MUCIN EXPRESSION PATTERNS IN THAI COLORECTAL CANCER

YUPA SRITHONGCHAI

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science in Anatomy
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Thesis entitled "Mucin expression patterns in Thai colorectal cancer "
By YUPA SRITHONGCHAI
has been approved by the Graduate School as partial fulfillment of the requirements
for the Master of Science in Anatomy of Naresuan University

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ABSTRACT

Colorectal cancer (CRC) is the third cause of death in cancer in the world etc. CRC is divided into 2 types; non-mucinous and mucinous types. The mucinous type of CRC is named by predominantly mucin feature secreted by goblet cells. Mucinous type was more frequently present in the proximal colon than in the distal colon and was mostly found in female and younger patients. Moreover, mucinous type was associated with poor prognosis and response to chemical therapies. In this study, we firstly aimed to investigate acid and neutral mucins expression patterns in the normal large intestine. Secondly, we were to compare the acid and neutral mucins, and MUC2 protein expression in mucinous and non-mucinous types of CRC early stage. All sample blocks were fixed and sectioned in 3 µm thickness. The sectioned was stained by Alcian blue (AB) pH2.5, Periodic acid Schiff (PAS), and Alcian blue combined with Periodic acid Schiff (AB-PAS) for examined acid and neutral mucins expression in normal large intestine and CRC tissues. The MUC2 protein expression in mucinous and non-mucinous CRC tissues was investigated by immunohistochemistry (IHC). In normal large intestine, we found the acid mucin showed significantly gradually increasing whereas the neutral mucin significantly gradually decreased from cecum to rectal parts of large intestine ($p<0.001$). In the non-mucinous type of CRC, the acid and neutral mucins, and MUC2 protein expression were significantly decreased in the cancer area compared to the normal area ($p<0.001$). The acid mucin and MUC2 protein expression were significantly increased but neutral mucin was significantly decreased in cancer area compared to the normal area of the mucinous type of CRC ($p<0.001$). Moreover,

both mucinous and non-mucinous types of CRC showed the positive association between acid and MUC2 protein ($p<0.001$). In conclusion, increased acid mucin and MUC2 protein are correlated to mucinous type of CRC and thus may first step in the prognostic study of mucinous CRC subtype.

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CHAPTER I
INTRODUCTION

Colorectal cancer (CRC) is the third cause of death in cancer in the world and mostly found in male (Arnold et al., 2017). About 10% of people in the world were affected by CRC which was mostly found in developing countries and Europe (Duineveld et al., 2016; Ferlay et al., 2015). Previous studies predicted that in 2030 will find CRC new cases increased by 20% and CRC recurrence by 17% (Duineveld et al., 2016). CRC is divided into 3 types including non-mucinous, mucinous, and signet-ring cell types by histological characteristics and genetic features. The most common subtype of CRC is non-mucinous, and mucinous is a secondary type (10% - 20%), Signet-ring cell (1%) is rare in CRC patients. Previous studies showed that mucinous type was found in poor stage and prognosis. Risk factors of CRC are age, gender, and genetics which associated with bowel diseases such as Lynch syndrome, Familial adenomatous polyposis (FAP), inflammatory bowel disease, and ulcerative colitis. Other risk factors are nationality, obesity, lifestyle, smoking behavior, alcohol behavior, acromegaly, and diabetes mellitus. Previous studies have presented that mucinous type was associated with ulcerative colitis and hereditary non-polyposis colorectal cancer (HNPCC) diseases, and related to anti-oncogenes p53 and p16 (Ionilă, Mărgăritescu, Pirici, & Mogoantă, 2011; Song et al., 2009). Previous study reported that the non-mucinous type occurred in the elderly and male, which found originated in the rectosigmoid part (Arnold et al., 2017). However, mucinous type can be differentiated from non-mucinous type. Mucinous type was mostly found in younger and females, and originated in the proximal part of colon (Luo, Cen, Ding, & Wu, 2019). Histological morphology of mucinous type was composed of mucin 50% of tumor volume (Song et al., 2009).

Mucin is the large glycoprotein that was produced by goblet cells. Mucins are built into mucus layers for lubricant and protection of epithelial cells (Grondin, Kwon, Far, Haq, & Khan, 2020; Kim & Khan, 2013). Major component of mucins is carbohydrate that plays a role in cell-cell signaling, protein folding, protein trafficking, and cell signaling (Kasprzak et al., 2019; Sterner, Flanagan, & Gildersleeve, 2016). Mucins are divided into acid and neutral mucins. Basic components of mucins are carbohydrate and sugar chains, named neutral mucin. In addition, the acid mucin component is added to sialic acid and sulfate molecules (Kim & Khan, 2013). Functions of acid mucin are control normal flora balance, anti-pathogenic cells, and trap of parasites (Alves et al., 2017; Truter, Strijdom, Everson, & Kotzé, 2017; Tsubokawa et al., 2015). Moreover, acid mucin plays a role in cell-cycle control and cancer cell growth inhibition (Danquah et al., 2017). Neutral mucin functions are epithelial cells protection, toxic and pH neutralization (Boonzaier, Van der Merwe, Bennett, & Kotzé, 2013; Truter et al., 2017). Previous studies have been reported that mucins expression was associated with cancer development (Danquah et al., 2017; Ionilă et al., 2011; Kasprzak et al., 2019). Core protein is the central structure of mucin, named MUC protein. Major MUC protein in large intestine was MUC2 protein (Hansson, 2020; Y. Liu et al., 2020; Yamashita & Melo, 2018; Yao, Dai, Dong, Dai, & Wu, 2021). Previous

studies showed that MUC2 was degraded in non-mucinous type but overexpressed in mucinous type of CRC (Byrd & Bresalier, 2004; Li et al., 2018; O'Connell, Reynolds, McNamara, Burke, & Prehn, 2021). However, the association and correlation of acid and neutral mucins, and MUC2 protein have not been reported. Therefore, we aimed to investigate the association between acid and neutral mucins, and MUC2 protein in non-mucinous and mucinous types of CRC. These data will provide basic knowledge of acid and neutral mucins, and MUC2 protein expression patterns in CRC.

Objectives

1. To quantify of acid and neutral mucins expression in differential parts of normal large intestine.
2. To quantify of acid and neutral mucins expression in non-mucinous and non-mucinous types of CRC

3. To investigate correlation of acid and neutral mucins and MUC2 protein expression in non-mucinous and mucinous types in CRC.

Keywords

The large intestine, acid mucin, neutral mucin, MUC2 protein, non-mucinous type, mucinous type, CRC

Scope of this study

This study investigates the quantity of acid and neutral mucins expression in differential parts of normal large intestine, and observes acid and neutral mucins, and MUC2 protein expression in non-mucinous and mucinous types of CRC. Acid mucin was detected by alcian blue (AB) pH 2.5 staining. Neutral mucin was detected by Periodic acid Schiff's (PAS) staining. The coexistence of acid and neutral mucins was investigated by alcian blue combined with Periodic acid Schiff's (AB-PAS) staining. Amount of acid and neutral mucins expressions were quantified by the ImageJ Fiji program. MUC2 protein was detected by the immunohistochemistical (IHC) technique. The area of positive immunoreactivity of MUC2 protein was quantified by ImageJ Fiji program. The mean value in all parts of normal large intestine and all areas of CRC were statistically calculated in IBM SPSS statistics program version 26.0 at $^{**}p<0.001$ and $^{*}p<0.05$. SPSS program was used for t-test and Analysis of Variance (ANOVA) with post hoc Tukey calculation in acid and neutral mucins, and MUC2 protein expressions.

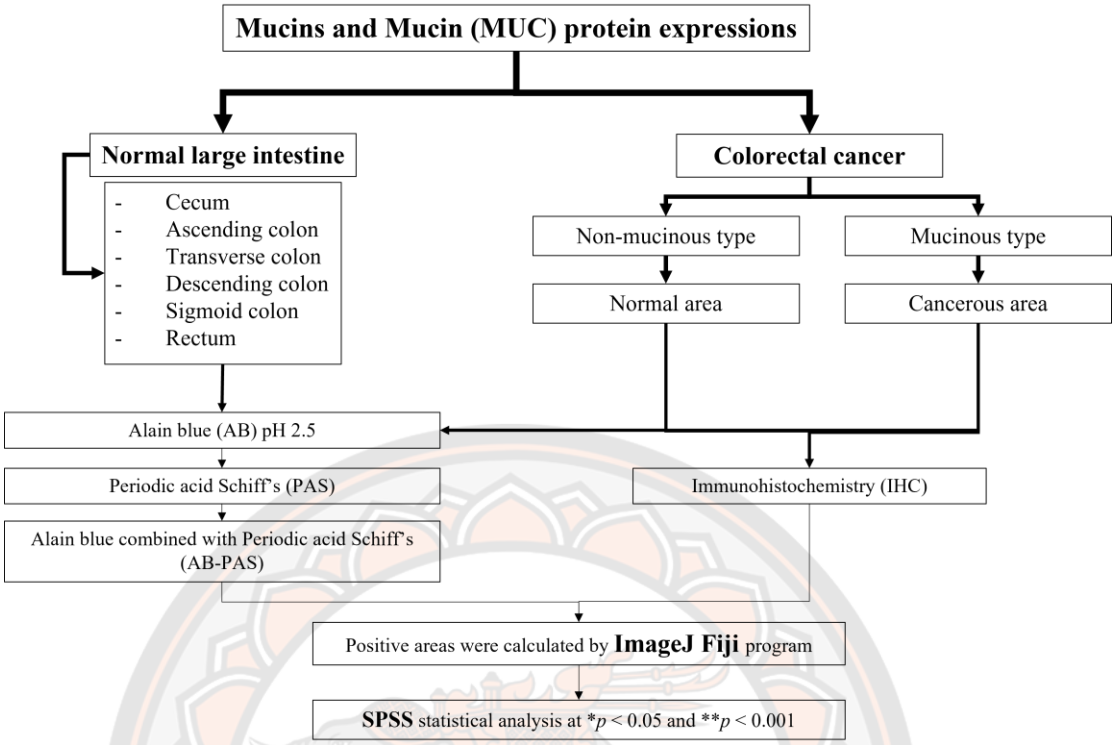

Figure 1 Scope of this study

Hypothesis

1. Acid and neutral mucins expression may be increased or decreased in rectum compared to the cecum in normal large intestine.

2. Acid and neutral mucins expression may be increased or decreased in mucinous type compared to non-mucinous type.

3. Acid and neutral mucins may be correlate to MUC2 protein expression in mucinous and non-mucinous types.

CHAPTER II
REVIEW LITERATURE

The normal large intestine

The large intestine is one of the gastrointestinal (GI) organs that is the last part of GI tract. Functions of large intestine are water, vitamin, and mineral absorptions, and building feces to chunk. Large intestine is divided into six parts including cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum (Figure 1A). Intestinal walls are divided into mucosa, submucosa, muscularis externa and serosa layers (Figure 1B). Mucosal layers are divided into epithelial, submucosal, and muscularis mucosae layers (Figure 1C). The epithelial layer is covered by simple columnar epithelium with goblet cells (Figure 1D). Epithelial cell function is nutrient absorption and goblet cell (Figure 1E) functions are mucus production and secretion forming into mucus layers. A major component of mucus is mucin (Figure 1F) (Azzouz & Sharma, 2018; Kahai, Mandiga, Wehrle, & Lobo, 2017).

In the GI tract, several normal florae and pathogen were found in the large intestine more than in other organs in the GI system (Canny & McCormick, 2008; Srikanth & McCormick, 2008). However, large intestinal cell system can control normal flora and pathogen balance. Moreover, it can protect epithelial cells from toxic agents because it has mucus barriers. Mucus barriers comprise inner and outer mucus layers. The inner mucus layer was built by dense mucin connection for protecting epithelial cells. The outer mucus layer was formed by loose mucin connection for the living of normal flora and trapping pathogenic cells (Figure 3) (Donaldson, Lee, & Mazmanian, 2016; Herath, Hosie, Bornstein, Franks, & Hill-Yardin, 2020; Johansson & Hansson, 2016).

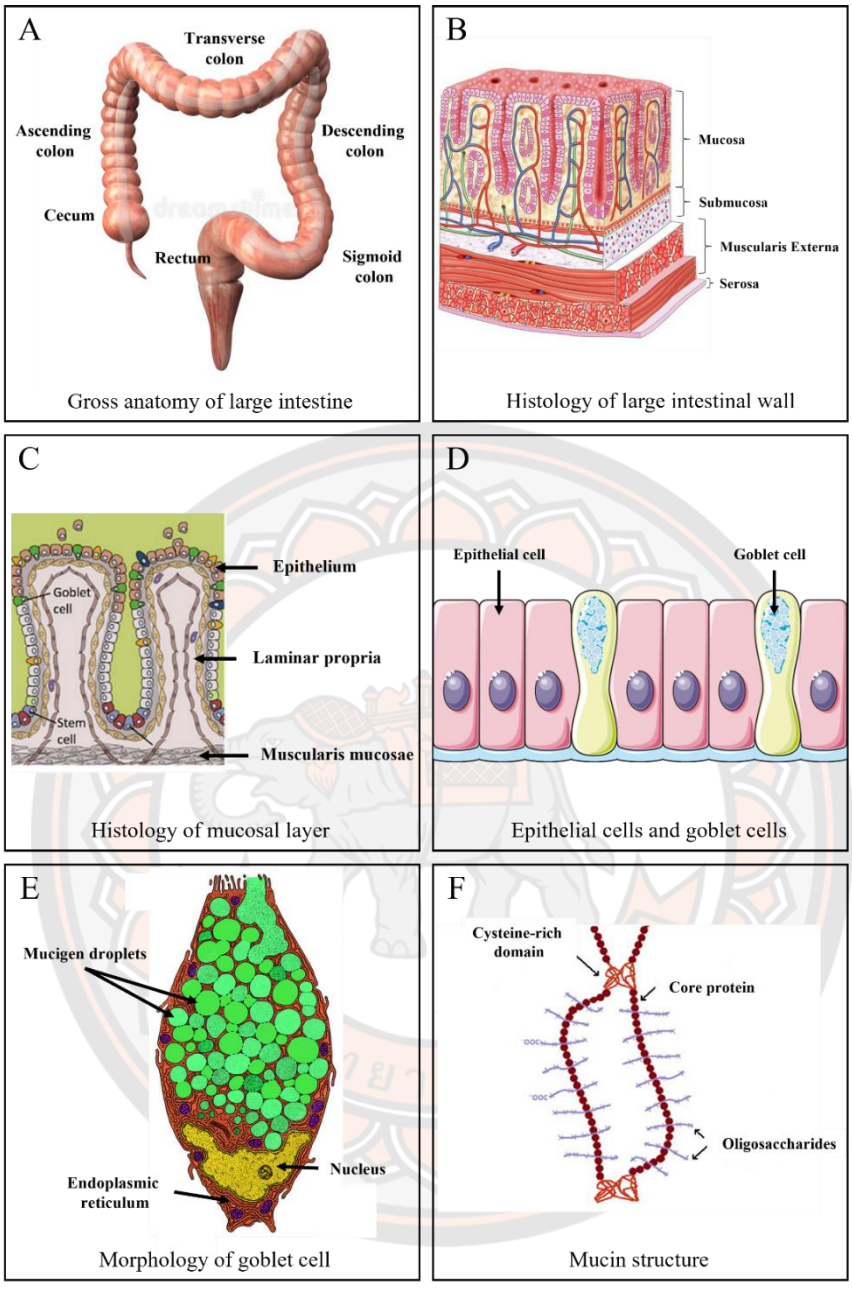

Figure 2 Components of large intestine organ

Differential parts of normal large intestine consisted of cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum parts (**A**). Intestinal walls of large intestine composed of mucosa, submucosa, muscularis externa, and serosa layers (**B**). Sublayer of mucosal layer included epithelium, lamina propria, and muscularis mucosae (**C**). Epithelial layer covered by simple columnar epithelium with goblet cells (**D**). Mucins dropped and another organism contained in goblet cell (**E**). Mucin structure contents composed of cysteine-rich domain, core protein, and oligosaccharide chains (**F**).

