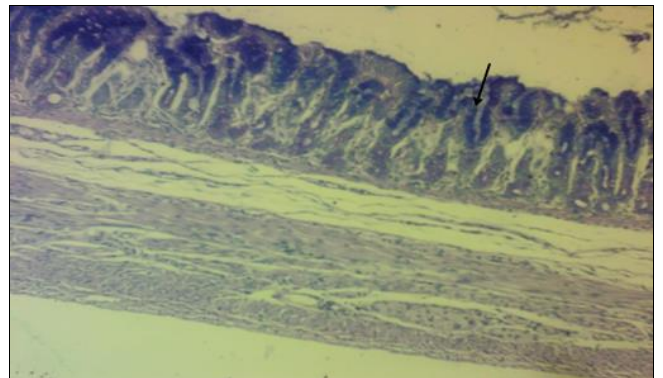


Fig 7: Photomicrograph of G-I (6 day) piglet stomach showing fundic region with positive PAS-AB reaction in luminal surface and gastric pits of fundic region (arrow). PAS-AB 10x

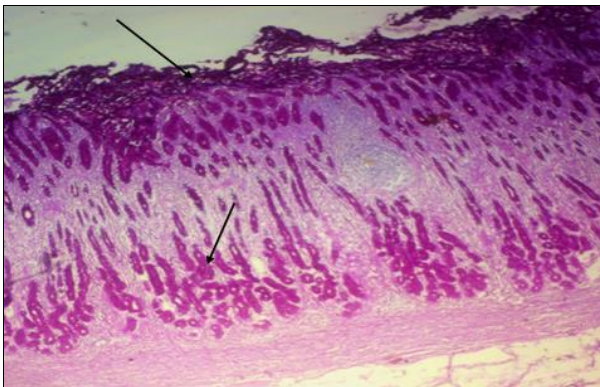


Fig 4: Photomicrograph of G-II pig stomach showing pyloric region with positive PAS reaction in epithelial cells, pits and base of the pyloric glands (arrow). PAS 10x

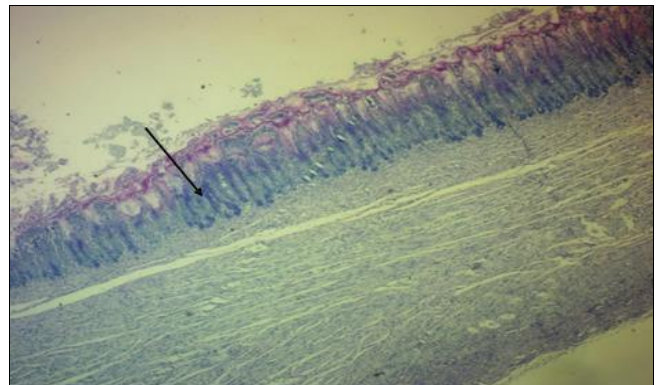


Fig 8: Photomicrograph of G-I (6 day) piglet stomach showing pyloric region with positive PAS-AB reaction in epithelial cells and gastric pits of pyloric region (arrow). PAS- AB 4x

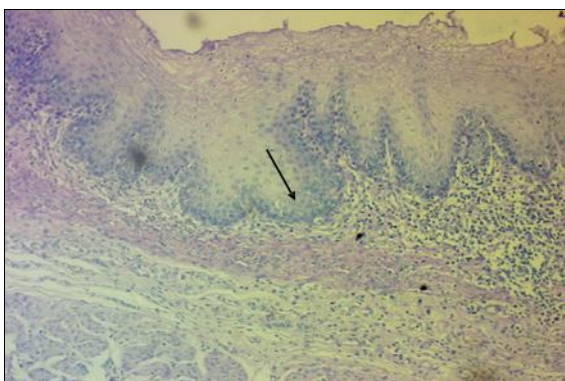


Fig 5: Photomicrograph of G-I (6 day) piglet stomach showing non glandular region with positive PAS reaction in lower layer epithelial cells, (arrow). PAS-AB 10x

Conclusion

The histochemical reactions indicate variations in glycoprotein content in different regions of the pig stomach, and these variations are influenced by age. The distribution and intensity of reactions suggest differences in the composition of mucins and other glycoproteins in different parts of the stomach across age groups.

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