Phenotype characteristics of gastric epithelial mucus in patients with different gastric diseases: from superficial gastritis to gastric cancer

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Background Gastric gland mucin is important for maintaining the basic function of the gastric mucosa, protecting it from foreign substances and reducing the occurrence of gastric diseases. Exploring the phenotype of gastric gland mucus changes during the progression of gastric disease is of great clinical significance. **Methods** A total of 483 patients with different gastric diseases were collected in this study, including 82 superficial gastritis (SG), 81 atrophic gastritis (AG), 168 dysplasia (GD), and 152 gastric cancer (GC). Mucin staining was performed using HID-ABpH2.5-PAS method and was further grouped according to the mucin coloration. **Results:** The phenotypic characteristics of mucin during disease progression were divided into neutral, acidic, and mucus-free types. Furthermore, acidic mucus can be divided into type I, type II, and type III. The SG group was dominated by neutral mucus (100%), and the AG was dominated by acid mucus (81.48%), which gradually increased with the severity of atrophy ($P<0.05$). The GD and GC groups were dominated by mucus-free (43.45%, 78.29%), and as the degree of GD worsened, neutral and acidic mucus gradually decreased and mucus-free increased ($P<0.001$). From the SG, AG, GD, and GC progression, neutral and acidic mucus gradually decreased, and mucus-free gradually increased. Acidic mucin revealed that type III (red-brown black) mucin was predominant in AG, GD, and GC, and increased with the degree of AG, GD, as well as the biological behavior of GC. In the lesion adjacent to high-grade GD or GC, type III acid mucin is predominant. **Conclusion** There were three mucin phenotypes in the process of gastric diseases. With the disease progression, the trend of phenotypic change was that neutral and acidic mucus gradually decreased and mucus-free increased. The appearance of type III mucin suggested a relatively serious phase of gastric diseases and may be a more suitable candidate for follow-up monitoring of patients with GC risk.

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2 **gastric diseases: from superficial gastritis to gastric cancer**

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31 **Abstract**

32 **Background**:Gastric gland mucin is important for maintaining the basic function of the gastric

33 mucosa, protecting it from foreign substances and reducing the occurrence of gastric diseases.

34 Exploring the phenotype of gastric gland mucus changes during the progression of gastric disease

35 is of great clinical significance.

36 **Methods**:A total of 483 patients with different gastric diseases were collected in this study,

37 including 82 superficial gastritis (SG), 81 atrophic gastritis (AG), 168 dysplasia (GD), and 152

38 gastric cancer (GC). Mucin staining was performed using HID-ABpH2.5-PAS method and was

39 further grouped according to the mucin coloration.

40 **Results:** The phenotypic characteristics of mucin during disease progression were divided into

41 neutral, acidic, and mucus-free types. Furthermore, acidic mucus can be divided into type I, type

42 II, and type III. The SG group was dominated by neutral mucus (100%), and the AG was dominated

43 by acid mucus (81.48%), which gradually increased with the severity of atrophy ($P<0.05$). The

44 GD and GC groups were dominated by mucus-free (43.45%, 78.29%), and as the degree of GD

45 worsened, neutral and acidic mucus gradually decreased and mucus-free increased ($P<0.001$).

46 From the SG, AG, GD, and GC progression, neutral and acidic mucus gradually decreased, and

47 mucus-free gradually increased. Acidic mucin revealed that type III (red-brown black) mucin was

48 predominant in AG, GD, and GC, and increased with the degree of AG, GD, as well as the

49 biological behavior of GC. In the lesion adjacent to high-grade GD or GC, type III acid mucin is

50 predominant.

51 **Conclusion**:There were three mucin phenotypes in the process of gastric diseases. With the

52 disease progression, the trend of phenotypic change was that neutral and acidic mucus gradually

53 decreased and mucus-free increased. The appearance of type III mucin suggested a relatively

54 serious phase of gastric diseases and may be a more suitable candidate for follow-up monitoring

55 of patients with GC risk.

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57   **Key words:** gastric mucus,gastric disease,HID-ABpH2.5-PAS

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60   Mucins, a family of large and heavily glycosylated proteins, represent the main component of this

61   hydrophilic gel-like mixture, and act as key molecules in the maintenance of gastrointestinal

62   homeostasis [1, 2] and protect the gastric mucosa from the damage from external environment[3, 4].

63   These mucins are divided into two subtypes: surface mucin and gland mucin[5, 6]. The former is

64   secreted from the surface cells of the gastric mucosa, while the latter is located in the lower layer

65   of the gastric mucosa from the gland mucous cells. The stomach wall covered with surface mucus

66   will not be directly exposed to the gastric juice environment with strong acid and proteases. Under

67   normal circumstances, surface mucin can reduce gastric mucosal irritation caused by food friction,

68   resist pepsin damage to gastric mucosa and reduce H^+ penetration to maintain the mucus-

69   bicarbonate barrier function[7]. Surface mucus is also important in drugs absorption process,

70   namely for those that are gastric pH-sensitive[8]. Gastric gland mucins are derived from pyloric

71   glands, mucus neck cells, and fundus gland cells[9]. These mucus are important for maintaining the

72   basic function of the gastric mucosa, protecting against foreign substances, and reducing the

73   occurrence of gastric diseases[10].