Source: (Balabushevich et al., 2019; Fellows & Varga-Weisz, 2020)

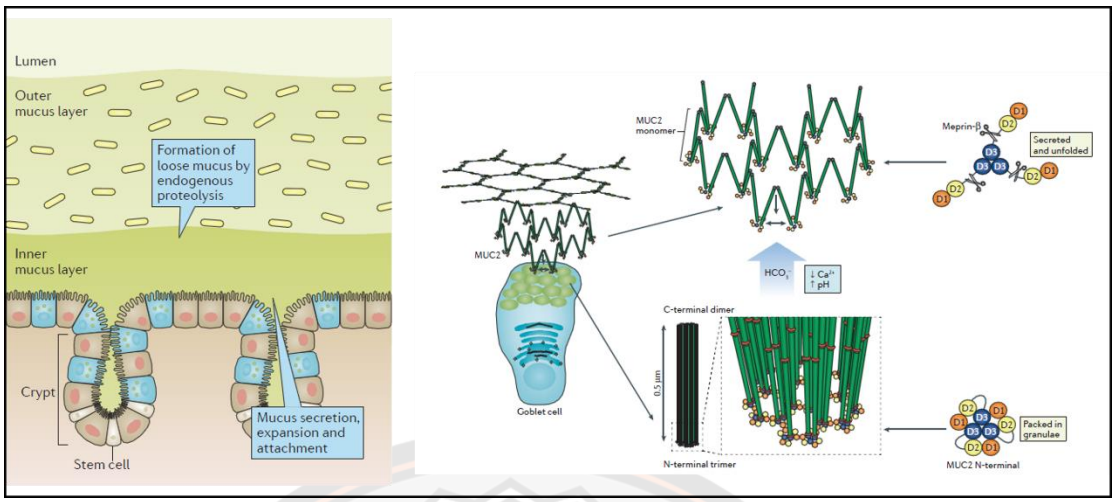

Figure 3 Mucus layers and mucus production
Inner and outer mucus layers were formed by mucin complexes. Mucins were produced and secreted by goblet cells in large intestine. Mucins contents were composed of core protein (MUC2 protein) connecting with carbohydrate chains.

Source: (Johansson & Hansson, 2016)

Mucins in large intestine

Mucins are large glycoproteins which are the main component of mucus. Mucins are produced and secreted by goblet cells for building mucus layers in the intestinal lumen (Figure 4A) (Johansson & Hansson, 2016). The mucus structure includes a lot of mucin networks that were composed of C- and N- terminals for network connection. In mucin structure, the mucin subunit is connected to mucin monomer, connected to mucin dimer, and connected to mucin networks. The mucin subunit was composed of core protein, oligosaccharides, and Cysteine-rich domain (Figure 4B). At cysteine-rich domain of each mucin subunit was blinded by a disulfate bond (s-s bond). Core protein was richly composed of serine (Ser) and threonine (Thr) which are locations of oligosaccharide connections. Between Ser or Thr and *N*-acetylgalactosamine were blinded by the *O*-glycosidic bond. Oligosaccharides were formed by five sugars including *N*-acetylgalactosamine (GalNAc), *N*-acetylglucosamine (GlcNAc), galactose, fucose, and sialic acid (Figure4D) (Grondin et al., 2020; Kim & Khan, 2013). Oligosaccharide chain structure divides mucin into acid and neutral mucins. Neutral mucin is not composed of sialic acid (Figure 4E). Acidic mucin is composed of sialic acid (Figure 4F) (Grondin et al., 2020; Kasprzak et al., 2019). Common mucin expression in large intestine is acidic mucin (Grondin et al., 2020).

Intestinal mucin carbohydrate contents were mostly investigated by histological technique. Acid mucin was detected by Alcian blue (AB) staining that was shown in dark blue color (Figure 5A). Neutral mucin was detected by Periodic acid Schiff's (PAS) that was presented in magenta color (Figure 5B) (Ali, Nagi, Naseem, & Ullah,

2012; Boonzaier et al., 2013; Kasprzak et al., 2019; Mandal, Chakrabarti, Ray, Chattopadhyay, & Das, 2013; Truter et al., 2017). Mucins are the frontline of the innate immune system and act as barrier for epithelial cells protection (Kim & Khan, 2013). Acid mucin controls the quantity of normal flora, inhibits pathogenic cells growth, movement and feeding into the vascular system of parasites (Alves et al., 2017; Grondin et al., 2020; Kasprzak et al., 2019; Kim & Khan, 2013; Tsubokawa et al., 2015). Moreover, acid mucin has an important role in cell division control and cancer cell growth inhibition (Danquah et al., 2017). Neutral mucin has an important role in the pH neutralization of the digestive system. Moreover, neutral mucin can neutralize toxic and chemical agents for dangerous reduction (Boonzaier et al., 2013; Truter et al., 2017). Previous studies were found that levels of mucins expression associated with various diseases such as ulcerative colitis, inflammation, and cancer (Ali et al., 2012; Boonzaier et al., 2013; Danquah et al., 2017; Gajendran et al., 2019; Grondin et al., 2020; Ionilă et al., 2011; Kasprzak et al., 2019; Kim & Khan, 2013; Luo et al., 2019; Mandal et al., 2013; Song et al., 2009).

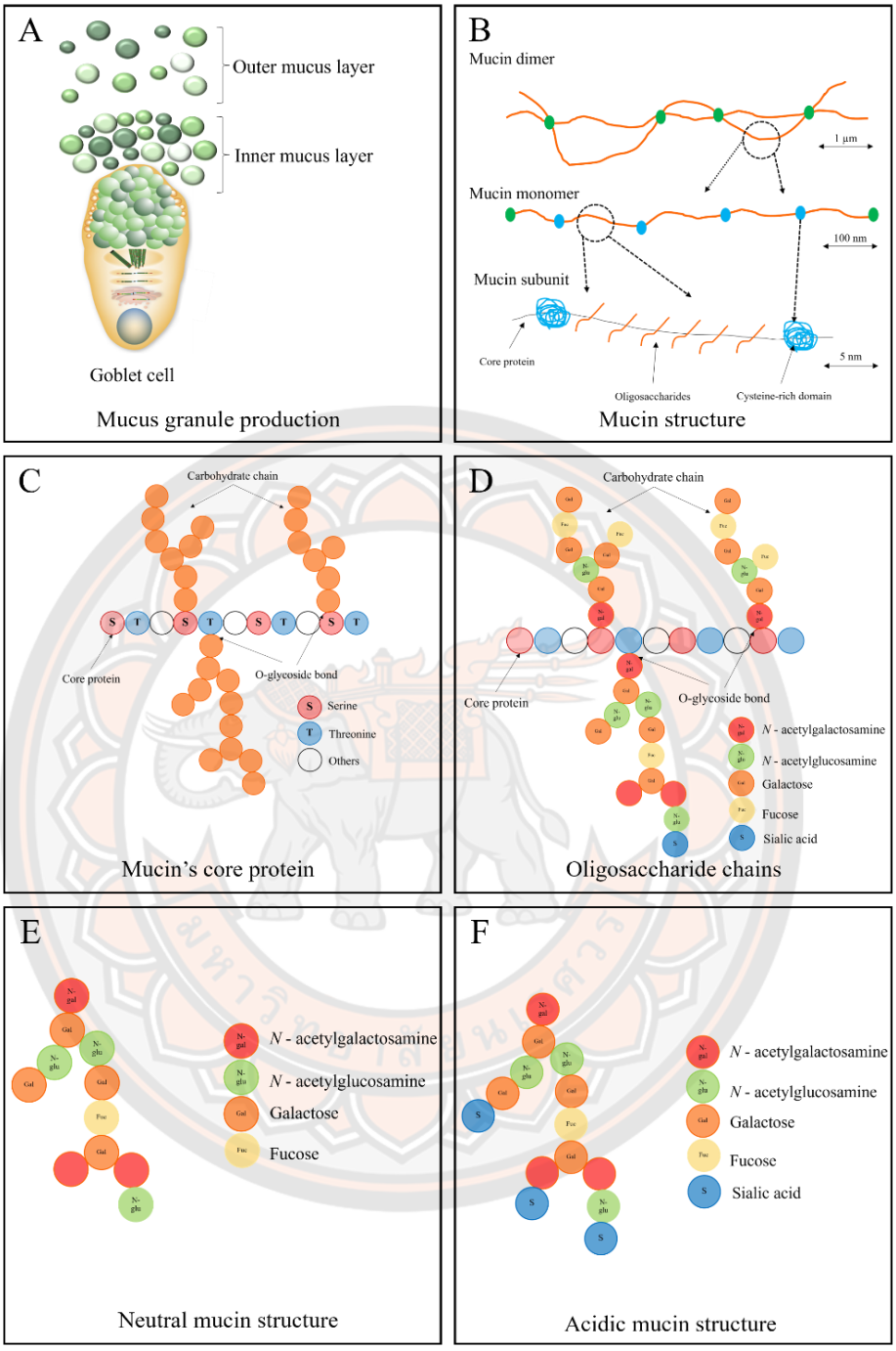

Figure 4 Mucin structure
Mucus granule production and secretion (**A**), mucin structure including mucin subunit, mucin monomer, mucin dimer (**B**), core protein components in mucin structure (**C**), oligosaccharide chain components (**D**), neutral mucin (**E**), and acidic mucin structures (**F**).

Source: (Authimoolam & Dziubla, 2016; Birchenough, Johansson, Gustafsson, Bergström, & Hansson, 2015)

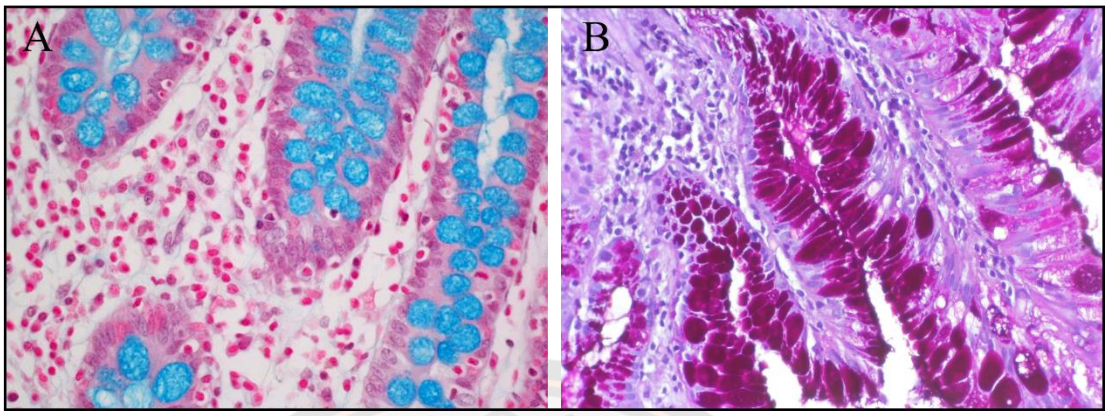

Figure 5 Color of acid and neutral mucins staining
Alcian blue (AB) staining for acid mucin detection showing blue color (**A**) and Periodic acid Schiff's (PAS) stainings for neutral mucins detection showing magenta color (**B**).

Source: (Bhagavathula, Dame, Aslam, & Varani, 2009; Osho, Wang, Horn, & Adeola, 2017)

Mucin 2 (MUC2) protein

Mucin composes of glycoproteins which are formed by core protein and the *O*-glycan family's connection. Mucin monomer is composed of 20% core protein and 80% *O*-glycan (figure 6A) (Hansson, 2020). Core protein composes of Thr and Ser more than other amino acids. Thr and Ser are located for *O*-glycan connection that was connected by the glycosidic bond. In core protein, Thr and Ser connection is followed by Proline (Pro), named PTS (Figure 6B) (Bäckström, Ambort, Thomsson, Johansson, & Hansson, 2013; Desseyn, Aubert, Porchet, & Laine, 2000; Lang et al., 2016). MUC protein is divided into 2 types including secretory and trans-membrane mucins groups (Hijikata et al., 2011; Pallesen, Berglund, Rasmussen, Petersen, & Rasmussen, 2002). In secretory mucins, mucins are produced and secreted for mucus layer building in the lumen, called gel-forming. Previous studies, members of secretory mucins were composed of MUC2, MUC5AC, MUC5B, MUC6, MUC7 MUC8, and MUC19. Mucins in the secretory mucins group are secreted by exocytosis processes (Figure 6C). In trans-membrane mucins, the C-terminal of mucin core protein connects with the cytoplasmic tail in the apical membrane of the cell membrane (Figure 6D). The members of trans-membrane mucins were include MUC1, MUC3A, MUC3B, MUC4, MUCH-13, MUC15, MUC16, MUC17, MUC20, MUC21, and MUC22 (Bhatia et al., 2019; Chaturvedi et al., 2008; Chen, Hung, Wang, Paul, & Konstantopoulos, 2013; Hansson, 2020; I Lakshmanan et al., 2016; Imayavaramban Lakshmanan et al., 2015; Moniaux, Andrianifahanana, Brand, & Batra, 2004; Ramasamy et al., 2007; Senapati et al., 2011).

