**Quantitative Study of Brunner’s Glands in the Human Duodenal Submucosa**

Maria Inez Marcondes Macéa; José Rafael Macéa & José Humberto Tavares Guerreiro Fregnani

**SUMMARY:** The existence of Brunner’s glands (BGs) in the duodenal submucosa is uncontestable, but their exact distribution along the full extent of the duodenal wall is unknown. Objective: To verify the BGs distribution along the human duodenum. Material and method: Twenty normal duodenums were examined. Two samples were removed from each of the four anatomical portions of the duodenum using a scalpel, in such a way that the whole circumference of each portion was excised. Sections were prepared and stained with hematoxylin-eosin. Twelve microscope fields were examined on each duodenal section. The mean numbers of glandular points per field were computed and compared, for the 12 microscope fields of each duodenal section examined. Results: The first duodenal portion presented large quantities of BGs in all of the fields examined. The second duodenal portion also showed the presence of BGs in all the fields examined, albeit in smaller quantities than in the first portion. In the third duodenal portion, BGs were present in six of the duodenums examined. In the fourth duodenal portion, there was a minimal quantity of glands, all located in only ten of the duodenums studied. Conclusions: BGs are present in the submucosa of all duodenal portions, with the greatest concentration in the first portion. Their concentration decreases significantly in the second portion of the duodenum. Furthermore, they become even fewer in number in the third portion and are minimally present in the fourth portion.  

**KEY WORDS:** Duodenum; Brunner’s duodenal glands; Duodenal submucosa; Duodenal histology.

**INTRODUCTION**

The existence of Brunner’s glands (BGs) in the duodenal submucosa is uncontestable (Bloom & Fawcett, 1994; Botros et al., 1990; Burkitt et al., 2000; Cormark, 1987). However, there remain doubts as to their exact location along the full extent of the duodenal wall, given that the opinions in the specialized literature regarding this are often incomplete or difficult to interpret (Coutinho et al., 1996; Farkas & Gero, 1989; Gartner & Hiatt, 2003).

Most studies on digestive system histology (and, more specifically, on the duodenum) regard BGs as mucous (and at times serous) glands located in the initial portions of the duodenal submucosa (Bloom & Fawcett; Botros et al.; Burkitt et al.; Cormark). Other authors situate them solely in the submucosa of the first few millimeters of the duodenum (Coutinho et al.; Farkas & Gero). Yet others consider the possibility of the glands being occasionally situated in the more distal parts of the duodenum (Giacosa, 1989; Junqueira & Carneiro, 2004). From the histophysiological point of view, BGs should produce an alkaline secretion (pH = 8.0-9.5) (Farkas & Gero) that is capable of, on the one hand, neutralizing the chymo acid that originates in the stomach, and on the other, supporting favorable pH conditions for adequate action by pancreatic juice enzymes (Krause, 2000; Leeson & Leeson, 1977). As it happens, the bile eliminated through the ductus choledochus, and also pancreatic juices eliminated through the principal and accessory pancreatic ducts, are disposed of in the second portion of the duodenum (Krause), where there are fewer BGs than in the first portion, thereby reinforcing the hypothesis that the main function of the BGs is to neutralize the gastric acidity (Leeson & Leeson; Lipski et al., 1992; Lockhart et al., 1959). Bearing in mind this apparent paradox, we sought to develop a study aimed at locating and quantifying the BGs, to enable better understanding of duodenal histophysiology.

**MATERIAL AND METHOD**

Our material consisted of 20 whole duodenums (from the pylorus to the duodenal-jejunal transition). These had been...
removed from 20 fresh adult cadavers, of both sexes, during the course of necropsies carried out in the Department of Pathology, School of Medical Sciences of Santa Casa de São Paulo. None of these cadavers presented any illness that might compromise the morphological integrity of the digestive system, as indicated through analysis of the clinical records and confirmed by the necropsy. After removal, each duodenum was immediately immersed in 10% formalin and taken to the Department of Morphology, where it was subsequently cut open and washed with running water. After complete inspection of the duodenal mucosa, which needed to be whole, small duodenal wall samples were removed with a scalpel (2 cm). The samples were obtained from each of the four anatomical portions of the duodenum (superior, descending, horizontal and ascending), in such a way that a segment encompassing the entire circumference of each duodenal portion was sampled. Each segment removed was then put back into 10% formalin, where it remained for 24 hours. The segments were then embedded in paraffin blocks. Following this, sections of five micrometers in thickness were cut and stained with hematoxylin-eosin. The BGs were analyzed quantitatively by means of the point counting technique, using a screen of 176 equidistant points, projected via a monitor showing the field to be quantified. Each sample was examined under 400x magnification. Twelve microscope fields were examined on every representative slide of each duodenal portion and underwent morphometric study through point counting, thus totaling 240 microscopic fields examined from each portion of the duodenum. The final result was expressed as the number of gland points per field (GPF), i.e. the number of points in each microscope field that were located over gland units. All results were tabulated for subsequent statistical study.