74   The occurrence of GC is due to the chronic inflammation of the gastric mucosa under the long-

75   term effect of pathogenic factors, which can further cause AG, IM(intestinal metaplasia), GD and

76   GC[11] following the Correa's Model. Exploring the changes of mucus phenotype during the above

77   pathological process is of great significance to reveal the occurrence and development of gastric

78   diseases. It has been reported that expression and glycosylation of mucins are associated with

79   gastric carcinogenesis including invasion, proliferation and regulation of tumor cells[12-15]. Several

80   studies have suggested that the type of mucin expressed in early and advanced gastric carcinomas

81   is of clinical significance, alluding to biologic differences in precursor lesions and/or pathways of

82   malignant transformation[16-19]. Accumulating evidence has indicated that different mucin

83 phenotypes of GC have distinct clinical characteristics and exhibit specific genetic and epigenetic

84 changes [20]. Thus, mucin phenotype classification is useful to understand GC pathogenesis.

85 However, it is not exactly clear how the phenotype of gastric gland mucus changes during the

86 progression of gastric disease.

87 In this study, we used AB-PAS mucus staining method to explore the changes of mucus

88 phenotype during the occurrence and development of gastric diseases, which would provide clues

89 and help to reveal the occurrence and development of gastric diseases[21].

90 **Material and Methods:**

91 **1) The subjects**

92 A total of 483 patients with different gastric diseases were included in this study, among them

93 283 patients underwent gastroscopic biopsy and 118 patients underwent endoscopic submucosal

94 dissection (ESD) surgery at the Endoscopy Center of the First Affiliated Hospital of China Medical

95 University from July 2014 to March 2018. In addition, 82 cases from the on-site GC screening

96 project in Zhuanghe county, Liaoning province from January 2014 to December 2014. They were

97 diagnosed as 82 SG, 81 AG, 168 GD, 152 GC, including 299 males and 184 females, with an

98 average age of 60.25 years old. The *H. pylori* infection was detected using ASSURE *Hp* rapid test

99 (MP Diagnostics[TM]). The histopathological diagnostic criteria of the subjects referred to the

100 updated Sydney classification system. This study was approved by the Ethics Committee of the

101 First Affiliated Hospital of China Medical University, and the subjects signed informed consents

102 ([2013]135 and 2016[161]).

103 **2)HID-ABpH2.5-PAS mucin histochemical staining**

104 The specific steps of HID-ABpH2.5 PAS mucin histochemical staining followed the

105 instructions of the kit (kit was purchased from Beijing Regen Biotechnology Co., Ltd.). In short,

106 after dewaxing to water, the slides were placed in the solution (A1:A2=50:3), away from light for

107 18-24 hours at 24℃-28℃. After that, stained with Alcian blue for 20 minutes, and next incubated

108 with periodic acid for 30 seconds, and then schiff reagent was added for 2 minutes, and at last the

109 slides were acidified, dehydrated and mounted. Neutral mucus material is red, acidic mucus

110 material is blue, mixed mucus material is blue-purple or purple-blue, and the nucleus is brown.

111 **3)The phenotypes of different acid mucus**

112  In acid mucus, sialic acid mucus is blue, and sulfuric acid mucus is brownish black. Further,

113 according to the different coloration of acid mucin, the gastric mucosa is divided into type I (blue

114 or brown-black), type II (red-blue) and type III (red-brown black). If there is more than one mucus

115 phenotype in the lesion, the most important mucus phenotype is classified.

116 **4) Statistical analysis**

117  SPSS 16.0 software was used for statistical analysis. $P<0.05$ was statistically significant. The

118 descriptive statistics of continuous variables are mean, standard deviation, minimum and

119 maximum, while categorical variables are count and percentage. Chi-square test or Fisher's exact

120 test were used to determine the statistical association between categorical variables, and t test was

121 used to determine the statistical association between continuous variables.

122

123 **Results:**

124 **1) The baseline characteristics of the subjects**

125  A total of 82 SG, 81 AG, 168 GD, and 152 GC were included in this study, the average age

126 and gender composition of each group were shown in Table 1, of which the SG group was younger

127 compared with other groups, with statistical significance ($P=0.000$). In terms of male and female

128 composition, the difference between the groups was not statistically significant ($P=0.067$).

129 **2)Mucous phenotype characteristics of different gastric diseases**

130  We compared the mucin staining of mucosal tissues in different gastric diseases, as shown in

131 Table 2 and Figure 1. Of the total 483 patients, 135 (27.89%), 154 (31.82%), and 195 (40.29%)

132 were neutral, acidic mucus, and mucus-free respectively. Among them, 100% of the SG group was

133 neutral mucus. The patients with AG were mainly acid mucus, accounting for 81.48%, followed

134 by neutral mucus accounting for 14.81%, and the difference between the groups was statistically

135 significant ($P=0.000$). The patients with GD were mainly without mucus (43.45%), followed by

136 acidic mucus (35.12%) and neutral mucus (21.43%), the difference between the groups was

137   statistically significant (*P*=0.000); patients with GC were similar to patients with GD, and mainly

138   stained without mucus (78.29%), the rest were neutral mucus (2.63%) and acid mucus (19.08%).

139   The difference between the groups was statistically significant (*P*=0.000).

140   In the comparison between different disease groups, the expression of neutral mucus

141   gradually decreased from SG, AG to GD and GC, with a positivity of 100%, 14.81%, 21.43% and

142   2.63%, respectively, and the difference between the groups was statistically significant (*P* =0.000).

143   The expression of acidic mucus gradually decreased (81.48%, 35.12% and 19.08%) respectively,

144   the difference between the groups was statistically significant (*P*=0.000). Those mucus-free

145   expression gradually increased (3.71%, 43.45% and 78.29%) respectively, the difference between

146   the groups was statistically significant (*P*=0.000). It can be seen that SG was mainly composed of

147   neutral mucus, AG was mainly expressed by acidic mucus, and GD and GC tissues were mainly

148   expressed by mucus-free. With the progress of gastric disease, neutral and acidic mucus expression

149   gradually decreased, and those without mucus expression gradually increased.