Mucin 2 (MUC2) protein is one of the secretory mucins members family that is synthesized by chromosome 11. MUC2 core protein begins to start in the endoplasmic reticulum (ER) and transports into the Golgi apparatus for glycosylation. In the Golgi apparatus, core protein is connected with carbohydrate chain become to a mucin network. After leaving form Golgi apparatus, MUC2 is packed into mucin granules and exocytosis to mucus layers forms (Figure 6E) (Godl et al., 2002; Y. Liu et al., 2020). MUC2 gene and protein were mostly found in large intestine (Figure 7) (Fagerberg et al., 2014). In normal conditions, MUC2 protein was slowly and continuously produced for compensation mucin degrading (Figure 8A) (Davis & Dickey, 2008; Y. Liu et al., 2020). In bacteria, virus, or parasite infection, MUC2 induces an inflammatory system that stimulates proinflammatory cytokine and chemokine such as TNF, IL-4, etc. for the infectious response. Inflammatory cytokine and chemokine stimulate NF-kB. NF-kB induces MUC2 protein production for pathogen control and anti-inflammation (Figure 8B) (Davis & Dickey, 2008; Iwashita et al., 2003; Kebouchi et al., 2016; Y. Liu et al., 2020). Repeated inflammation becomes chronic inflammation which is one cause of cancer. Carcinogenesis may cause defective mucin production. Previous studies found that MUC2 degradation promoted invasion and metastasis of cancer cells (Byrd & Bresalier, 2004; Li et al., 2018). However, frequently previous studies found that MUC2 overexpression promoted tumor cells growth and speed invasion. Additionally, in normal conditions, MUC2 protein was highly expressed in the large intestine whereas low expressed in other organs (Huang et al., 2021; Luo et al., 2019; O'Connell et al., 2021).

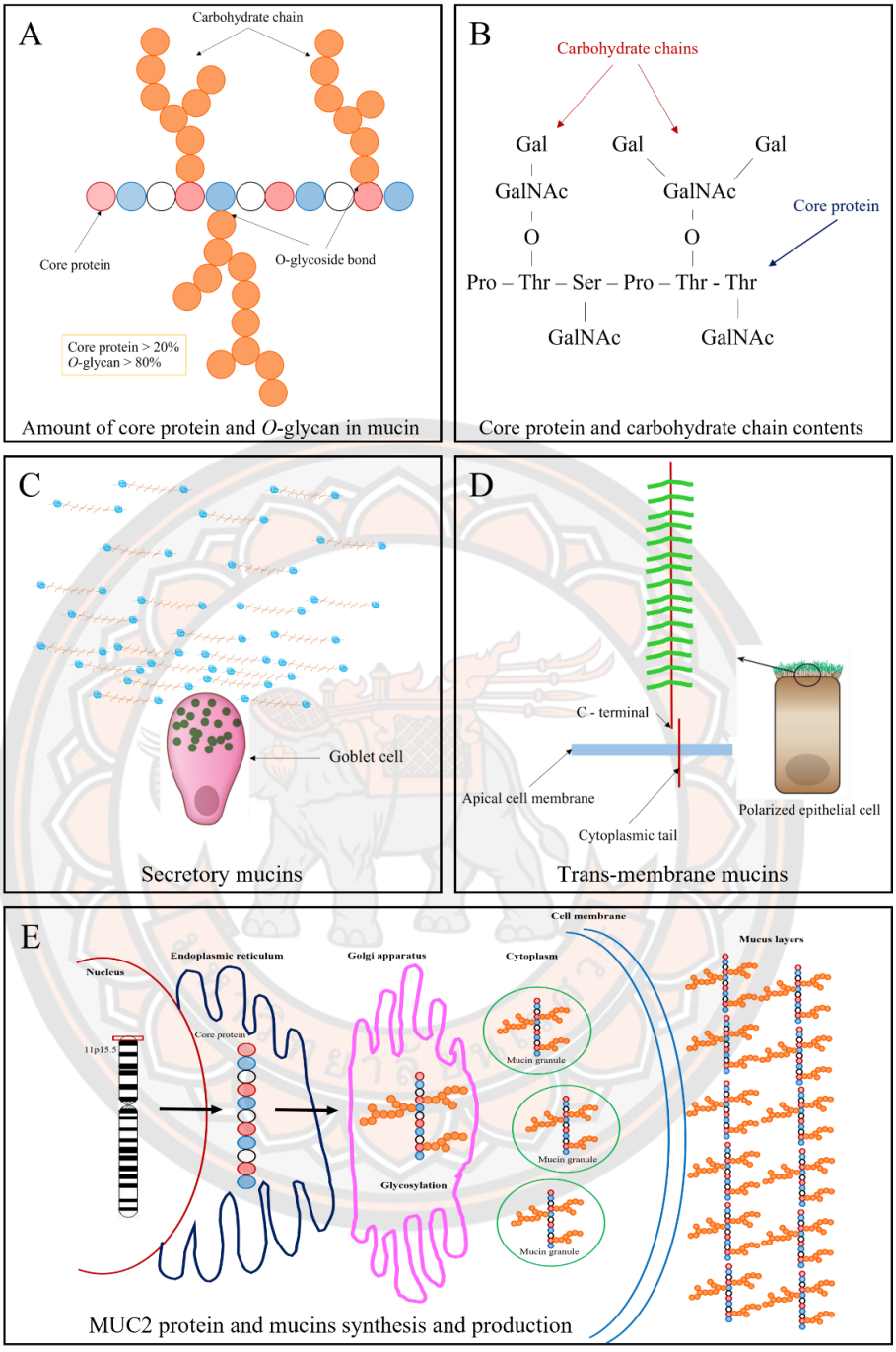

Figure 6 MUC2 structure and synthesis

The amount of core protein and carbohydrate chain in MUC2 protein (**A**), component of core protein and carbohydrate chain (**B**), secretory mucin production (**C**), trans-membrane mucin production (**D**), and MUC2 protein production and secretion (**E**).

Source: Adapted from (Hansson, 2020; Y. Liu et al., 2020)

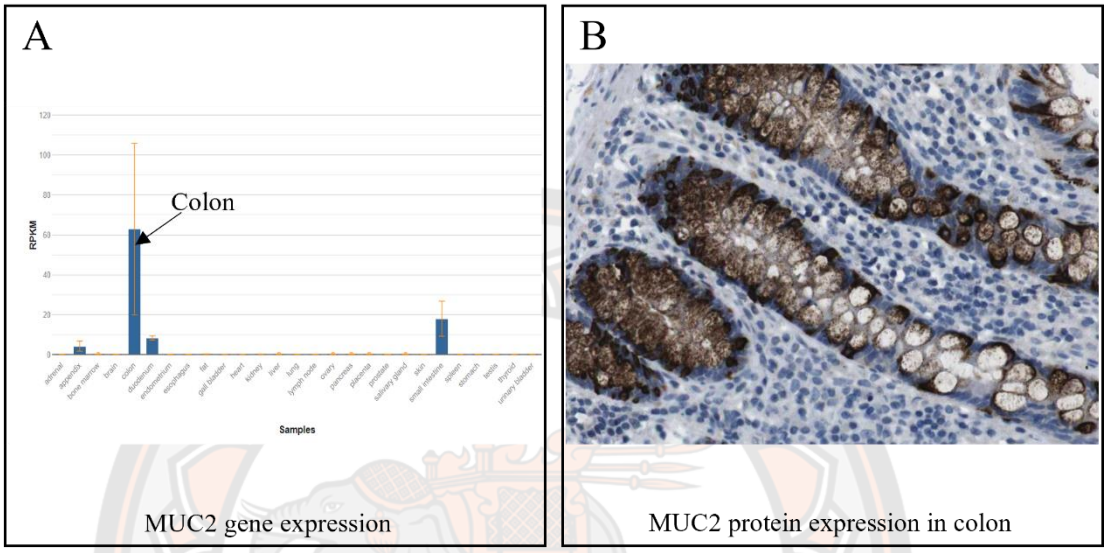

Figure 7 MUC2 gene and protein expression in large intestine
MUC2 gene (A) and protein (B) expressions in normal large intestines.

Source: (Fagerberg et al., 2014) and protein atlas database

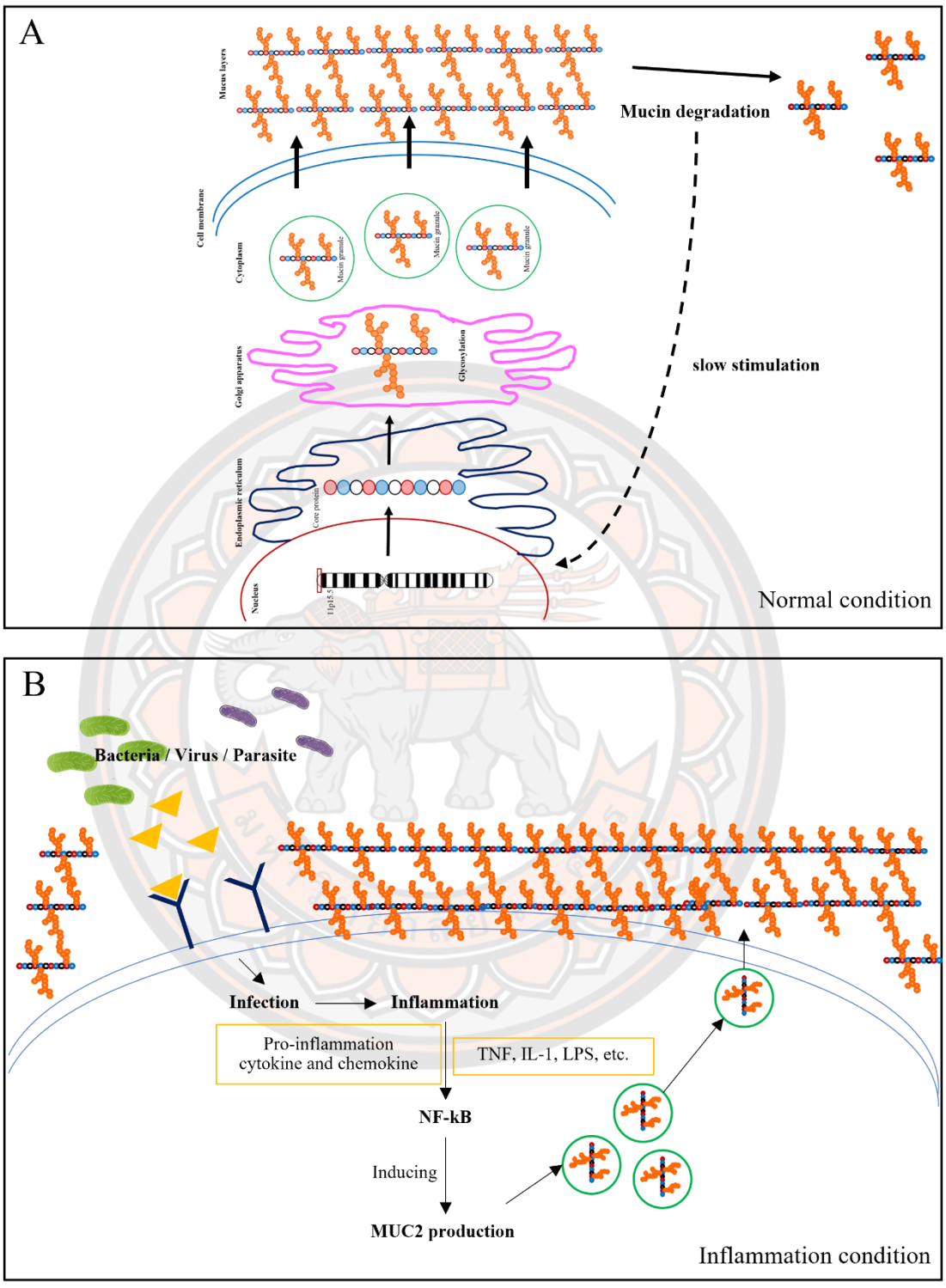

Figure 8 MUC2 protein function in normal and inflammation conditions MUC2 protein function in normal is lubricants and epithelial protection, (**A**) and inflammation (**B**) conditions is pathogenic response.

Source: Adapted from (Bhatia et al., 2019; Y. Liu et al., 2020)

Colorectal cancer (CRC)

CRC is the third most common diagnosis and the leading cause of death in all cancer. In 2012, CRC new cases were found 1.4 million people, and death of 700,000 people (Arnold et al., 2017). The highest incidence of CRC was found in the high developing countries such as the USA, Australia, New Zealand, and Western European than in Asia (Figure 9) (Center, Jemal, Smith, & Ward, 2009). Previous studies predicted that CRC would increase in 2030. CRC new cases were increased by 60% (more than 2.2 million people) and the death of 1.1 million people (Arnold et al., 2017).

