Original Research Article

Histology and Histochemical study of Human Brunner's glands in comparison with few mammals

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Abstract

Background: Mucins are complex composition of carbohydrates and may be present as a mixture of different types. Mucins have been classified into neutral and acidic types, the latter being subdivided into sulphomucins and sialomucins. Normal distribu tion of mucin and its alteration in various inflammatory, benign and malignant lesions of gastrointestinal tract has aroused interest in the field of histochemistry **Objective:** Histology and Histochemical study of Human Brunner's glands in comparison with few mammals.. **Methods:** Samples were taken from duodenum of human, sheep and guinea pig, from dissected fresh specimens. The samples were washed in normal saline, fixed in 2% calcium acetate in 10% formalin. The tissues were routinely processed and paraffin blocks were prepared. 6µ sections of these blocks were taken for histological and different histochemical staining. **Results:** The H & E preparation showed the secreting cells of Brunner's glands in case of human, sheep and guinea pig are typically mucous in nature. The Brunner's glands of human duodenum secretes neutral mucosubstances, that of sheep secretes scanty neutral mucin and predominantly acid mucin. Guinea pig Brunner's glands secrete mixture of acid and scanty neutral mucins. The duodenal goblet cells in human, sheep and guinea pig secrete predominantly acid mucin (sulphomucin). **Conclusion**: The neutral mucosubstance is the predominant type in human Brunner's glands. Sheep Brunner's glands mainly secrete acid mucins., Guinea pig secrete both scanty neutral mucin and acid mucin. Goblet cells in human, sheep and guinea pig secrete acid mucin (sulphomucin).

Keywords: Mucins, Mucin histochemistry, Brunner's glands.

Introduction

Brunner's glands are regarded as being unique to mammalian species. Kuczynski (1890) classified mammalian species into three categories depending upon the extent of these glands whether short, moderate or long. Carnivores in general have short, omnivores have the moderate and herbivores have the long category.[1]Mucins are complex carbohydrate secreted by different types of epithelial cells and glandular tissues of respiratory and alimentary tract. There has been growing recognition in recent years that the demonstration of these substances is difficult, complex and affected by types of mucins present. Much of the interest in the duodenum is attributed to the frequency of the incidence of duodenal ulcer disease and duodenal carcinomas.

The several types of epithelial cells in the human duodenal mucosa produce different mucosubstances. Histochemical techniques provide a means of studying directly the cells of the duodenal mucosa and glands which produce the mucus protective barrier.

Mucins of the human gastrointestinal tract can no longer be thought of merely as a mechanical lubricant or an inert protective barrier. The increasing interest in mucins shared by molecular biologists, oncologists and pathologists led during the last few years to the accumulation of vast knowledge about the biological significance of these secretions. Mucins reflect in their composition changes in the functional state of the mucosa in health and disease. [2]

The composition, secretion and topographical characteristics of duodenal and colonic mucins have been studied extensively. There are, however only few works, which give detailed characteristics of mucins secreted in different segments of gastrointestinal tract. [3]

Although there have been many efforts of isolation and chemical characterization of mucin, lacunae still persist in many areas and the field is wide open for further exploration.

The combination of available histochemical and biochemical information should make it possible to relate changes at the cellular level with those which take place at the molecular level. Hence a correlation will be made, whenever possible, between histochemical and biochemical knowledge about gastrointestinal mucins in health and disease. [4]

Histochemical methods have proved to be adjuvant to the routine pathological diagnosis. The significance of these techniques is not only for diagnostic purposes but also reveals the physiological process in human secretory epithelial cells and their variations in disease.

The main purpose of the present work is histology and histochemical study of Human Brunner's glands in comparison with few mammals

Materials & Methods

Collection of specimens

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1) 10 Fresh specimens of duodenum obtained from mortuary, Chigateri General Hospital, Davangere.
2) 8 Surgically resected specimens of duodenum collected from the department of Pathology, J.J.M. Medical College, Davangere.
3) 16 Specimens from mammal like Guinea pig obtained from Animal House, J.J.M. Medical College, Davangere, after the death of animal.
4) 16 Specimens from mammal like sheep collected from Slaughter house, Davangere.