150   **3)Subgroup analysis of mucous phenotype characteristics of different gastric diseases**

151   Further, we analyzed mucin staining in different subgroups of AG, GD, and GC. The results

152   were shown in Table 3. In AG, there was no statistically significant difference in the percentage

153   of neutral mucus and acid mucus in the antrum, corpus and angle of stomach (*P*=0.874). In mild

154   atrophy, neutral and acid mucus accounted for 33.33 and 53.33%, respectively. The acid mucus of

155   moderate and severe AG were 85.11% and 94.74%, respectively, higher than that of mild AG, and

156   the difference between the groups was statistically significant (*P*=0.02). In the low-grade of GD,

157   neutral, acidic mucus, mucus-free were 34.38%, 50.00%, 15.62%, and in the high-grade were

158   4.17%, 15.28%, 80.56%, the difference between the groups was statistically significant (*P*=0.000).

159   In GC, mucus-free was the highest in intestinal-type, followed by diffuse-type, unclassifiable type

160   and mixed-type, but the difference was not statistically significant (*P*=0.5). It can be seen that in

161   AG, acid mucus gradually increases with the severity of gastric disease, in GD, as the degree

162   increasing, neutral and acid mucus gradually decreased, and no mucus increased. Among various

163   histological types of GC, those with no mucus were the highest.

164 **4) The analysis of mucous phenotype characteristics of different *H. pylori* status**

165   Among the AG, GD, and GC samples included in this study, there were 151 patients with *H.*

166   *pylori* infection information. We compared the relationship of *H. pylori* infection status and

167   different mucus phenotypes in the overall disease group and among the three disease groups,

168   respectively. As shown in table 4, the results indicated that in the overall disease group, there was

169   no difference in mucus phenotype between the *H. pylori* positive and negative groups (*P*=0.724).

170   However in AG subgroup,the *H. pylori* positive group was mainly acidic mucus type (72.97%),

171   and the *H. pylori* negative group was mainly neutral mucus (100%). There was no difference in

172   mucus phenotype between *H. pylori* positive and negative groups in GD, GC subgroups

173   (*P*=0.879,0.819).

174 **5) The distribution of acidic mucus phenotype in different diseases**

175   We further conducted a comparative analysis of acidic mucus phenotypes among different

176   gastric diseases, including AG, GD and GC, as shown in Table 5 and Figure 2. The results showed

177   that the acidic mucus phenotype can be further subdivided into three subtypes: type I, sialic acid

178   or mucin sulfate; type II, neutral and acidic sialic acid mucin, occasionally, mucin sulfate, or both;

179   type III, neutral and sulfuric acid mucus mainly, occasionally, sialic acid mucin, or both.

180   Among 66 cases of AG, there was 11 cases of type I (16.67%), 10 cases of type II (15.15%)

181   and 45 cases of type III (68.18%); in 59 cases of GD, there was 3 cases of type I (5.08), 12 cases

182   of type II (20.34%) and 44 cases of type III (74.58%); in terms of 29 cases of GC, there were 2

183   cases of type I (6.9%), 6 cases of type II (20.69%) and 21 cases of type III (72.41%).

184   Further stratified according to location and degree in AG, there was no statistically significant

185   difference in mucin subtype (*P*=0.348, *P*=0.505). In the 59 cases of GD, including 48 cases of low-

186   grade and 11 cases of high-grade, type III were the mainly subtype, especially with 100% in the

187   high-grade (*P*=0.000). There were 12 cases of intestinal-type GC and 11 cases of diffuse-type GC

188   with mucin subtype, all of which were mainly type III, and the difference between the groups was

189   not statistically significant (*P*=0.091).

190 **6)The comparation of acid mucus phenotypes in the lesion adjacent to high-grade GD or**

191 **GC**

192   There were 112 cases of IM nearby the above-mentioned high-grade GD and GC. In order to

193 further analyze the correlation between different mucus phenotypes and high-grade GD or GC. As

194 shown in Table 6 and Figure 3, in high-grade GD, intestinal and diffuse GC, type III acid mucin

195 is predominant, without statistically significant difference between groups (P=0.714).

196

197 **Discussion:**

198 Current research findings suggest that mucin subtypes and clinical relevance are reported

199 differently in the literature, but most of the data always support that different mucin subtypes are

200 associated with the risk of intestinal gastric adenocarcinoma [16-19]. That is to say, the progression

201 of gastric precancerous lesions to GC is a slow and gradual development process, at least targeted

202 monitoring can be performed by mucin typing, and there is an opportunity to detect and remove

203 tumorous lesions early. Although there are currently no clinically deterministic predictive

204 biomarkers in clinical use, the use of mucin staining for subtype analysis can further stratify people

205 who are already at risk[22]. In this study, we explored the changes of different mucus phenotypes

206 during the progression of gastric disease.

207   Usually, the gastric epithelium, pyloric gland, and duodenal gland mainly secrete neutral

208 mucus[23]. The goblet cells and intestinal glands of the small and large intestine mucosa mainly

209 secrete acidic mucus[24]. Under normal physiological conditions,the staining of gastric mucin

210 showed red neutral mucin, but in some pathological conditions, such as IM, carcinogenesis, etc.,

211 the characteristics of gastric mucin staining can change with the specific state. *Kazuhiro Yamanoi*

212 *et al*. reported that the reduction of gastric mucus is related to the high mitotic activity of tumor

213 cells, which represents an increase in malignant potential[25]. The results of our study showed that

214 in the SG, neutral mucus was dominant, but in AG with IM, acid mucus was mainly expressed,

215 which produced by intestinal metaplasia glands. In the GD and GC, it was mainly expressed as

216 mucus-free phenotype. During the progression of gastric diseases from SG, AG, GD, and GC, the

217   expression of neutral and acidic mucus gradually decreased, mucus-free expression gradually

218   increased. From this we can speculate that the disappearance of epithelial mucus phenotype may

219   be one of the high-risk factors for the progression of gastric disease.