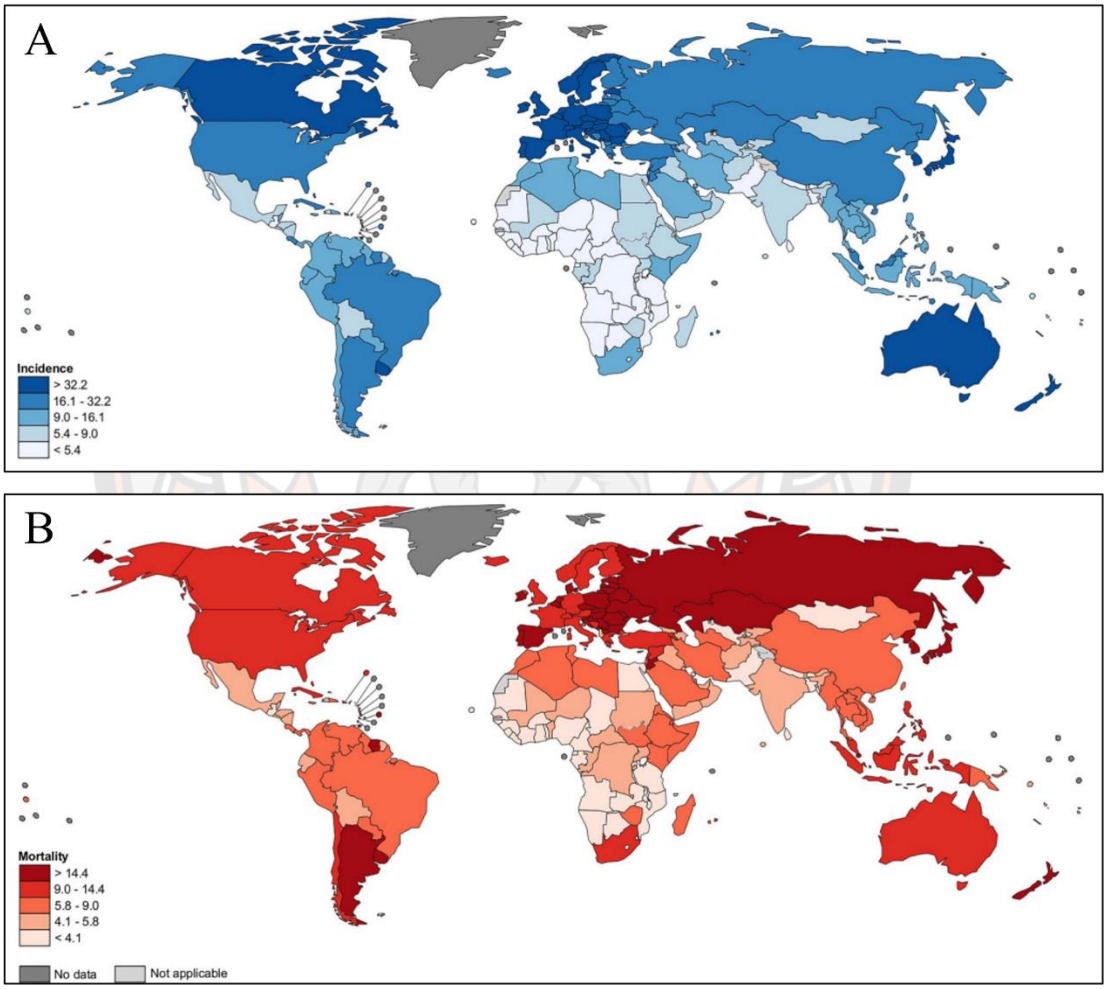

Figure 9 Incidence and motility of CRC

The incidence and motility of CRC in the world in 2012 databases. Dark blue to soft blue shows a level of incidence of CRC that shows high to low incidence (**A**). Dark red to soft red shows the level of motility of CRC (**B**).

Source: (Arnold et al., 2017)

CRC risk factors

Previous studies investigated the causes of CRC that were examined by oncogene inhibition or overexpression of the tumor suppressor gene and other methods. However, it is not clear of the causes of CRC. Nevertheless, previous studies were reported that CRC was stimulated by many factors composed of personal and environmental factors. Personal factors are older than 50 years of age, personal history of inflammatory intestinal conditions, or family history of CRC. Environmental factors are the lack of physical exercise, a low-fiber diet, and use of alcohol and tobacco (Figure 10). All risk factors above can induce normal cells to cancer cells (Gausman et al., 2020; Johnson et al., 2013).

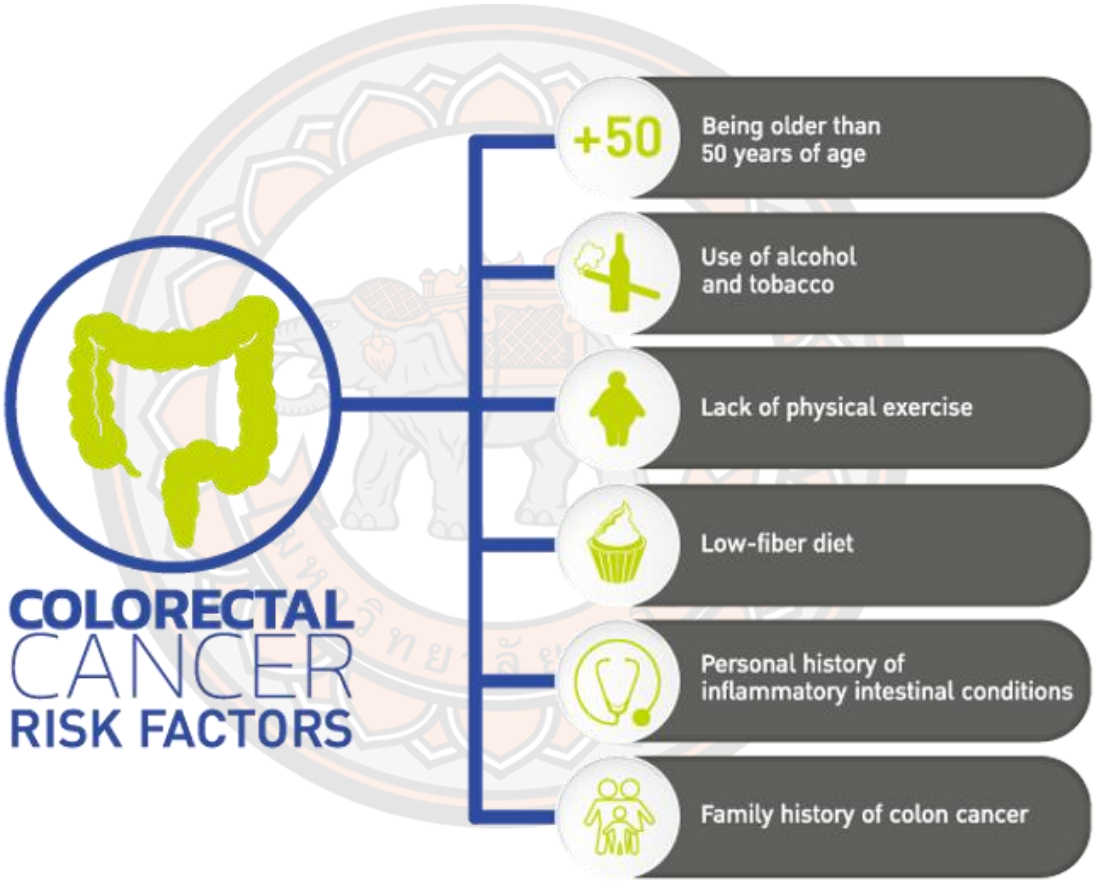

Figure 10 Risk factors of CRC

Colorectal cancer risk factors including older than 50 years of age, use of alcohol and tobacco, lack of physical exercise, low-fiber diet, personal history of intestinal conditions, and family history of colorectal cancer.

Source: (Keum & Giovannucci, 2019)

Symptoms of CRC

CRC symptoms in the early-stage present is still unclear. CRC symptoms are associated with tumor sizes, location, and stage of the disease. Common symptoms include bowel habits changing, anus bleeding, abdominal pain, and unexplained weight loss (Figure 10) (Astin, Griffin, Neal, Rose, & Hamilton, 2011; John, George, Primrose, & Fozard, 2011).

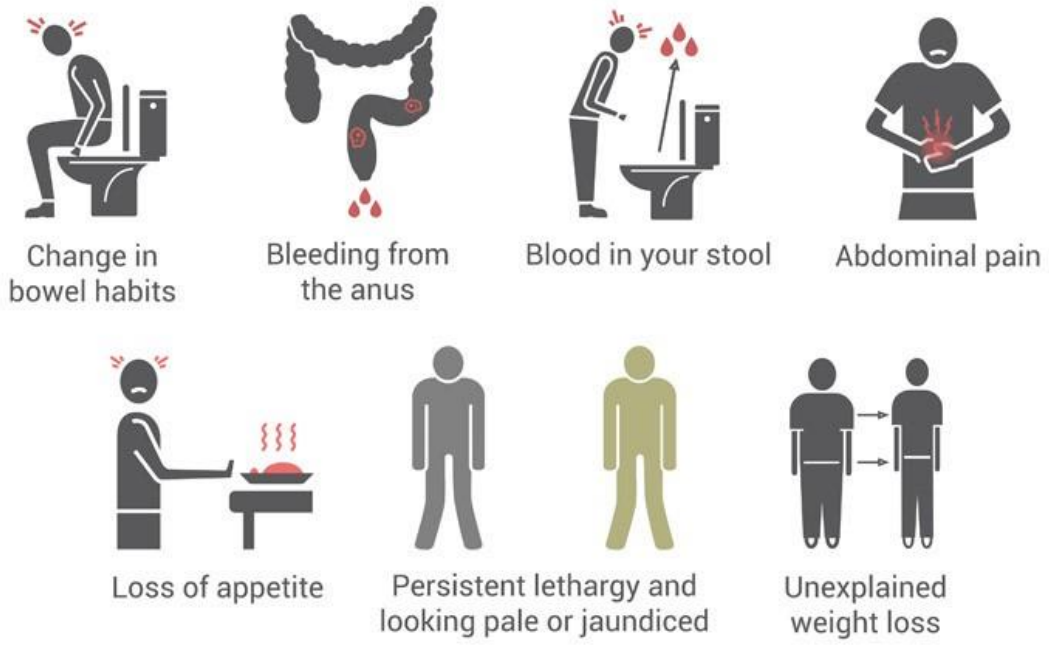

Figure 11 Symptoms of CRC

Symptoms of CRC. It is associated with the size, location, and stage of the disease. And common symptoms of CRC compose of changes in bowel habits, bleeding from the anus, blood in your stool, abdominal pain, loss of appetite, persistent lethargy, looking pale or jaundiced, and unexplained weight loss.

Source: (Biller & Schrag, 2021)

Screening and diagnosis of CRC

Getting a diagnosis of CRC in the early stage improves treatment and survival rate. Thus, CRC screening is important. The first step for CRC screening is risk factors evaluation including family history, history of intestinal inflammation, age, gender, and other risk factors. Next, CRC is diagnosed by digital rectal exam for tumor mass detection, followed by lab tests including complete blood count (CBC), liver function test (LETs), and tumor markers and tumor biopsy (Figure 11) (Astin et al., 2011; Shaukat et al., 2021).

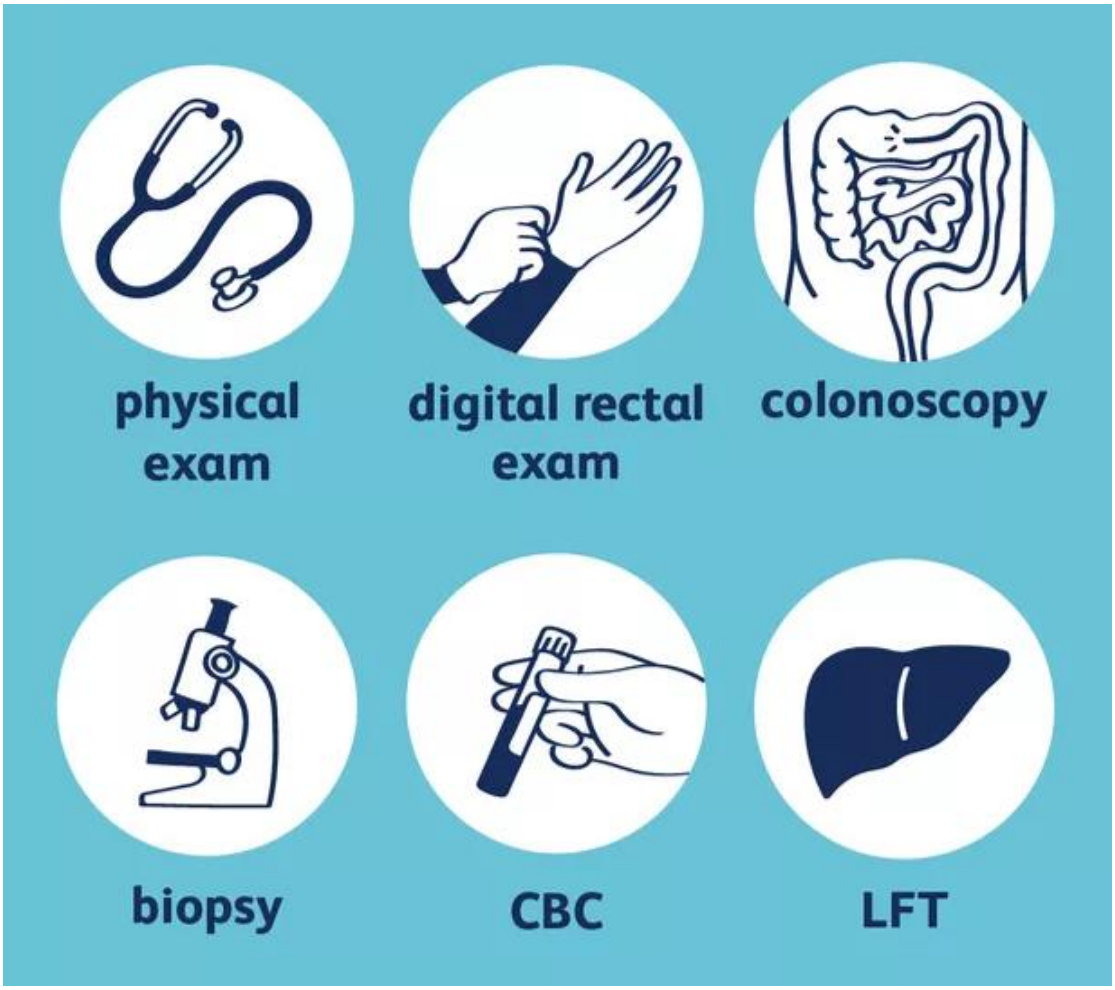

Figure 12 CRC diagnosis

Methods for diagnosis of CRC including physical exam, digital rectal exam, colonoscopy, biopsy, Complete blood count (CBC), Liver function tests (LFT), and tumor maker methods.