Inclusion criteria

1) Specimens from postmortem cases of hanging and road traffic accidents.
2) Normal duodenum.

Exclusion criteria

1) Person died after prolonged hospital stay
2) Postmortem cases of poisoning, snake bite and burns.
3) Ulcerated and inflammed areas of duodenum

Methods

- The samples were taken from different sites of duodenum like first, second, third and fourth part of duodenum.
- The samples were washed in normal saline and put into 2% calcium acetate in 10% formalin for fixation.
- From fixed samples tissue bits were taken.
- The bits were placed in tissue capsules with the label and then processed by standard method. First they were subjected to a process of dehydration by serial passage through ascending grades of alcohol.
- The dehydrated bits were then cleared in xylol. Finally they were put in paraffin bath kept at 65°C for filtration.
- The paraffin infiltrated bits were then embedded in paraffin wax using L blocks. The paraffin blocks were then trimmed.
- After trimming the blocks, they were cut serially at 4 µ thickness and mounted on slides.
- The slides were incubated at 60°C for 1-2 hours, 2 slides from each bit were stained with routine haematoxylin and eosin method and the rest with special stains.

Staining methods used in the study:

1) Haematoxylin and Eosin Staining method:

- Sections deparaffinised by putting in Xylol.
- Hydrated by using descending grades of alcohol (100%, 90%, 70%) for 1 minute each.
- Washed with distilled water
- Stained 4-8 minutes in Harris hematoxyline solution.
- Washed in running tap water for 1 hour until the blue colour developed.
- Stained in eosin for 25 to 45 seconds.
- Dehydrated using ascending grades of alcohol (70%, 90%, 100%) for 1 minute each.
- Cleared with xylol.
- Sections then mounted in DPX.

Results : Nucleus – Blue
Cytoplasm – Pale pink.[5][43]

2) Alcian Blue staining

- More specific for acid mucins
- Basis for AB staining is by salt linkage with the acidic groups of acid mucopolysaccharide.

Alcian Blue at pH 1:

- Specific stain for sulphate groups

Solutions :
Alcian Blue 8GX – 1 g
0.1 M hydrochloric acid – 100 ml

Alcian Blue at pH 2.5 :

Solution :
Alcian Blue 8GX – 1g
3% glacial acetic acid – 100 ml

3) Periodic Acid Schiff (PAS)

- To identify neutral mucopolysaccharides.

Schiff reagent : Dissolve 1gm of basic fuchsin in 200ml of boiling distilled water, removing the flask of water from the Bunsen flame just before adding the basic fuchsin.
Allow the solution to cool to 50°C. Add 2g of sodium metabisulphite. Add 2gm of activated charcoal and leave overnight in the dark at room temperature. Solution should be clear or pale yellow. Filter and store the solution at 0-4°C.

Solutions :

a. Periodic acid solution
Periodic acid - 1g
Distilled water – 200ml
b. Schiff reagent

4) Combined Alcian Blue pH 2.5 – PAS

- Acid and neutral mucins are clearly separated by this technique.
- The rationale is that by first staining all acid mucins with Alcian Blue, those acid mucins which are also PAS – positive will not react in the subsequent PAS reaction, only the neutral mucins will.

Solutions :

- Alcian Blue pH 2.5 solution
- 1% aqueous Periodic Acid
- Schiff's reagent

5) Aldehyde Fuchsin technique

Aldehyde Fuchsin solution

- Basic Fuchsin – 1 gm
- Paraldehyde – 2ml
- Concentrated HCl – 1ml
- Ethanol – 60ml
- Distilled water – 40ml

Dissolve the basic Fuchsin in the alcohol – distilled water. Add hydrochloric acid and paraldehyde. Allow to 'ripen' for 2-7 days at room temperature, then filter. Store at 4°C.