220   *H. pylori* persistent infection of the normal gastric mucosa triggers a chronic inflammatory

221   process designated by chronic gastritis. The presence of virulent *H. pylori* strains together with

222   host immune vulnerability can lead to severe mucosal atrophy with focal loss of gland architecture

223   and disease progression[26]. In fact, the development of IM originates multiple foci where

224   superficial foveolar cells with neutral mucin expression are gradually replaced by acidic producing

225   cells with an intestinal phenotype[27]. In our study, we found in AG subgroup,the *H. pylori*

226   positive group was mainly acidic mucus type and the *H. pylori* negative group was mainly neutral

227   mucus. There was no difference in mucus phenotype between *H. pylori* positive and negative

228   groups in GD, GC subgroups. It was the truth that with the progress of IM, the colonization of *H.*

229   *pylori* gradually decreased, especially in the stage of GC, there was basically no colonization[28],

230   so AG was dominant with acid mucus in *H. pylori* positive.

231   The main feature of intestinal epithelium is acid mucin staining while once IM occurs in the

232   gastric mucosa, acidic mucin can be shown in gastric epithelium. Acid mucin can be divided into

233   sialic acid or sulfated mucin, which can be used to evaluate two types using combined HID-AB

234   staining,and the latter is brown stained with ferric diamine (HID). *Filipe et al.*[29]used this method

235   to distinguish mucin phenotypes:type I expressed only salivary mucin, type II expressed a hybrid

236   form of a mixture of gastric mucosa and intestinal mucin, and type III expressed neutral and

237   sulfated mucin. It is still not clear whether there is a chronological relationship between the

238   expression of these three types of mucins[24, 30, 31]. During the initial development of gastric

239   diseases with IM, the neutral mucin in normal mucosa gradually decreased, while salivary mucin

240   appeared and became the main type of mucin. In the serious stages of IM, sulfate mucin appeared

241   and may become the main ingredient. In this study, we detected acid mucin in AG, GD, and GC,

242   and found that type III mucin was the main type in the three types of lesions, and increased with

243   the change of the IM degree, the GD grade and the biological behavior of GC. In lesion next to

244 high-grade GD, intestinal-type and diffuse GC, the type III acidic mucin is predominant. The

245 emergence of type III mucin is a progressive stage of IM, which can become the best candidate for

246 follow-up monitoring of patients with GC risk. In addition, based on a meta-analysis of 7 studies,

247 including patients with GIM in 2014 and no accompanying dysplasia (929 patients with type II or

248 III mucin, and 1112 patients with type I mucin), these patients ranged from 3-12.8 years of GC

249 developed during the follow-up period. Compared with individuals of type I, the risk of GC was

250 3.33 times higher (95% confidence interval, 1.96-5.64)[32, 33]. Consistent with the AGA (American

251 Gastroenterological Association) guideline[34], the recent ESGE(European Society of

252 Gastrointestinal Endoscopy) guideline also mentioned that mucin staining has prognostic value,

253 which is contrary to the 2012 ESGE guideline that opposes GIM subtype guidance for prognosis[35,

254 36]. Considering the potential prognostic value and the opportunity to generate stronger clinical and

255 epidemiological evidence, as well as the pathologist's minimum cost and effort, recommendations

256 for routine typing of mucin are advisable.

257   From a histochemical point of view, the pH value of normal gastric mucin is neutral, and they

258 are dyed red with periodate-Schiff (PAS). Chronic AG is histologically characterized by chronic

259 inflammation of the gastric mucosa, with decreasing number of gastric gland cells and increasing

260 intestinal metaplastic glands, therefore acid mucin replaces the original gastric mucin and gets blue

261 stained with pH 2.5 Alcian blue. Therefore, AB-PAS combined staining can distinguish normal

262 epithelium with IM. Recently, the expression profile of different mucins has been analyzed with

263 antibodies. MUC5AC, MUC6, MUC2, and CD10 have been found to be specifically expressed in

264 the gastric foveolar epithelium, pyloric gland cells, goblet cells, and brush border, respectively[17].

265 However, this antibody staining method cannot further distinguish GI mixed type mucin into

266 salivary mucin and sulfated mucin, that is, it cannot distinguish between type II and type III.

267 Moreover, compared with antibody staining, the HID-AB-PAS method is more time-saving,

268 efficient, and cheaper and cost-effective.

269   However, the current study was limited that although we have seen the dynamic changes of

270 gastric mucus phenotype during the progression of gastric disease, whether the disappearance of

271 mucus phenotype is "cause" or "effect", the conclusions are not consistent, and further study is

272 needed in the future such as functional assays with relevant study models (cell lines, mice…).

273 Although we found that *H. pylori* positive and negative groups have different dominant mucus

274 phenotype, however, in AG, GD, GC subgroups, the difference was not obvious. And due to the

275 limitation of the number of cases, we did not further analyze the acidic mucus subtypes between

276 *H. pylori* status, which has yet to be further studied.