Source: (Biller & Schrag, 2021)

Treatment of CRC

CRC treatments are associated with stage, tumor size, and type of cancer. Standard treatments of CRC are surgery, chemotherapy, and radiotherapy which are used in combination with some patients. However, radiotherapy and chemotherapy cause the side effects on the body. Thus, previous studies the investigated new treatments such as clinical trials or immunotherapy for reduced side effects from radioactivity and drug chemical (Figure 13) (Biller & Schrag, 2021; Johdi & Sukor, 2020)

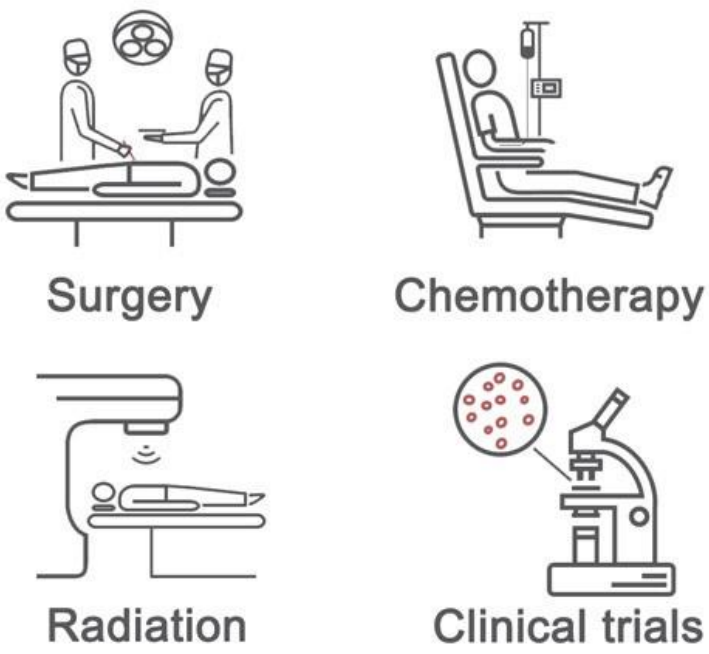

Figure 13 CRC treatments
Methods for the treatment of CRC including surgery, chemotherapy, radiation, and clinical trials.

Source: (Biller & Schrag, 2021)

CRC TNM staging

Stage of cancer is used for invasion and violence estimation which is utilized for treatment planning and prognosis of cancer. The stage of cancer can change following knowledge of scientific research. TNM staging is usually used for colorectal cancer stage evaluation; T (tumor), N (lymph node), and M (metastasis) (Obrocea, Sajin, Marinescu, & Stoica, 2011; Weiser, 2018).

T (tumor): tumor invasion

- **Tis**: Involvement of lamina propria with no extension through muscularis mucosae
- **T1**: Tumor invades the submucosa
- **T2**: Tumor invades the muscularis externa
- **T3**: Tumor invades through the muscularis externa into per colorectal tissues
- **T4a**: Tumor invades through the vascular peritoneum (including gross perforation of the bowel through the tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum))
- **T4b**: Tumor directly invades or adheres to adjacent organs or structure

N (lymph node): lymph node metastasis

- **N0**: No regional lymph node metastasis
- **N1a**: Lymph node metastasis (+1 node)
- **N1b**: Lymph node metastasis (2 or 3 nodes)
- **N1c**: No positive nodes, but tumor deposits in the subserosa mesentery nonperitonealized pericolic, or perirectal/ mesorectal tissues
- **N2a**: Lymph node metastasis (4 – 6 nodes)
- **N2b**: Lymph node metastasis (7 – more nodes)

M (metastasis): other organs metastasis

- **M0**: No metastasis
- **M1a**: Metastasis to one site or organ is identified without peritoneal metastasis
- **M1b**: Metastasis to two or more sites or organs is identified without peritoneal metastasis
- **M1c**: Metastasis to the peritoneal surface is identified alone or with other sites or organ metastases (Figure 14)

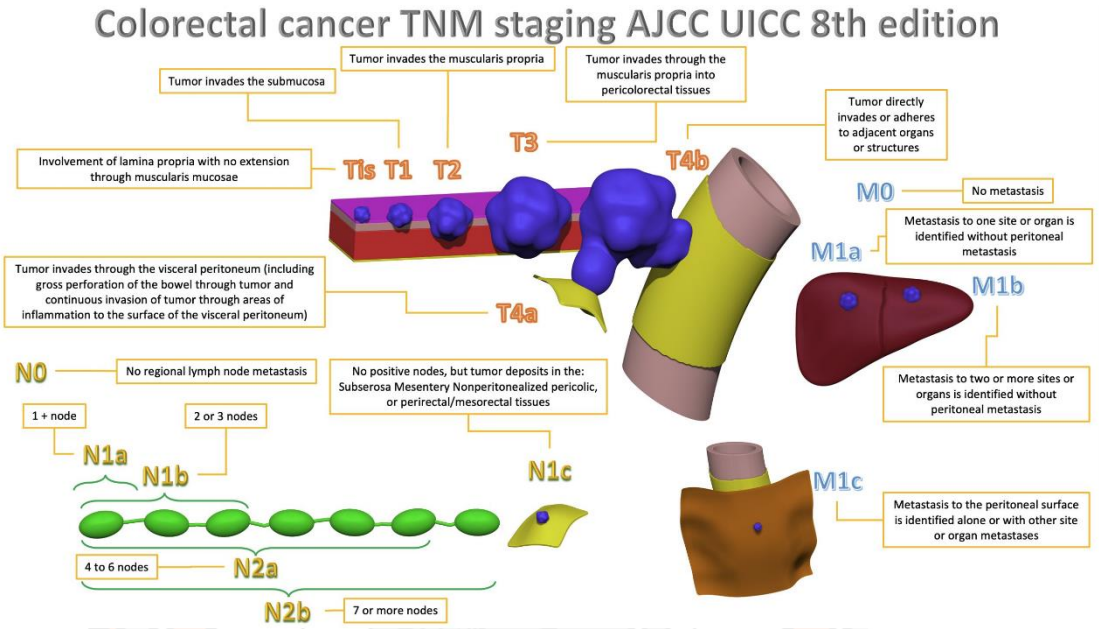

Figure 14 TNM staging of CRC

TNM staging AJCC UICC 8th edition of CRC; T = tumor invasion, N = Lymph node metastasis, and M = metastasis.

Source: Enrique de-Madaria, 2021

Pathological stage of CRC

Pathological grading is used for the prognosis of cancer which observed by morphological structure in cancer tissues. Pathological grading is used for gland forming evaluation in CRC including well-differentiated adenocarcinoma (gland forming more than 95%), moderately differentiated adenocarcinoma (gland forming 50% - 95%), and poorly differentiated adenocarcinoma (gland forming less than 50%) (Figure 15) (Dumitrescu et al., 2015).

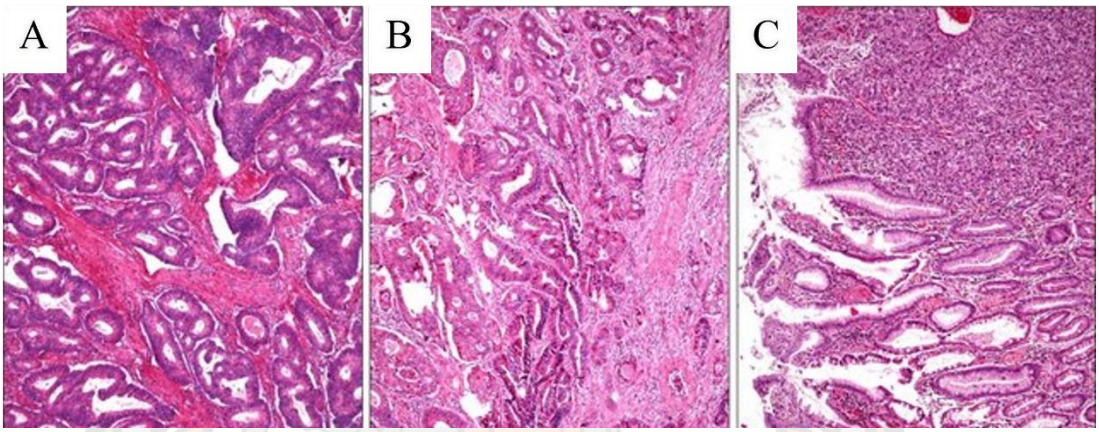

Figure 15 Pathological grading of colorectal cancer

This figure shows pathological grading of CRC including well-differentiated adenocarcinoma (A), moderately differentiated adenocarcinoma (B), and poorly differentiated adenocarcinoma (C).

Source: (Dumitrescu et al., 2015)

Mucinous and non-mucinous types of colorectal cancer

The most common type of CRC is the non-mucinous type (colorectal adenocarcinoma). Previous studies were found that the non-mucinous type was caused by oncogene and tumor suppressor genes mutation such as the *APC* gene and *P53* gene that is commonly mutated in the non-mucinous type of CRC but a less common mutation in mucinous type (Aust et al., 2002). In mucinous type was found that *BRAF* and *KRAS* genes mutation were associated with cell division inducing and apoptosis reduction. The mucinous type was negatively related to *P53* gene mutation (Reynolds et al., 2019). Thus, the histological structure expression in the mucinous type is different from the non-mucinous type (O'Connell et al., 2021).

Previous studies have found that MUC2 expression in non-mucinous and mucinous types of CRC was different. In normal conditions, MUC2 is highly expressed in the normal large intestine but loses expression in the non-mucinous type for anti-cancer pathway inhibition (Imai et al., 2013). However, previous studies were reported that MUC2 overexpresses in mucinous type. It is believed that MUC2 protects cancer cells from pathogenic cells and chemical reagents. Therefore, cancer cell in the mucinous type is chemotherapy resistant (Huang et al., 2021; Ionilă et al., 2011; Luo et al., 2019; O'Connell et al., 2021; Reynolds et al., 2019; Song et al., 2009). Moreover, histopathological structure in mucinous differently expresses from non-mucinous. Non-mucinous losses express mucin and goblet cells in intestinal glands (Figure 16B). In contrast, mucin is overexpressed in the extracellular in mucinous type (Figure 16A). Nevertheless, mucinous have followed treatment and stages scoring of non-mucinous methods because the mucinous pathway is unclear and not yet understood of mucinous causing. Some previous studies suggested that mucinous may associated with inflammatory conditions (O'Connell et al., 2021).

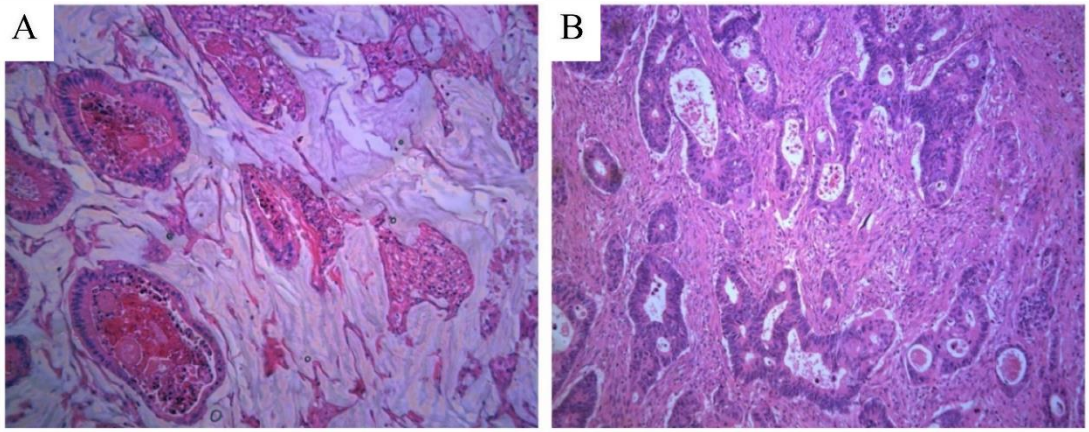

Figure 16 Histological structure of mucinous and non-mucinous types of CRC

This figure shows the histological structure of mucinous (A) and non-mucinous (B) types of colorectal cancer.

Source: (Dumitrescu et al., 2015; O'Connell et al., 2021)

CHAPTER III
RESEARCH METHODS

Conceptual framework

We investigated acid and neutral mucins expression patterns in differential parts of the normal large intestine including cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum in cadavers. Acid mucin was detected by Alcian blue (AB) pH2.5 staining showing to dark blue color. Neutral mucin was detected by Periodic acid Schiff (PAS) staining showing a magenta color. The coexistence of both acid and neutral mucins in goblet cells was detected by Alcian blue combined with Periodic acid Schiff (AB-PAS) staining. Acid and neutral mucins staining slides were photographed by the ZEISS ZEN program under a microscope. Positive stain areas of both acid and neutral mucins expression were calculated by the ImageJ Fiji program. All positive areas data were statistically analyzed by IBM SPSS 26 software.

In CRC, we investigated acid and neutral mucins, and MUC2 protein expression patterns in normal and cancer areas in non-mucinous and mucinous types. Acid and neutral mucins expression were stained by AB pH2.5, PAS, and AB-PAS staining, respectively. MUC2 protein was detected by IHC technique showing brown color in positive areas. Acid and neutral mucins and MUC2 protein staining slides were photographed by the ZEISS ZEN program under a microscope and were calculated by the ImageJ Fiji program. Quantitative data of positive areas were statistically analyzed by IBM SPSS 26 software. Finally, we evaluate the correlation between acid and neutral mucins, and MUC2 protein by Pearson's correlation method (Figure 17).