6) Combined Aldehyde Fuchsin – Alcian Blue pH 2.5

- This technique is a reliable mean of separating sulphated from carboxylated mucins.
- The rationale depends on the greater affinity of aldehyde Fuchsin for sulphated mucins, so that by first staining with this solution they are stained purple and by subsequently counterstaining with Alcian Blue, the carboxylated forms only will be stained blue.

Solutions :

- Aldehyde Fuchsin solution
- Alcian Blue pH 2.5 solution

Statistical methods used: The results of present study were expressed as number and percentage to compare. Analysis was done using SPSS (statistical presentation system software) package version 16.

Results: Duodenal villi with intestinal glands staining H and E (Crypts of Lieburkuhn) seen. Submucosa consists of Brunner's glands, lined by columnar cells with basal flattened nuclei. The ducts of few glands are opening into lumen of crypts. Fig 1

Submucosal branched tubuloalveolar Brunner's glands seen, lined by columnar cells with eosinophilic cytoplasm and basal flattened nuclei. Fig 2

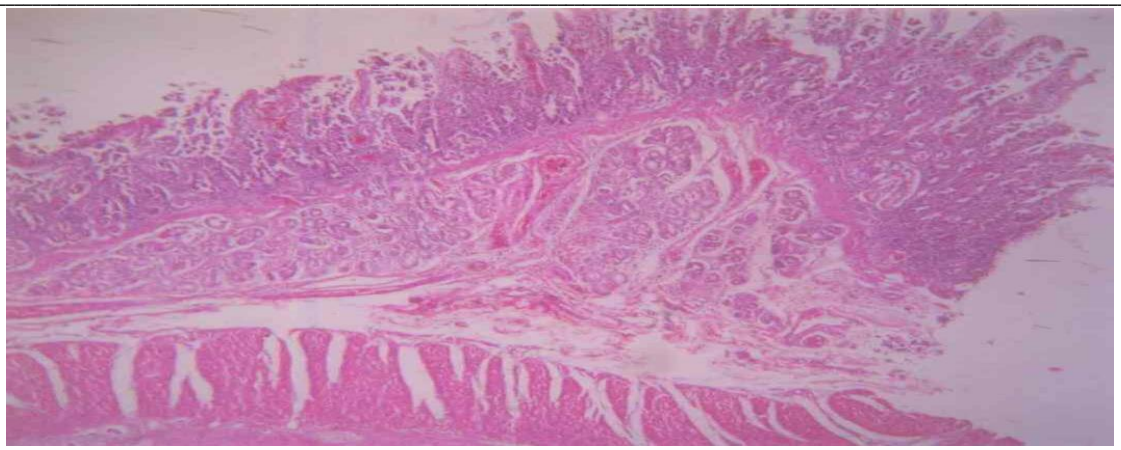

Fig.1: Human duodenum stained with H&E (Magnification X 40)