277   In summary, this study explored the phenotype of gastric gland mucus changes during the

278 progression of gastric disease and found that the disappearance of mucus phenotype is one of the

279 high-risk factors for the progression of gastric diseases. The appearance of type III mucin is a

280 relatively serious phase of gastric diseases and may be a more suitable candidate for follow-up

281 monitoring of patients with GC risk. In China, there is currently still no effective method for

282 clinical management and prognosis follow-up of patients with IM and dysplasia. With the

283 continuous improvement of our understanding of mucin typing, the expansion of research samples

284 and the increasing awareness of clinicians, we will get more evidence to support, continue to

285 improve our clinical management of GIM and dysplasia patients, and ultimately improve the early

286 detection rate of GC and reduce its mortality.

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457   **Figure legends:**
458   **1. Mucous phenotype characteristics of different gastric diseases**
459   A, B. Representative picture of neutral mucus (red) in the SG group. C, D. Representative picture
460   of acid mucus (blue) in the AG group. E, F. Representative picture of mucus-free in GC group.
461   Images on the left panel were made at ✖□100 magnification; the images on the right were
462   magnified images (✖□400) of the boxed sections depicted at left.
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465   **2. Acidic mucus phenotype in AG**
466   A, B. Representative picture of type I (blue). C, D. Representative picture of type II (red-blue).
467   E, F. Representative picture of type III(red-brown black). Images on the left panel were made at ✖□100

468   magnification; the images on the right were magnified images (✖□400) of the boxed sections
469   depicted at left.
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472   3. **Acid mucus phenotypes in the lesion adjacent to GC**
473   A,B. Acid mucus of type II can be seen in the lesion adjacent to GC. C,D. Acid mucus of type III can be seen in
474   the lesion adjacent to GC. Images on the left panel were made at ✖□100 magnification; the images on
475   the right were magnified images (✖□400) of the boxed sections depicted at left.
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Table 1. Baseline characteristics of 483 subjects

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Characteristcs	SG	AG	GD	GC	*P* value
Total cases	82	81	168	152	
Age (years)	52.09	61.46	61.73	62.39	0.000
Sex (%)					
Male	42(51.22)	46(56.79)	112(66.67)	99(65.13)	0.067
Female	40(48.78)	35(43.21)	56(33.33)	53(34.87)	

Table 2. Mucous phenotype characteristics of different gastric diseases

Table 2. Mucous phenotype characteristics of different gastric diseases

Diseases	Cases	neutral mucus(%)	acidic mucus(%)	non-mucus(%)	*P* value
Total	483	135(27.89)	154(31.82)	195(40.29)	
SG	82	82(100)	0	0	0.000
AG	81	12(14.81)	66(81.48)	3(3.71)	0.000
GD	168	36(21.43)	59(35.12)	73(43.45)	0.000
GC	152	4(2.63)	29(19.08)	119(78.29)	0.000
P value		0.000	0.000	0.000	

Table 3. The analysis of mucous phenotype characteristics of different gastric diseases

Table 3. Subgroup analysis of mucous phenotype characteristics of different gastric diseases

Diseases	Cases	neutral mucus (%)	acidic mucus (%)	non-mucus (%)	*P* value
AG	81	12(14.81)	66(81.48)	3(3.71)	
Position					
Corpus	15	2(13.33)	13(86.67)	0(0)	
Angle	13	2(15.38)	10(76.92)	1(7.69)	0.874
Antrum	53	8(15.09)	43(81.13)	2(3.78)	
Degree					
Mild	15	5(33.33)	8(53.33)	2(13.34)	
Moderate	47	6(12.77)	40(85.11)	1(2.12)	0.020
Severe	19	1(5.26)	18(94.74)		
GD	168	36(21.43)	59(35.12)	73(43.45)	
Low-grade	96	33(34.38)	48(50.00)	15(15.62)	
High-grade	72	3(4.17)	11(15.28)	58(80.56)	0.000
GC	152	4(2.63)	29(19.08)	119(78.29)	
Intestinal type	78	4(5.13)	12(15.38)	62(79.49)	
Diffuse type	46	0(0)	11(23.91)	35(76.09)	0.500

Mixed type	3	0 (0)	1 (33.33)	2 (66.67)
Unclassifiable	25	0 (0)	5 (20.00)	20 (80.00)

Table 4. Subgroup analysis of mucous phenotype characteristics of different H.pylori status

Table 4. Subgroup analysis of mucous phenotype characteristics of different Hp status

Diseases	Hp	Cases	neutral mucus (%)	acidic mucus(%)	mucus-free(%)	*P* value
Total	Positive	97	15(15.46)	41(42.27)	41(42.27)	0.724
	Negative	53	7(13.21)	20(37.73)	26(49.06)	
AG	Positive	37	8(21.62)	27(72.97)	2(5.41)	0.000
	Negative	8	8(100)	0(0)	0(0)	
GD	Positive	33	6(18.18)	10(30.30)	17(51.52)	0.879
	Negative	26	6(23.08)	8(30.77)	12(46.15)	
GC	Positive	27	1(3.71)	4(14.81)	22(81.48)	0.819
	Negative	19	1(5.26)	4(21.05)	14(73.69)	

Table 5. The distribution of acidic mucus phenotype in different diseases

Table 5. The distribution of acidic mucus phenotype in different diseases

Diseases	Cases	Type I(%)	Type II(%)	Type III(%)	*P* value
CAG	66	11(16.67)	10(15.15)	45(68.18)	0.000
Position					
Corpus	13	0(0)	2(15.38)	11(84.62)	
Angle	10	1(10)	2(20)	7(70)	0.348
Antrum	43	10(23.26)	6(13.95)	27(62.79)	
Degree					
Mild	8	3(37.5)	1(12.5)	4(50)	
Moderate	40	5(12.5)	7(17.5)	28(70)	0.505
Severe	18	3(16.67)	2(11.11)	13(72.22)	
GD	59	3(5.08)	12(20.34)	44(74.58)	0.000
Low-grade	48	3(6.25)	12(25)	33(68.75)	0.014
High-grade	11	0(0)	0(0)	11(100)	
GC	29	2(6.90)	6(20.69)	21(72.41)	0.000
Intestinal type	12	0(0)	3(25)	9(75)	
Diffuse type	11	0(0)	2(18.18)	9(81.82)	0.091
Mixed type	1	0(100)	0(0)	1(100)	
Unclassifiable	5	2(40)	1(20)	2(40)	