The SPSS program for Windows, version 10.0, was utilized for all statistical analyses. Comparison of the mean GPF values was performed via analysis of variance (ANOVA), utilizing Dunnet’s test for the post-hoc analysis. The level of significance was 5% for all tests.

RESULTS

The same analysis was performed on the second (descending) anatomical portion of the duodenum and BGs were found on every section studied. However, out of the 240 microscopic fields examined, BG were only observed in 214 of them (89.0%). Nonetheless, for 10 duodenums there were BGs in all of the 12 microscope fields examined. The mean GPF value for the second portion of the duodenum was 76.7 (sd = 25.9), thus indicating a lower concentration of mucous alveoli on the walls of the second portion of the duodenum (Fig. 2).

For the third (horizontal) portion of the duodenum, BGs were observed in 65 out of the 240 microscope fields examined (27.0%). None of the duodenums had BGs in all of the 12 microscope fields. On the other hand, there were no BGs at all, in any of the sections examined, for six duodenums. These results show the clear reduction in the concentration of gland alveoli in the third portion of the duodenum. The mean GPF value for the third portion of the duodenum was 19.8 (sd = 19.2) (Fig. 3).

The analysis of the fourth (ascending) portion of the duodenum showed the presence of BGs in only 28 of them (12.0%). As in the third portion, none of the duodenums had BGs in all of the 12 fields. BGs were only found in 10 of the duodenums. In one of these, BGs were present in eight of the 12 fields and, in another, in six of the 12. In the other eight duodenums with BGs, they

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were only present in one or two microscope fields. On the other hand, 10 of the duodenum had no BGs in any of the microscope fields. The mean GPF value for the fourth portion of the duodenum was 7.4 (sd = 11.4), thus indicating a clear reduction in the presence of mucous alveoli in this portion (Fig. 4). All these findings are summarized in Table I.
Differences in mean GPF were observed between the groups (p<0.001). The mean GPF for the first portion of the duodenum was significantly greater than for the second (p<0.001), third (p<0.001) and fourth (p<0.001) portions.

Likewise, the mean GPF for the second portion was significantly greater than for the third (p<0.001) and fourth (p<0.001) portions. There was no statistically significant difference in mean GPF between the third and fourth portions (p=0.103).
DISCUSSION

BGs are tubuloalveolar glands, with a secreting portion that is formed by mucous alveoli or, less frequently, serous alveoli (Bloom & Fawcett; Botros et al.; Burkitt et al.). Its excretory ducts penetrate the muscle mucosa and reach the base of the intestinal crypts, where they eliminate their secretion (even though they are able to eliminate their secretion through their own ducts, which reach the duodenal surface by going through its mucosa) (Morikawa et al., 1993). From this point, the secretion is discharged into the duodenal lumen. BG secretions are alkaline and fluid, and respond to parasympathetic vagal stimuli (Moore et al., 2000). Their alkalinity helps neutralize the chymo acid that originates in the stomach, and also provides optimal conditions for enzyme action on pancreatic juices (Morikawa et al.; Olsen et al., 1994). Some studies have demonstrated that somatostatin is able to inhibit BG secretions (Kirkegaard et al., 1984; Olsen et al.).

Most authors accept that BGs secrete bicarbonate. Nevertheless, because enterocytes of the duodenal mucosa also secrete bicarbonate, the part that BG plays in bicarbonate production is hard to quantify (Underwood, 1996).

The quantity of BGs in the first portion of the duodenum is sometimes so large that they warp the architecture of the villous pattern of the mucosa (Junqueira & Carneiro; Leeson & Leeson). Variation in the duodenal mucosa may lead to erroneous diagnosis in digestive endoscopy processes, and even in histopathological interpretations of biopsies performed in these areas.

Another possible BG function would be in the production of epidermal growth factor (EGF/urogastrone), which is a powerful gastric acidity inhibitor (Farkas & Gero) and also a stimulator for intestinal epithelium proliferation, thereby contributing towards post-traumatic duodenal mucosa regeneration (Stevens & Lowe, 1995). In addition to reducing the gastric acidity when released in the duodenal lumen, EGF is thought to play an important role in accelerating the turnover of epithelial cells in the intestinal coating (Schumacher & Krause, 1995).