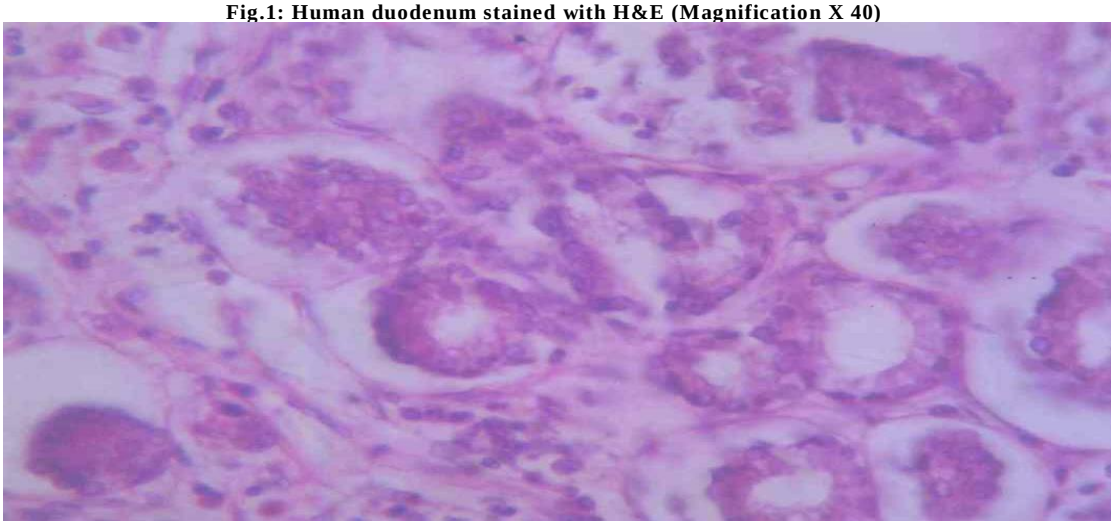

Fig.2: Human duodenal Brunner's glands stained with H&E(Magnification X 400)

Numerous villi with goblet cells are seen. Submucosa present tubuloalveolar Brunner's glands with basal flattened nuclei. Fig 3 Tubuloalveolar Brunner's glands seen. Lining epithelium of glands consists of columnar cells, their cytoplasm stained with eosinophil and basal flattened nuclei seen. Fig 4

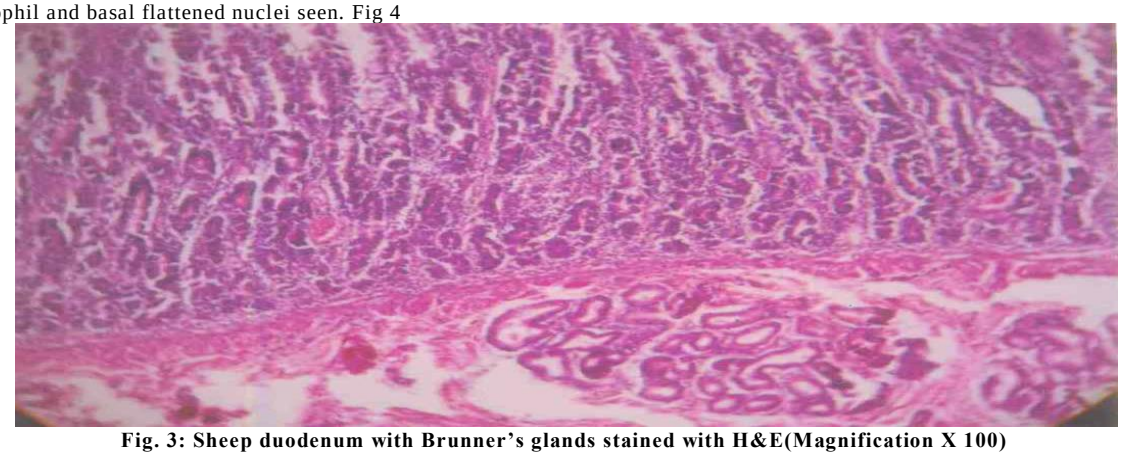

Fig. 3: Sheep duodenum with Brunner's glands stained with H&E(Magnification X 100)