Table 6. The comparation of acid mucus phenotypes in the lesion adjacent to high-grade GD or GC

Table 6. The comparation of acid mucus phenotypes in the lesion next to high-grade GD or GC

GD/GC	Cases	Type I(%)	Type II (%)	Type III(%)	*P* value
High-grade	42	4(9.53)	3(7.14)	35(83.33)	0.000
Intestinal type	49	4(8.16)	1(2.04)	44(89.8)	
Diffuse type	13	1(7.69)	0(0)	12(92.31)	
					0.980
Mixed type	2	0(0)	0	2(100)	
Unclassifiable	6	1(16.67)	0	5(83.33)	

Figure 1

1. Mucous phenotype characteristics of different gastric diseases

A, B. Representative picture of neutral mucus (red) in the SG group. C, D. Representative picture of acid mucus (blue) in the AG group. E, F. Representative picture of mucus-free in GC group. Images on the left panel were made at ×100 magnification; the images on the right were magnified images (× 400) of the boxed sections depicted at left.
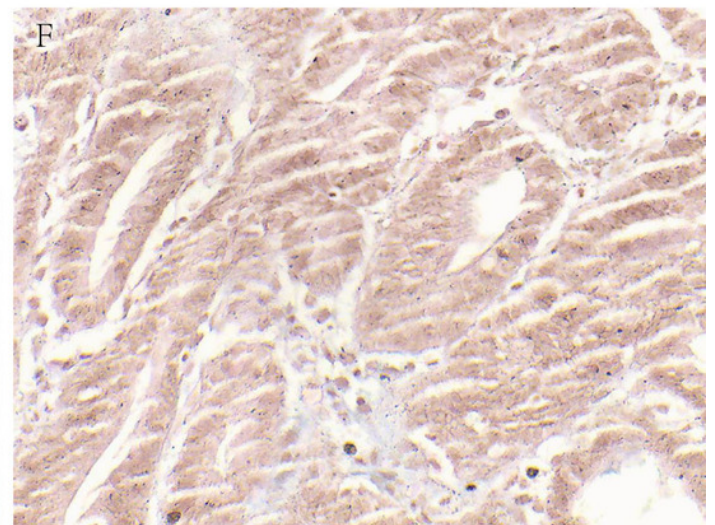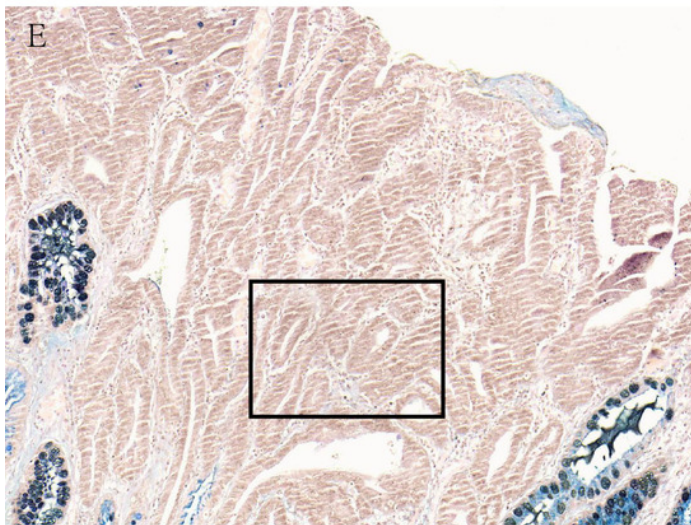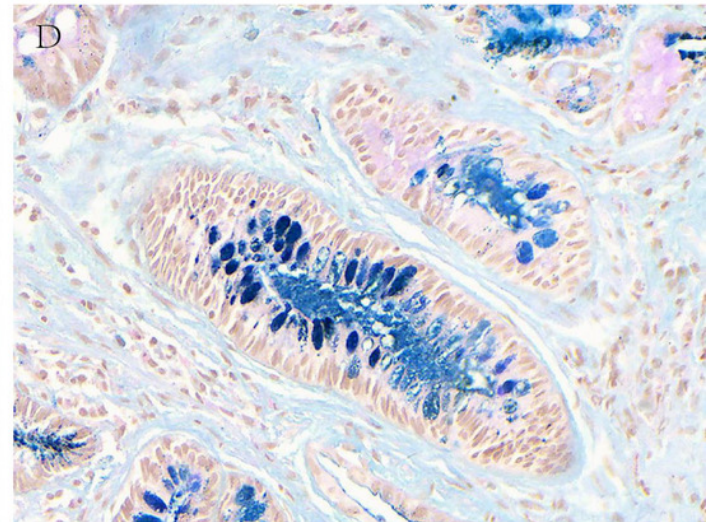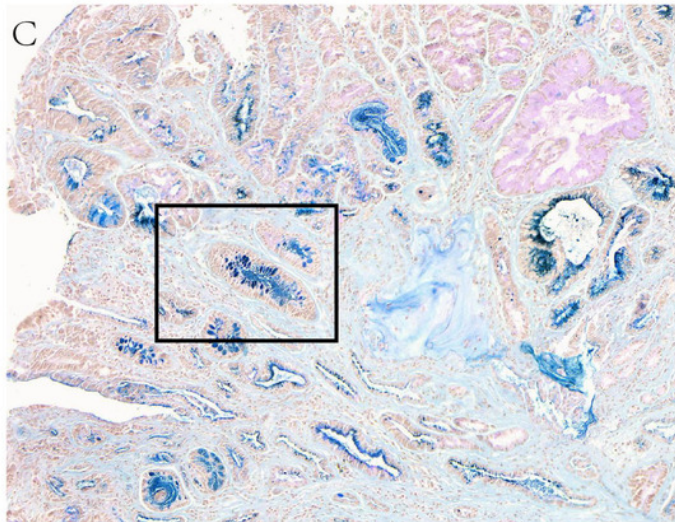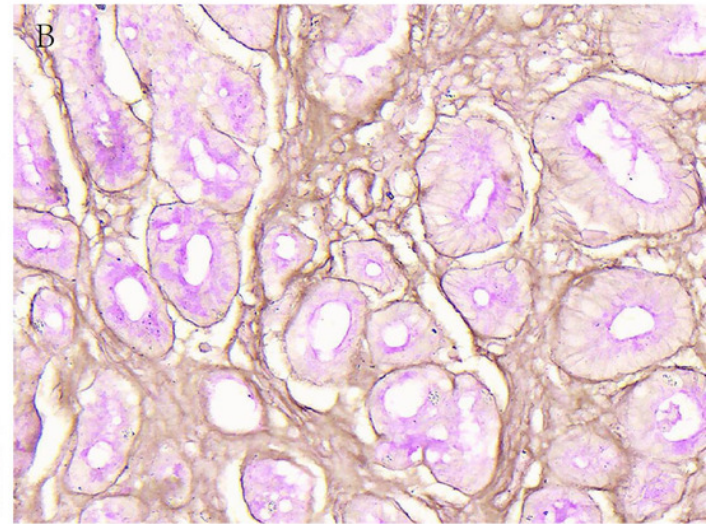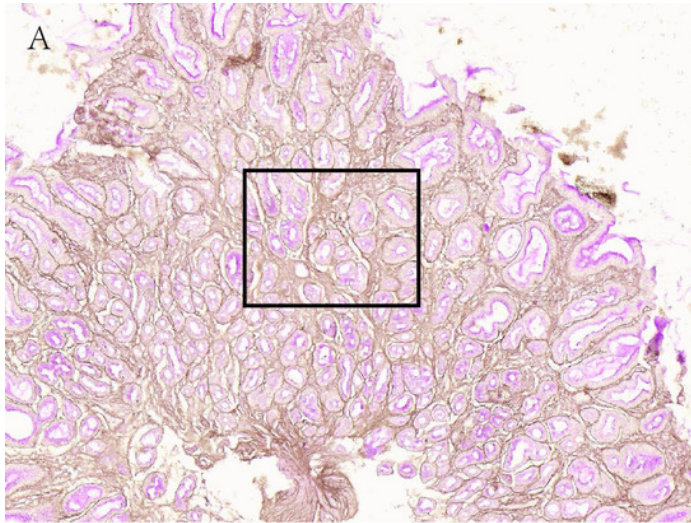
Figure 2