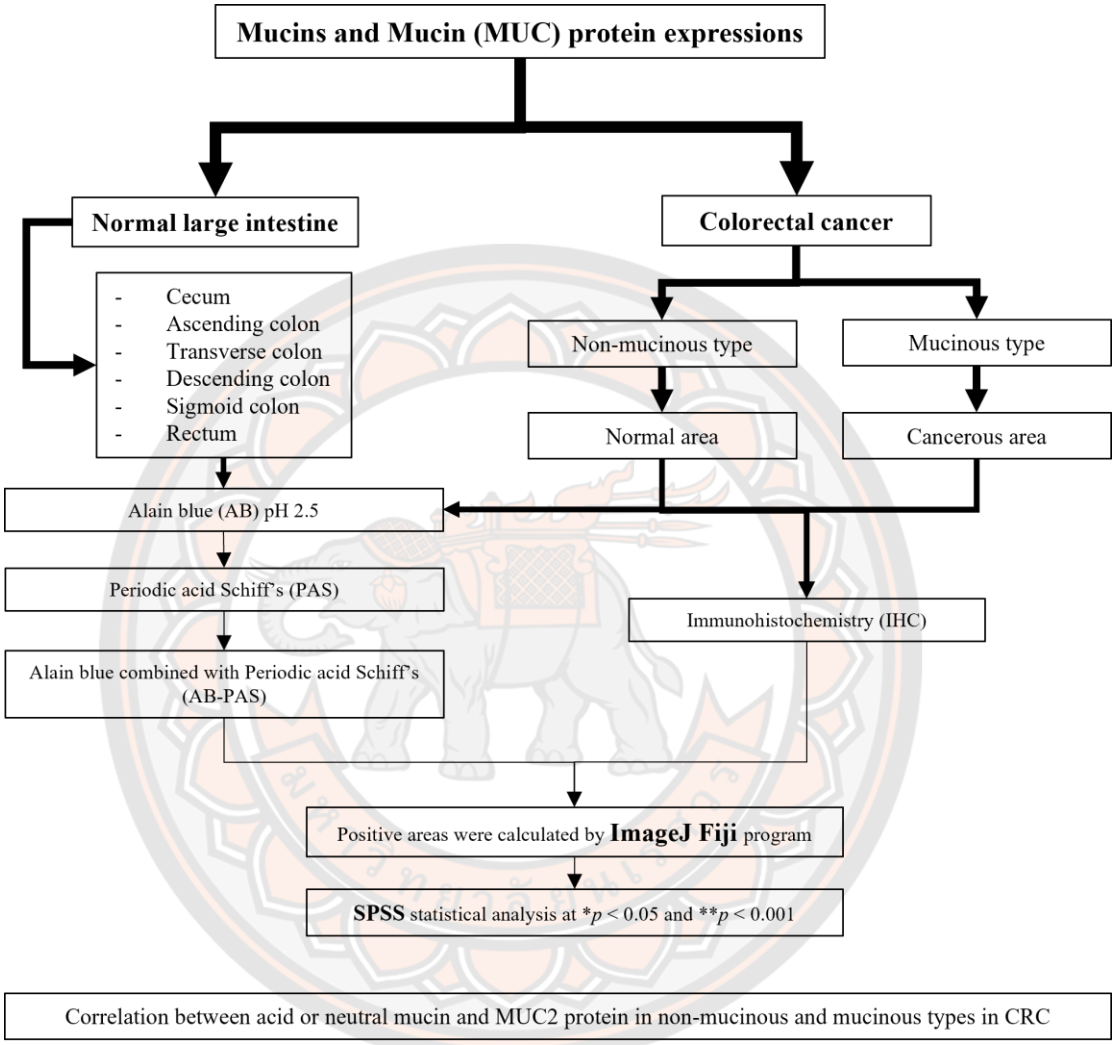

Figure 17 Conceptual framework

Samples collection

1. Normal large intestinal tissues collection

All parts of normal large intestinal tissues including cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum in thirty cadavers were collected in three pieces per part. This study was approved by Naresuan University Institutional Review Board (NUIRB) NO. 0940/62. Some tissues in rectum parts were received from the pathological unit of Sawanpracharak hospital, Nakhon Sawan province. All samples were fixed in a fixative solution (10% neutral buffer formalin: NBF) (Figure 18).

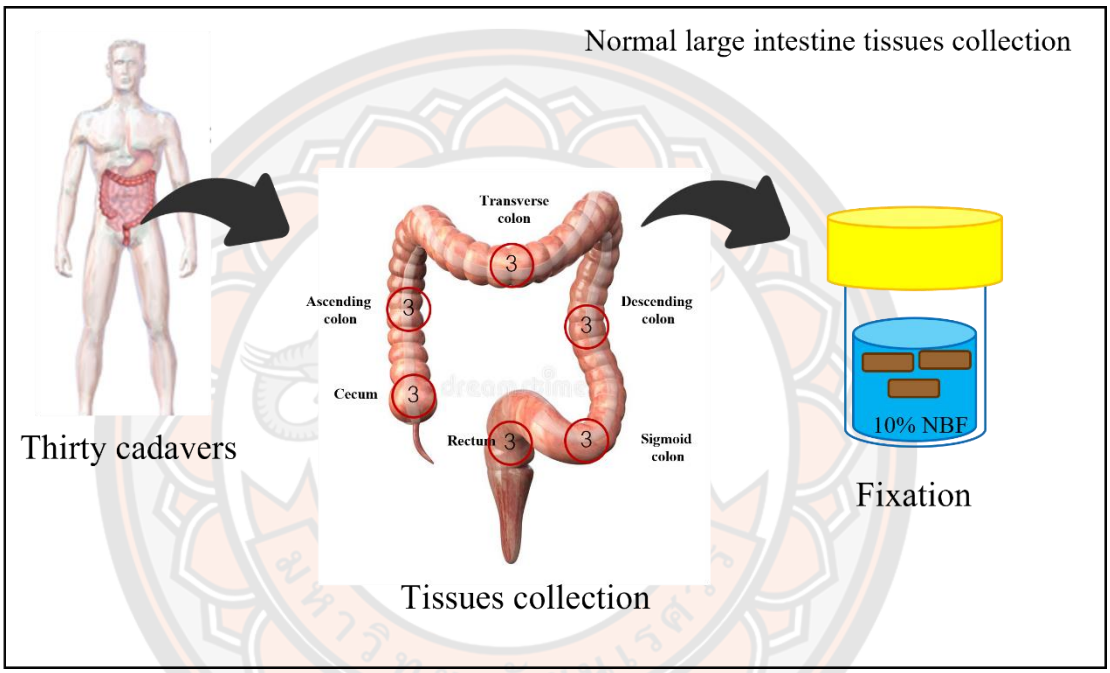

Figure 18 Collectingprocess of normal large intestinal tissues collection

2. Colorectal cancer tissues

CRC samples were divided into mucinous and non-mucinous types. Three of mucinous type and twenty of non-mucinous type paraffin block samples were collected in well-differentiated adenocarcinoma and the rectosigmoid parts.

Inclusion and exclusion criteria

1. Normal large intestine

Inclusion criteria: Large intestinal tissues of cadavers were selected by age between 50 - 80 years old, completely of all parts, not found diseases, not smoke, and not alcohol behaviors.

Exclusion criteria: Large intestine tissues of cadavers were not selected by age more than 80 years old, intestinal diseases, incompletely of all parts, smoker, and alcohol behaviors.

2. CRC tissues

Inclusion criteria: CRC paraffin blocks were selected by types of CRC including mucinous and non-mucinous, rectosigmoid parts, well-differentiated adenocarcinoma stage, and composed of normal and cancer areas

Exclusion criteria: CRC paraffin blocks were not selected by type unclear, besides rectosigmoid parts, moderately and poorly differentiated adenocarcinoma stages, composed only normal or cancer area.

Formalin-Fixed Paraffin-Embedded (FFPE) process

1. Formalin-Fixed process

All normal large intestinal tissues were preserved by 10% neutral buffer formalin (NBF) for more than 24 hours in the sampling container.

2. Tissue processing

After the formalin-fixed process, all normal large intestinal tissues were washed in distilled water for five minutes. All samples were packed in tissue embedding cassettes (three pieces per cassette). Finally, all tissues were dehydrated, cleared, and infiltrated by paraffin in the tissue processor.

3. Tissue embedding

After the tissues processing process, all samples were carried out from the tissue processor. After that, all tissues were embedded in the embedding mold and filled with the paraffin wax to perform Formalin-Fixed Paraffin-Embedded (FFPE) blocks (Figure 19).

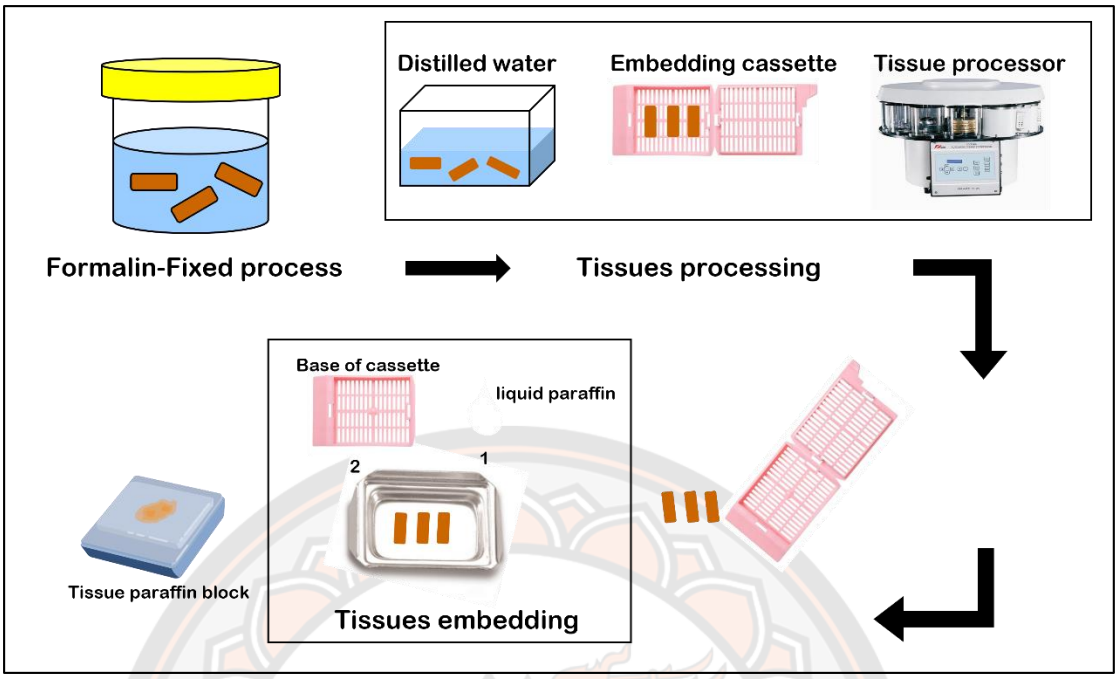

Figure 19 Formalin-Fixed Paraffin-Embedded (FFPE) process

Tissue sectioning

All paraffin blocks of normal large intestine and CRC were sectioned into ribbons at 3 µm thickness by a rotary microtome, and after that were floated at 37° C on water and picked up on the glass slide (Figure 20).

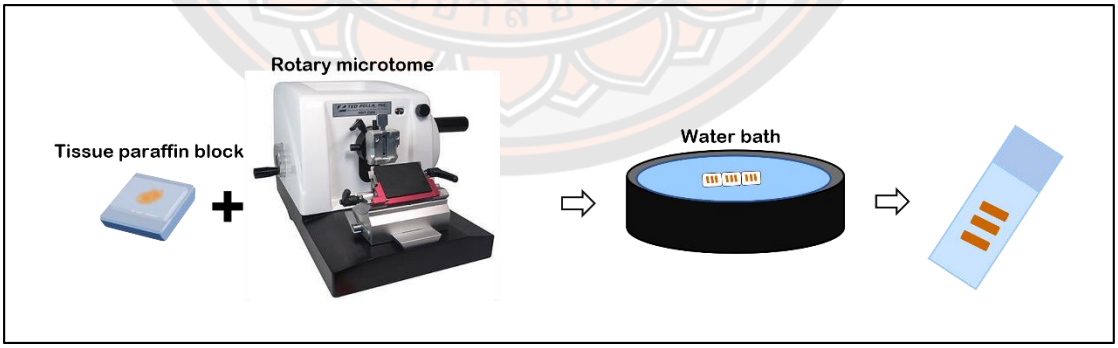

Figure 20 Tissues sectioning process.

Tissue staining

1. Hematoxylin and Eosin (H&E) staining

Normal large intestine tissues were stained by H&E for morphological structure detection. All tissue slides were deparaffined by xylene and rehydrated by serial graded alcohol (100% to 95%). The tissues slide was stained by Hematoxylin reagent for

nucleus detection and stained by Eosin reagent for cytoplasm and extracellular matrix detections. Finally, the tissues slide was dehydrated by serial alcohol (95% to 100%), and the slide was cleared by xylene (Figure 21).

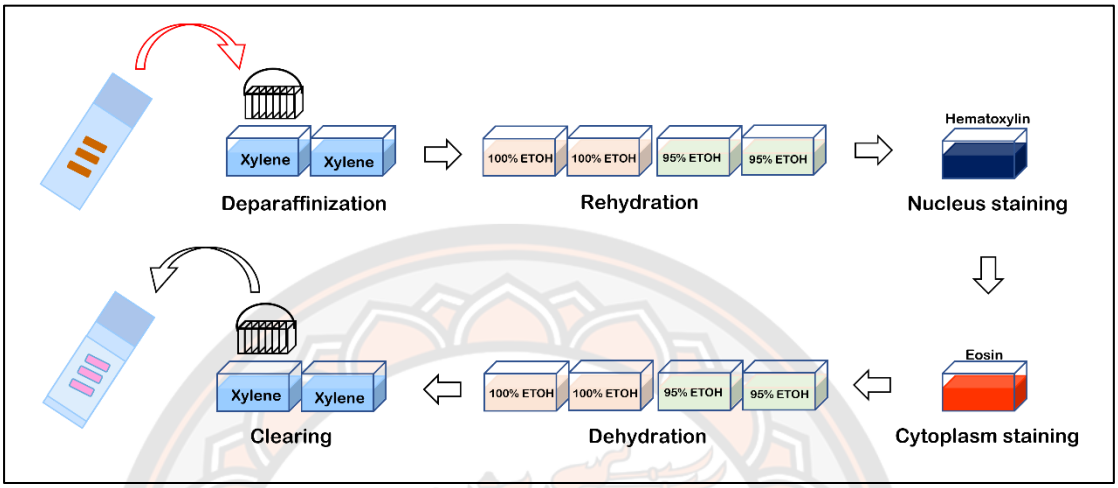

Figure 21 Hematoxylin and Eosin (H&E) staining

2. Periodic acid Schiff (PAS) staining

Normal large intestine and CRC slides were stained by PAS for neutral mucin detection. Briefly, all tissues slides were deparaffined by xylene and rehydrated by serial graded alcohol (100% to 95%). Tissue slides were stained with Periodic acid and Schiff reagent for neutral mucin detection. All tissues slides were counter-stained with Hematoxylin for nucleus detection, dehydrated by serial graded alcohol (95% to 100%), and cleared by xylene (Figure 22).