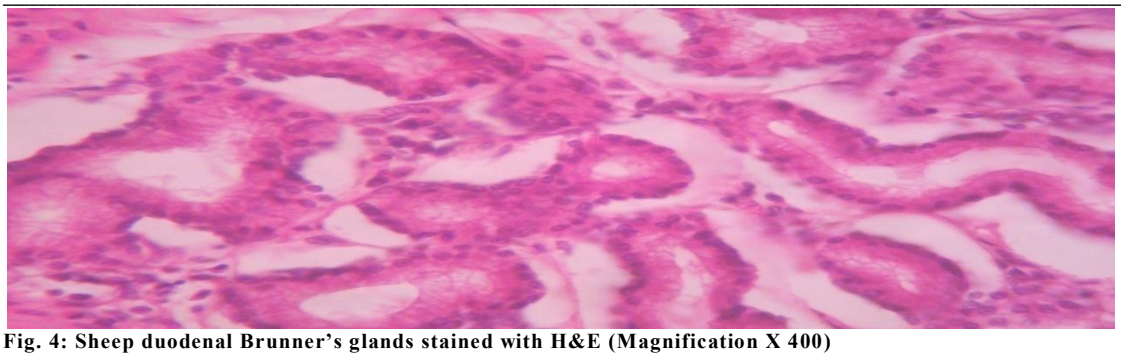

Fig. 4: Sheep duodenal Brunner's glands stained with H&E (Magnification X 400)

Table 1:Distribution of mucin in human duodenal brunner's glands and goblet cells of villi

Sl. No.	Histo tech	Brunner's glands	Goblet cells
1	PAS	+++ M	++ M
2	AB pH 1	-ve	+++ B
3	AB pH 2.5	-ve	++ B
4	AB pH 2.5 – PAS	+++ M	+++ B
5	AF	-ve	+± P
6	AF-AB pH 2.5	-ve	++ B

1)Goblet cells stained with magenta with PAS and showed alcinophilia indicating presence of PAS +ve substance i.e. acid mucin.
2)Brunner's glands showed PAS +ve substance.
3)Brunner's glands stained –ve with Alcian blue and AF, AF-AB pH 2.5, confirms presence of neutral mucin.
4)Brunner's glands stained with magenta with AB pH 2.5 – PAS

Table 2:Distribution of mucin in sheep duodenal brunner's glands and goblet cells of villi

Sl. No.	Histo tech	Brunner's glands (BG)	Goblet cells
1	PAS	++± M	++ M
2	AB pH 1	-ve	++ B
3	AB pH 2.5	+± B	+++ B
4	AB pH 2.5 – PAS	++± M ± P	+++ B
5	AF	± P	++ P
6	AF-AB pH 2.5	+++ BP	++ B

1) Goblet cells stained with magenta and showed alcinophilia, so goblet cells contain acid mucin.
2) Brunner's glands show presence of mixture of sulphomucin and sialomucin.
3) Glands also show presence of scanty amount of neutral mucin.

Table 3:Distribution of mucin in guinea pig duodenal brunner's glands and goblet cells of villi

Sl. No.	Histo tech	Brunner's glands (BG)	Goblet cells
1	PAS	± M	++ M
2	AB pH 1	++± B	++ B
3	AB pH 2.5	++± B	+++ B
4	AB pH 2.5 – PAS	+± B ± P	+++ B
5	AF	± P	++ P
6	AF-AB pH 2.5	+++ B ± P	++ B

1)Goblet cells staining with magenta and alcinophilia goblet cells mainly contain acid mucin.
2)Brunner's glands show mixture of acid and neutral mucin.
3)Brunner's glands show presence of mixture of sialomucin and sulphomucin, with predominance of carboxymucin.

Discussion: The present work is undertaken to study the Brunner's glands in human duodenum in comparison with Brunner's glands of sheep and guinea pig, to findout the mucins present in their secretions by employing various histochemical techniques. An attempt has also been made to include duodenal goblet cells in our study to determine the nature of mucins present in their secretions.

The finding of our study reveals that the secreting cells of Brunner's glands in human are typically mucous in nature (Fig. No.1). The findings are in agreement with that of Grossman MI (1958) and Daniel G. Sheahan et al., (1976). [6,7] This was also confirmed by Leeson and Leeson (1968), Riva A and Zaccheo, D (1968) by electron microscopic study. [8,9]

The Brunner's glands in human showed a magenta colour when they were treated with PAS, indicating the presence of PAS positive material in their secretions (Table No.1). The findings are in agreement with that of Leeson CR (1968).[8] The duodenal goblet cells showed a PAS positive reaction indicating the presence of PAS +ve material in their secretions.