2. Acidic mucus phenotype in AG

A, B. Representative picture of type I (blue). C, D. Representative picture of type II (red-blue).E, F. Representative picture of type III(red-brown black). Images on the left panel were made at $_{x}$100 magnification; the images on the right were magnified images ($_{x}$400) of the boxed sections depicted at left.
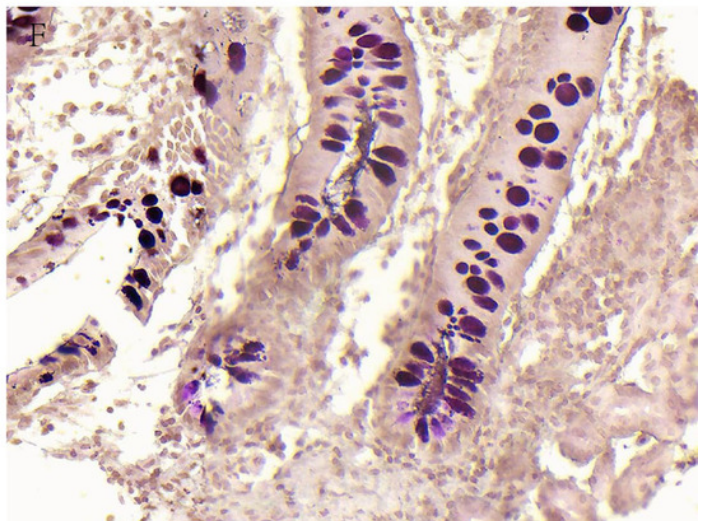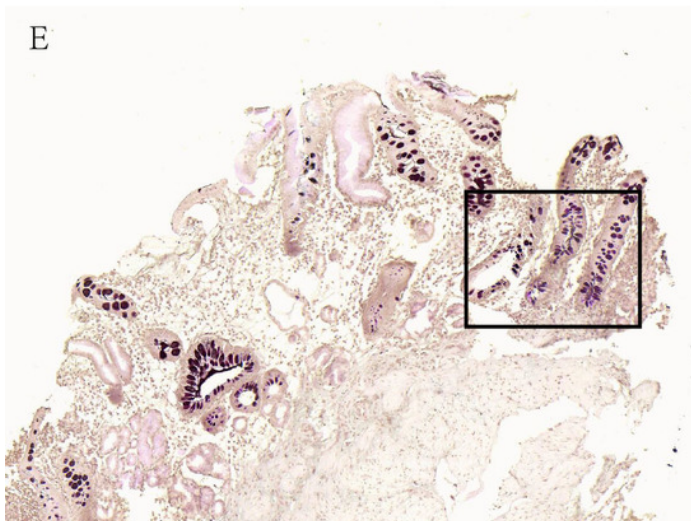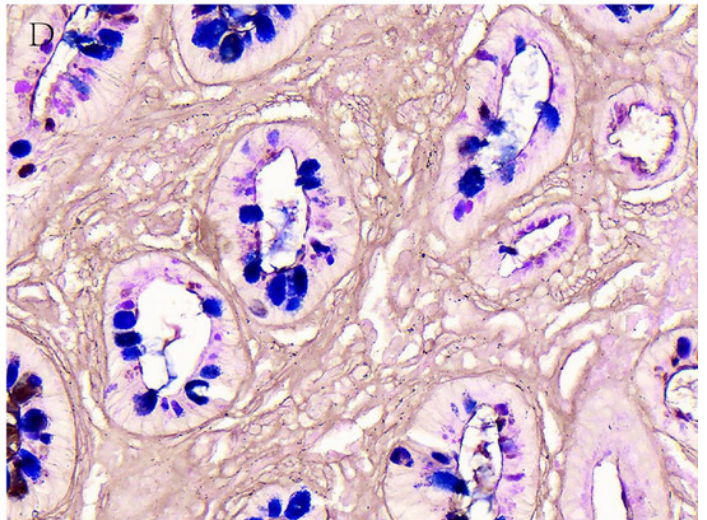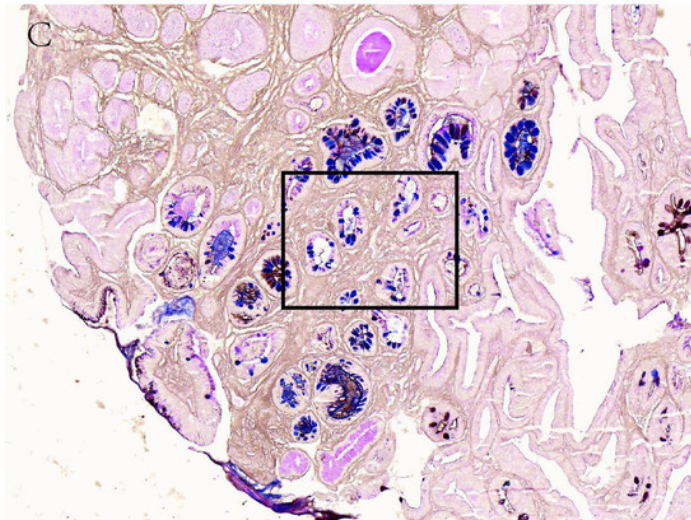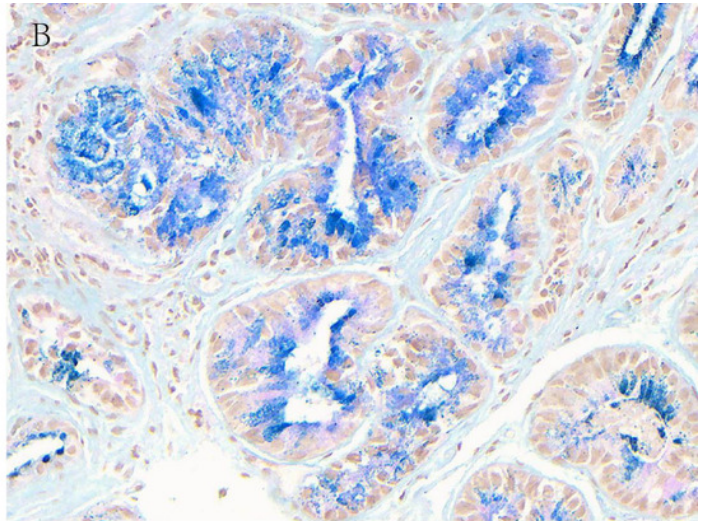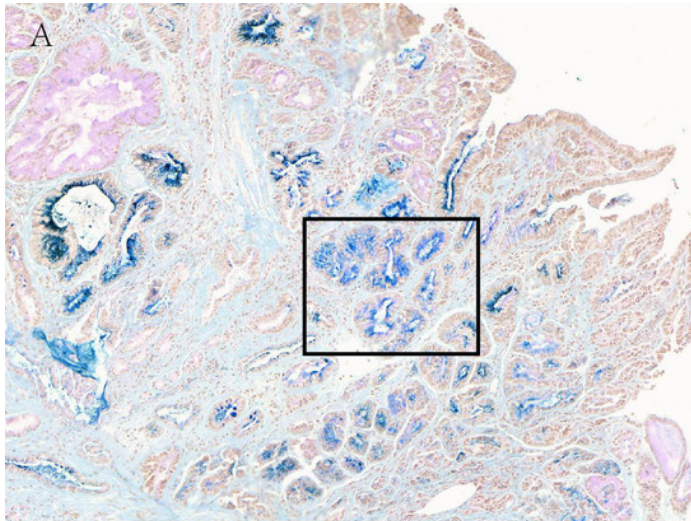
Figure 3

3. Acid mucus phenotypes in the lesion adjacent to GC

A,B. Acid mucus of type II can be seen in the lesion adjacent to GC. C,D. Acid mucus of type III can be seen in the lesion adjacent to GC.Images on the left panel were made at $\times$100 magnification; the images on the right were magnified images ($\times$400) of the boxed sections depicted at left.