Some authors have suggested that BGs secrete bactericidal enzymes (Coutinho et al.; Bohe et al., 1995) and could possibly be related to the transportation of immunoglobulins into the intestinal lumen, thereby contributing towards nonspecific defense mechanisms (Coutinho et al.).

A relatively recent study (Lipski et al.) using biopsies of duodenums from men aged between 22 and 70 years has indicated no significant change in BG morphometry with aging. However, some researchers have noted BG hyperplasia in peptic ulcer disease, chronic pancreatitis and chronic renal insufficiency, by means of endoscopic biopsy (Farkas & Gero; Fuse et al., 1990).

With regard to the location of BGs, there is a wide range of opinions, as already mentioned. Lockhart et al. located them from the pylorus to the papilla duodenal major, decreasing in frequency with increasing distance from the mouth. Leeson & Leeson situated them in the duodenal submucosa, mentioning that only occasionally were they to be found in the initial portion of the jejunum, while Cormack and Underwood advocated that BGs were located mostly in the initial portions of the duodenum. Bohe et al. reported that BGs in humans were located mainly in the proximal two-thirds of the duodenum. On the other hand, Stevens & Lowe regarded BGs as being characteristic of the first portion of the duodenum. Ross & Rowrell (1993), in their turn, stated that BGs were situated in the duodenal submucosa without, however, mentioning their distribution. The findings of Gartner & Hiatt supported the same opinion. Bloom & Fawcett stated that they started in the pyloric sphincter and diminished in size and number further along the duodenal wall, usually ceasing to be present in the third distal portion. Junqueira & Carneiro and Jansen et al. (2002) shared the same opinion. Burkitt et al. described BGs in the whole duodenum, without reference to their distribution within it. They also argued that BGs were not found anywhere else in the small intestine. Henken & Forouhar (1983) described a case with a BG tumor situated in the proximal ileum.

Our findings unequivocally show the presence of BGs all over the duodenal submucosa. In the first portion they are massively present, occupying the whole duodenal circumference. It is in this portion that the BGs are located in largest numbers. In the other portions of the duodenum, we saw there were statistically significant decreases in the quantities of gland alveoli in the duodenal submucosa towards the jejunum. In the second portion of the duodenum, the quantity of BGs is larger in the submucosa around the opening of the duodenal papilla and gradually diminishes towards the third portion. The greater quantities of BGs in the first two portions, especially in the first few centimeters of the duodenum, suggest the need for greater amounts of secretion from these glands, with the aim of neutralizing the greater acidity of food coming from the stomach.

Our results allow the following conclusions: 1) BGs are present in the submucosa of all portions of the duodenum. 2) The presence and quantity of BGs is greater in the first portion of the duodenum. Even though BGs are present in all the sections studied, the quantities found diminish significantly in comparison with the quantities in the first portion. 3) There is a gradual and significant decrease in the quantity of BGs in the third portion, and even more so in the fourth portion, where their quantity reaches a minimum.
RESUMEN: La presencia de las glándulas de Brunner en la submucosa duodenal es innegable, pero se desconoce su exacta distribución a lo largo de toda la extensión de la pared duodenal. El objetivo del presente estudio fue analizar la distribución de las glándulas duodenales de Brunner (GDB) en la submucosa de duodenos humanos. Para ello, se examinaron 20 duodenos normales en los que fueron seccionados 22 cm de cada porción duodenal, retirados con bisturí, de forma tal, que toda la circunferencia de cada segmento fuese extraída. Cada porción seleccionada fue preparada, teñida con hematoxilina-eosina y observada en 12 campos microscópicos diferentes. Las medias de los puntos glandulares por campo fueron computadas y comparadas para 12 campos microscópicos de cada porción del duodeno examinado. El primer segmento duodenal presenta un gran número de GDB en todos los campos microscópicos examinados. El segundo segmento también mostró la presencia de GDB, aunque el número encontrado fue menor. En el tercer segmento GDB fueron encontradas en 6 de los duodenos estudiados. En el cuarto segmento, el número de GDB fue aún menor y se encontraron solamente en 10 de los duodenos analizados. Así, la presencia de GDB ocurre en la submucosa de todos los segmentos duodenales y la mayor incidencia se encuentra en la primera porción, disminuyendo significativamente en la segunda, mucho más en la tercera, siendo mínima en la cuarta.

PALABRAS CLAVE: Duodeno; Glándulas duodenales de Brunner; Submucosa duodenal; Histología duodenal.

REFERENCES


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