The Alcian blue staining method is generally regarded as being specific for identifying acid mucosubstances. Our study on Brunner's glands in human (Fig. No.3&4) showed a negative staining reaction when treated with Alcian blue at pH1.0 and pH 2.5. It indicates the presence of neutral mucosubstances in their secretions. The findings are in agreement with that of Belanger (1963) who also obtained similar results in case of human duodenum. [10] The duodenal goblet cells showed alcinophilia i.e., stained with blue indicating presence of acid mucosubstances.

Neutral mucosubstance secreted by human Brunner's glands and small amount of acid mucin secreted by goblet cells play a important role in the protection of duodenal mucosa as pH changes from acid to alkali in duodenum (Gad A 1982).[4]

The Brunner's glands of human are stained magenta colour, when treated with AB pH 2.5 – PAS (Table No.1) indicating presence of neutral mucosubstances. This finding is correlating to that of Sirugu, P and Riva, A (1968), Berlin et al., (1970), S. Willems G et al., (1970), Daniel G. Sheahan et al., (1976).[11-13, 7] Goblet cells stained with blue indicating presence of acid mucosubstances.

The Brunner's glands in human showed a negative reaction when treated with AF technique (Table No.1) indicating the presence of neutral mucosubstances. The majority of the goblet cells are weakly stained with purple showing presence of nonsulphated acid mucosubstances. These findings are similar to that of S. Willems et al., (1970). [13]

When treated with AF-AB pH 2.5 technique (Table No.1), the Brunner's glands in human showed a negative reaction. It indicates the presence of neutral mucosubstances. Majority of the goblet cells in human are stained blue indicating the presence of nonsulphated, carboxylated.

When treated with AF-AB pH 2.5 technique (Table No.3). Brunner's glands of guinea pig showed moderate alcinophilia and weakly purple staining indicating mixture of sialomucin and sulphomucin with predominance of carboxylated mucin. Goblet cells showed alcinophilia. Earlier reports (Daniel G. Sheahan et al., 1976). [7] demonstrated qualitative differences in the content of mucosubstances between deeper and superficial glands. The deeper glands contain mixtures of neutral and sulphated (sulphomucin) acid mucosubstance correlating well with our finding.

By knowing normal histochemical staining in duodenum and changes in different diseases, may be valuable in the early detection for cancer and also help to identify primary and secondary metastasis. Gross alteration in intestinal flora and impairment of mucin degradation by association of drugs is important or challenging field for the histochemists, Pathologists and Pharmacologist. This work may help in the detection for the precancerous lesions. From the present work it is also evident that we must seek to develop a variety of controllable models for the study of functional parameters of mucus in physiological conditions, parasite rejection, neoplasia and inflammatory status.

Comparative study of mucins in humans, sheep and guinea pig may help to understand pathophysiology and its role in carcinogenesis. Different types of mucin in different parts of gastrointestinal tract may help to understand its role and dietary habitats, pathological changes. The study of histochemical mucin changes in different pathological condition may help in diagnostic medicine as an adjuvant technique and also give valuable information about pathogenesis.

Conclusion: The following conclusion can be drawn after observing the results of present study.

- The H & E preparation showed the secreting cells of Brunner's glands in case of human, sheep and guinea pig are typically mucous in nature.
- The Brunner's glands of human duodenum secretes predominantly neutral mucosubstances, no acid mucin made out. The goblet cells of villi showed predominant acid mucins (sulphomucins) .
- The cells of sheep Brunner's glands secretes scanty amount of neutral mucin and predominantly acid mucin, that is mixture of sialomucin and sulphomucin. Goblet cells of villi secretes predominantly acid mucin (sulphomucin) .
- The Brunner's glands of guinea pig duodenum secrete mixture of acid and scanty neutral mucins. The secreted acid mucin contain mixture of sialomucin and sulphomucin (Table No.9) with predominance of carboxymucin. The goblet cells of villi secrete acid mucin (sulphomucin).
- Knowledge about mucin secreted in gastrointestinal tract reveals the physiological process in human secretory epithelia and their variation in disease.

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