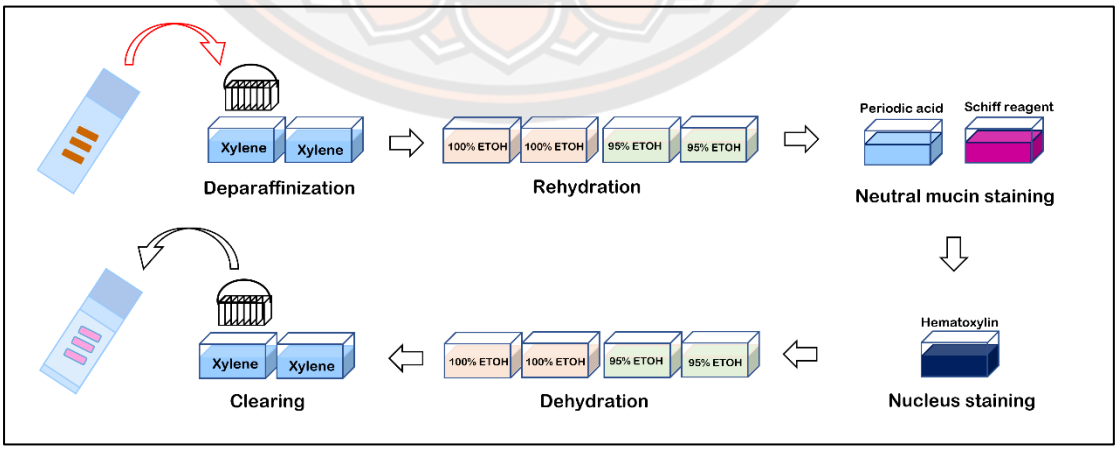

Figure 22 Periodic acid Schiff (PAS) staining

3. Alcian blue (AB) pH2.5 staining

Normal large intestine and CRC slides were stained by AB pH 2.5 for acid mucin detection. In summary, all sample slides were deparaffined by xylene and rehydrated in serial graded alcohol (100% to 95%). Tissue slides were stained by alcian blue pH2.5 reagents for acid mucin detection and nuclear fast red for nucleus detection. Tissue slides were dehydrated by serial graded alcohol (100% to 95%) and cleared by xylene (Figure 23).

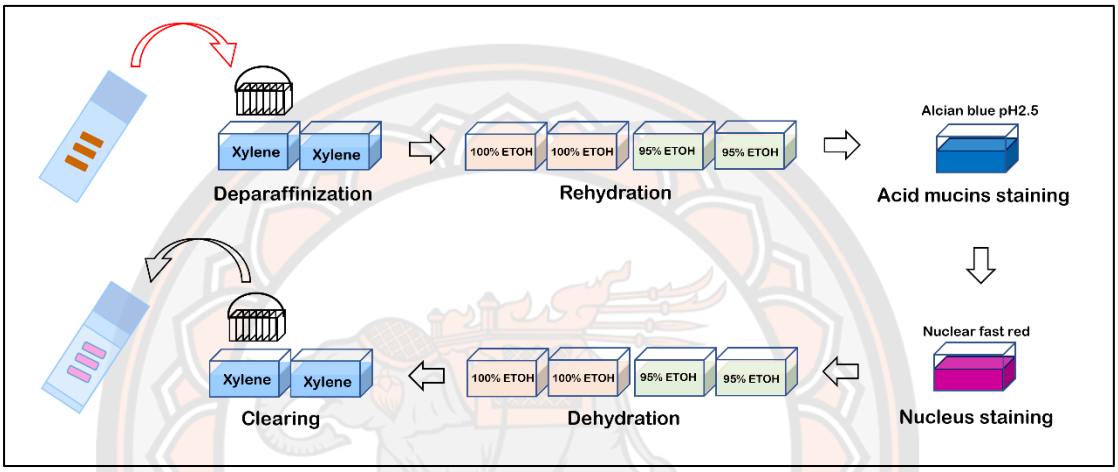

Figure 23 Alcian blue (AB) pH2.5 staining

4. Alcian blue pH2.5 combined Periodic acid Schiff (AB-PAS) staining

Normal large intestine and CRC slides were stained by AB-PAS for observed coexistence of acid and neutral mucins detection. All sample slides were deparaffined by xylene and rehydrated in serial graded alcohol (100% to 95%). Tissue slides were stained by alcian blue pH2.5 reagents for acid mucins detection and stained by PAS for neutral mucin detection. All sample slides were deparaffined by xylene and rehydrated in serial graded alcohol (100% to 95%). Tissue slides were dehydrated by serial graded alcohol (100% to 95%) and cleared by xylene (Figure 24).

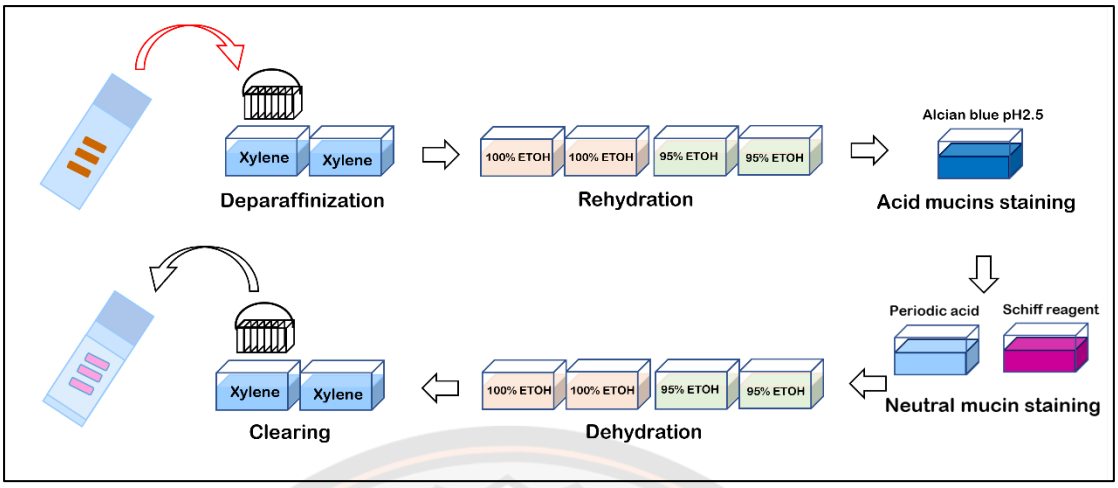

Figure 24 Alcian blue pH2.5 combined with Periodic acid Schiff (AB-PAS) staining

5. Immunohistochemistry (IHC)

IHC technique was used for specific protein detection in tissues. This study used the MUC2 antibody for MUC2 protein detection. Briefly, CRC slides were deparaffined by xylene, rehydrated by serial graded alcohol (100% to 95%), and antigen retrieved by citrate pH 6 solution. All slides were blocked with non-specific protein by hydrogen peroxidase (H_2O_2) and protein block solution and incubated in MUC2 antibody. After that, slides were incubated in secondary antibody and protein detected by 3, 3'-diaminobenzidine (DAB). Finally, all slides were counterstained by hematoxylin dry, dehydrated by serial graded alcohol (95% to 100%), and cleared by xylene (Figure 25) (Liu, Yan, & Tao, 2020).

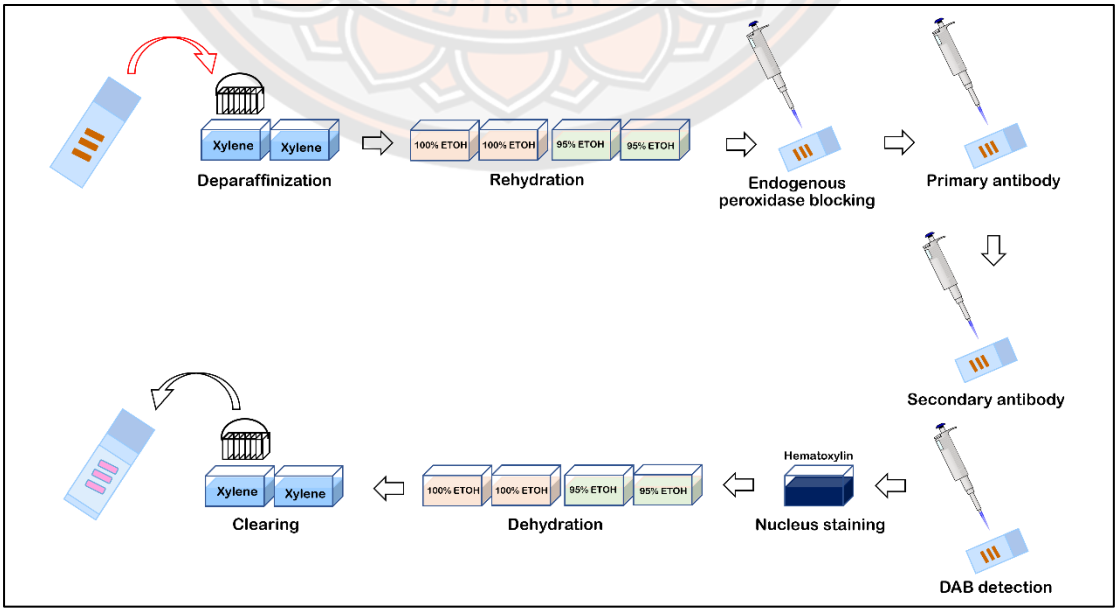

Figure 25 Immunohistochemistry (IHC)

Image analysis

1. Normal large intestine

All slides of AB pH 2.5, PAS, and AB-PAS staining in the normal large intestinal tissues were photographed by the ZEISS ZEN program under the CX31 Olympus microscope. The slide was selected in 10 areas in the mucosal layer at 200 magnifications (20X). The figure was selected in the longitudinal section of the intestinal gland for acid and neutral mucins calculation. The color of acid and neutral mucins was separated by the ImageJ Fiji program. The positive area of acid and neutral mucins was selected and calculated by the ImageJ Fiji program showing to %area (A%). In AB-PAS staining was calculated mix mucins in intestinal gland. This method was adapted by Kasprzak et al., 2019 (Figure 26) (Kasprzak et al., 2019).

"A% = area of histochemistry reaction (pixels)/tissue area (pixels) x 100%"

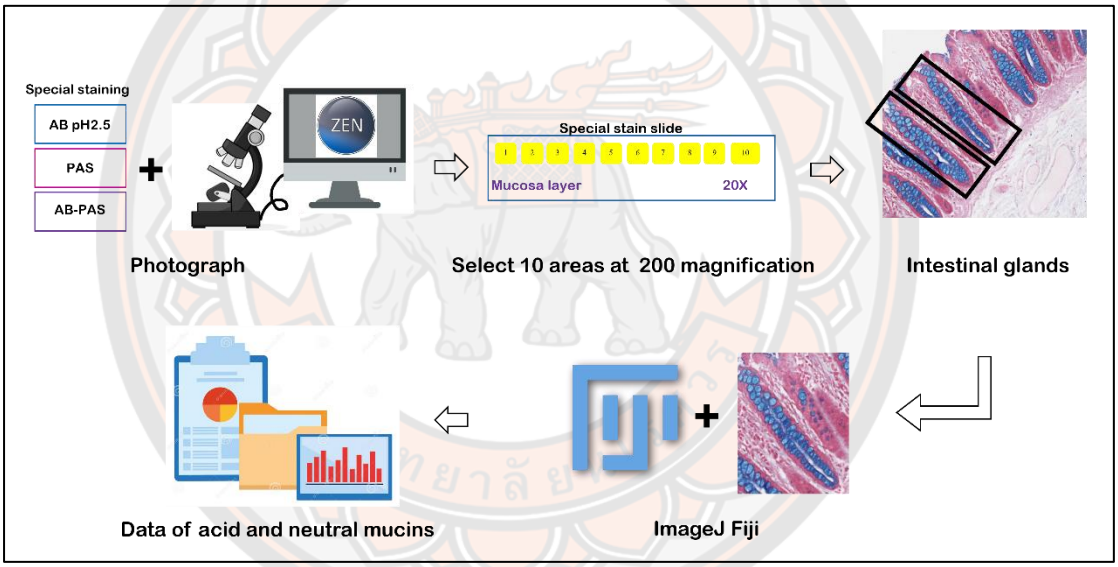

Figure 26 Acid and neutral mucins in normal large intestine analysis

2. Colorectal cancer

AB pH 2.5, PAS, and AB-PAS staining and IHC technique in CRC slides were photographed by the ZEISS ZEN program under the CX31 Olympus microscope. CRC samples were composed of mucinous and non-mucinous types and divided into normal and cancer areas in the same paraffin block. The slide was selected in 10 areas in the normal and cancer areas at 200 magnifications (20X). The figure was selected in the intestinal gland for acid and neutral mucins and MUC2 protein calculation. The positive area of acid and neutral mucins and MUC2 protein was selected and calculated by the ImageJ Fiji program showing to %area (A%). In AB-PAS staining was calculated mix mucins in intestinal gland. This method was adapted by Kasprzak et al., 2019 (Figure 27) (Kasprzak et al., 2019).

"A% = area of histochemistry reaction (pixels)/tissue area (pixels) x 100%"

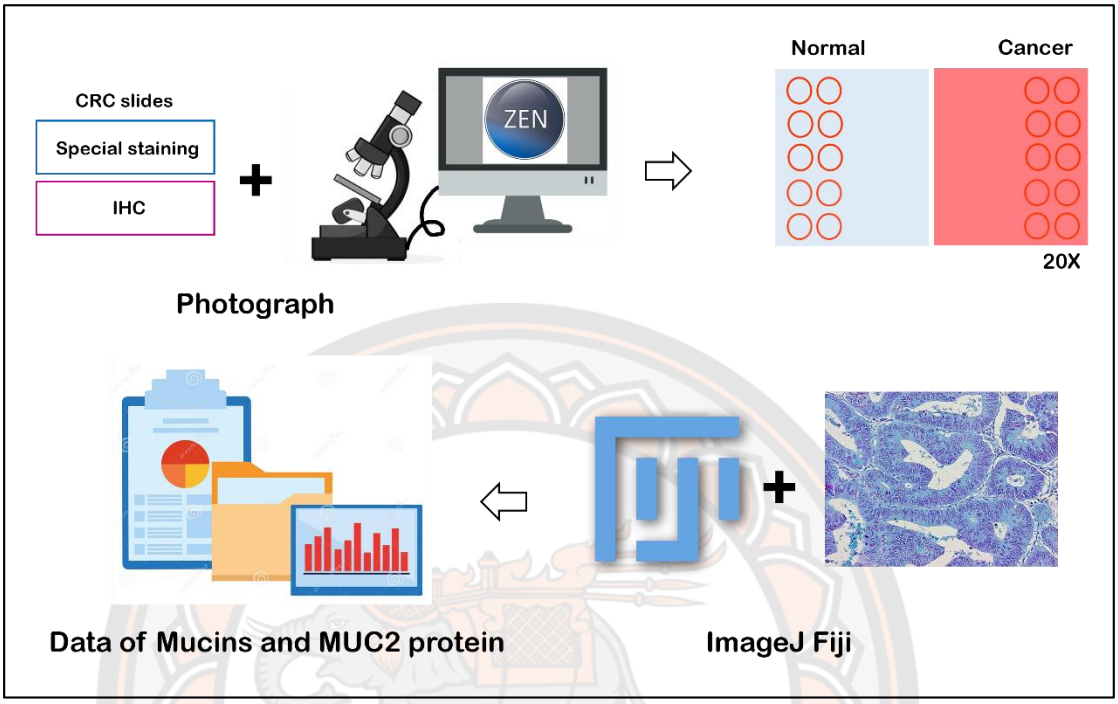

Figure 27 Acid and neutral mucins and MUC2 protein in CRC tissues analysis

Data and statistical analyses

Normal large intestine

Positive areas of acid and neutral mucins in differential parts of normal large intestine tissues were autos calculated by the ImageJ Fiji program showing to %area (A%). The A% in all parts was calculated to Mean ± SD in Microsoft excel software. The Mean in all parts was statistically calculated in IBM SPSS statistics program version 26.0 at $^{**}p<0.001$ and $^{*}p<0.05$. SPSS program was used for t-test and Analysis of Variance (ANOVA) with post hoc Tukey calculation in acid and neutral mucins expression in normal large intestine tissues.

CRC

Positive areas of acid and neutral mucins and MUC2 protein in mucinous and non-mucinous types of CRC tissues were automatically calculated by the ImageJ Fiji program showing to %area (A%). The A% in normal and cancer areas in mucinous and non-mucinous types was calculated to Mean ± SD in Microsoft excel software. The Mean in normal and cancer areas was statistically calculated in IBM SPSS statistics program version 26.0 at $^{**}p<0.001$ and $^{*}p<0.05$. SPSS program was used for t-test calculation in acid and neutral mucins and MUC2 protein expression in mucinous and non-mucinous types of CRC tissues. The mean of acid and neutral mucins, and MUC2

protein expression in mucinous and non-mucinous types were evaluated the correlation between acid or neutral mucins, and MUC2 protein estimated by Pearson's correlation method.

CHAPTER IV
RESULTS

Morphology of goblet cells in normal large intestine and in CRC tissues

Normal large intestine

Morphological detection in goblet cells was stained by Hematoxylin and Eosin (H&E) staining in differential parts of normal large intestine composing of cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum. Among of epithelial cells, goblet cell was resembled triangle-like shape and nucleus located at the basal part of cell. Most areas of goblet cells contained mucin observing as clear color. The normal large intestine showed large number of goblet cells in all part of colon. (Figure 28).

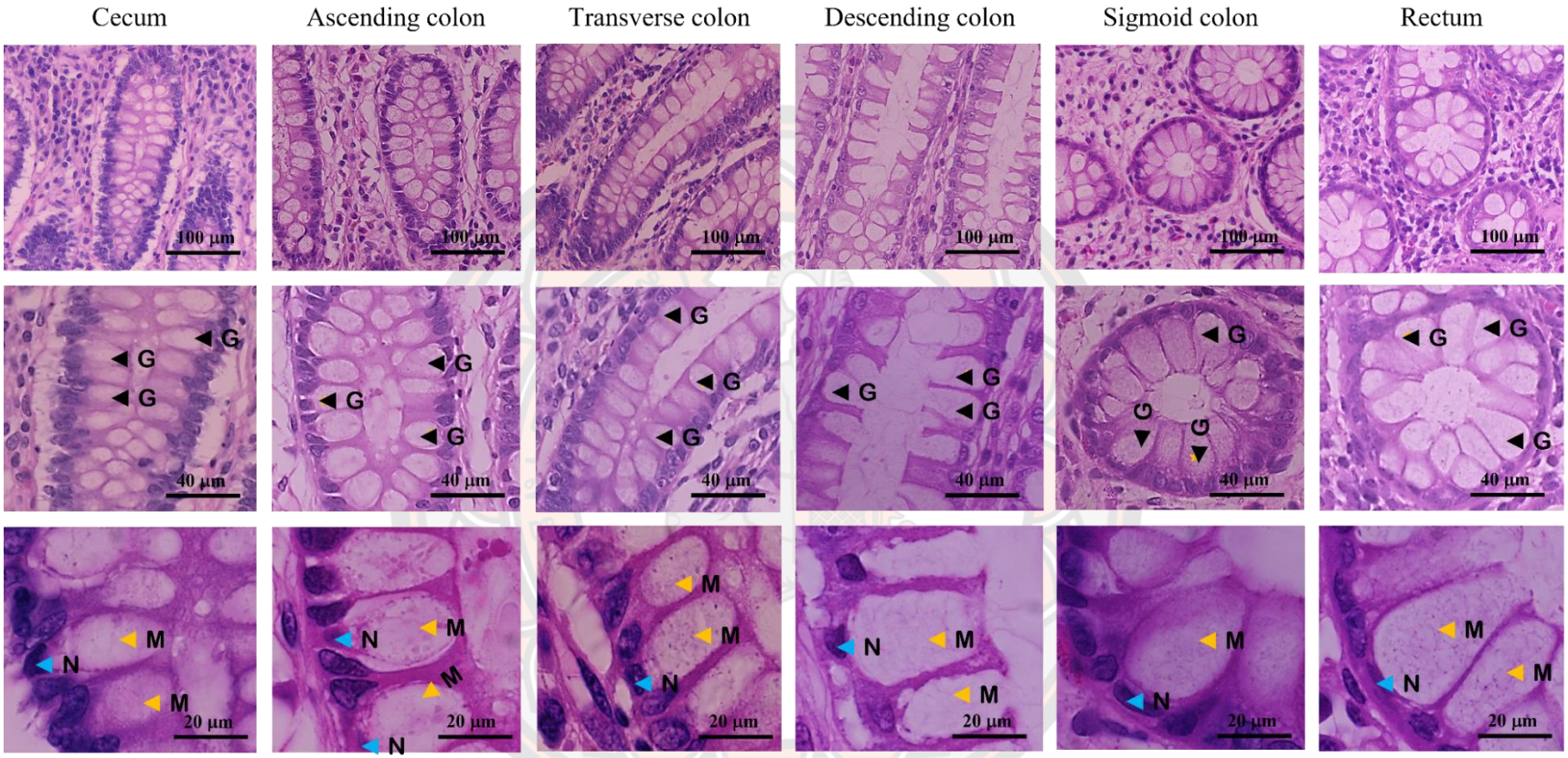

Figure 28 Morphological structure of goblet cells in various parts of normal large intestine tissues

This figure shows the morphology of goblet cells in 6 parts of normal large intestine tissues at 200, 400, and 1000 magnifications (20X, 40X, and 100X). Goblet cells resembled a triangle-like shape (◀G), clear color (◀M), and a nucleus showing dark blue (◀N). Most areas of goblet cells contain by mucins.

CRC

The morphological structure of goblet cells in mucinous and non-mucinous types in CRC was stained by H&E staining. Mucinous and non-mucinous types were divided into normal and cancer areas. In normal areas of mucinous and non-mucinous types, the goblet cell was resembled triangle-like shape and nucleus located at the basal part of cell (Figure 29A, C). In contrast, the goblet cell in the cancer area of mucinous type showed triangle-like shape, and high appearance (Figure 29B). In cancer areas of the non-mucinous type, goblet cells resembled the equivocal shape, large nucleus, obvious nuclear membrane, apparent nucleolus, and less appearance (Figure 29D).

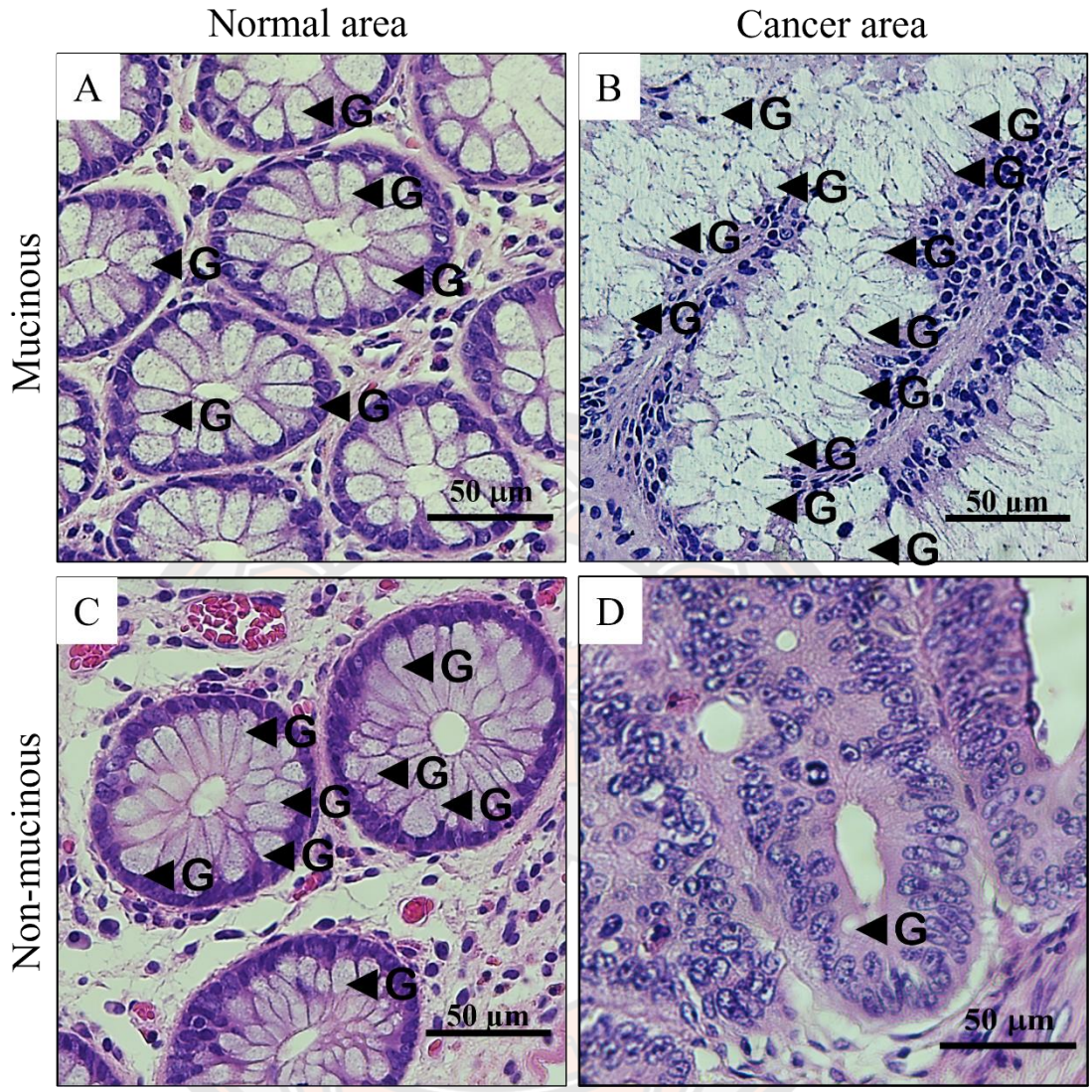

Figure 29 Morphological structure of goblet cells in normal and cancer areas in mucinous and non-mucinous types of CRC tissues.

This figure shows the morphology of goblet cells in mucinous and non-mucinous types in CRC tissues at 40X. In normal areas, goblet cells resemble a triangle-like shape (◄G) (A, C). In the cancer areas of mucinous type, goblet cells resemble tall triangle-like shapes and predominantly appearance (B). In cancer areas of the non-mucinous type, goblet cells resemble equivocal shapes. large nucleus and less appearance (D)

Acid, neutral and mixed mucins expression in normal large intestine

Acid and neutral mucins positive areas in differential parts of normal large intestine were stained by Alcian blue (AB) pH2.5, Periodic acid Schiff (PAS), and Alcian blue combined with Periodic acid Schiff (AB-PAS). Numbers of positive area were calculated by ImageJ Fiji program. Acid mucin presented blue color, and neutral mucin showed a magenta color (Figure 30). Acid mucin was commonly presented in normal large intestine and highly presented in the distal part (descending colon, sigmoid colon, and rectum). In this study, the number of acid mucins was represented as mean ± SD; cecum 16.15 ± 0.90, ascending colon 22.99 ± 1.72, transverse colon 28.61 ± 1.44, descending colon 32.60 ± 1.11, sigmoid colon 36.01 ± 1.14 and rectum 38.72 ± 1.61 (Table 1). Acid mucin expression was significantly increased compared to other parts and the cecum part (**$p < 0.001$). However, neutral mucin significantly decreased expression comparing other parts and cecum parts in a normal large intestine (**$p < 0.001$). The amount of neutral mucin was represented to the mean ± SD; cecum 10.74 ± 3.53, ascending colon 6.69 ± 1.71, transverse colon 6.35 ± 1.31, descending colon 3.87 ± 1.40, sigmoid colon 3.85 ± 1.09 and rectum 1.70 ± 0.51 (Table 1). Additionally, Mixed mucin were represented as present of %positive cells; cecum 38.34, ascending colon 25.23, transverse colon 8.78, descending colon 1.43, sigmoid colon 1.22 and rectum 1.15 (Table 2).

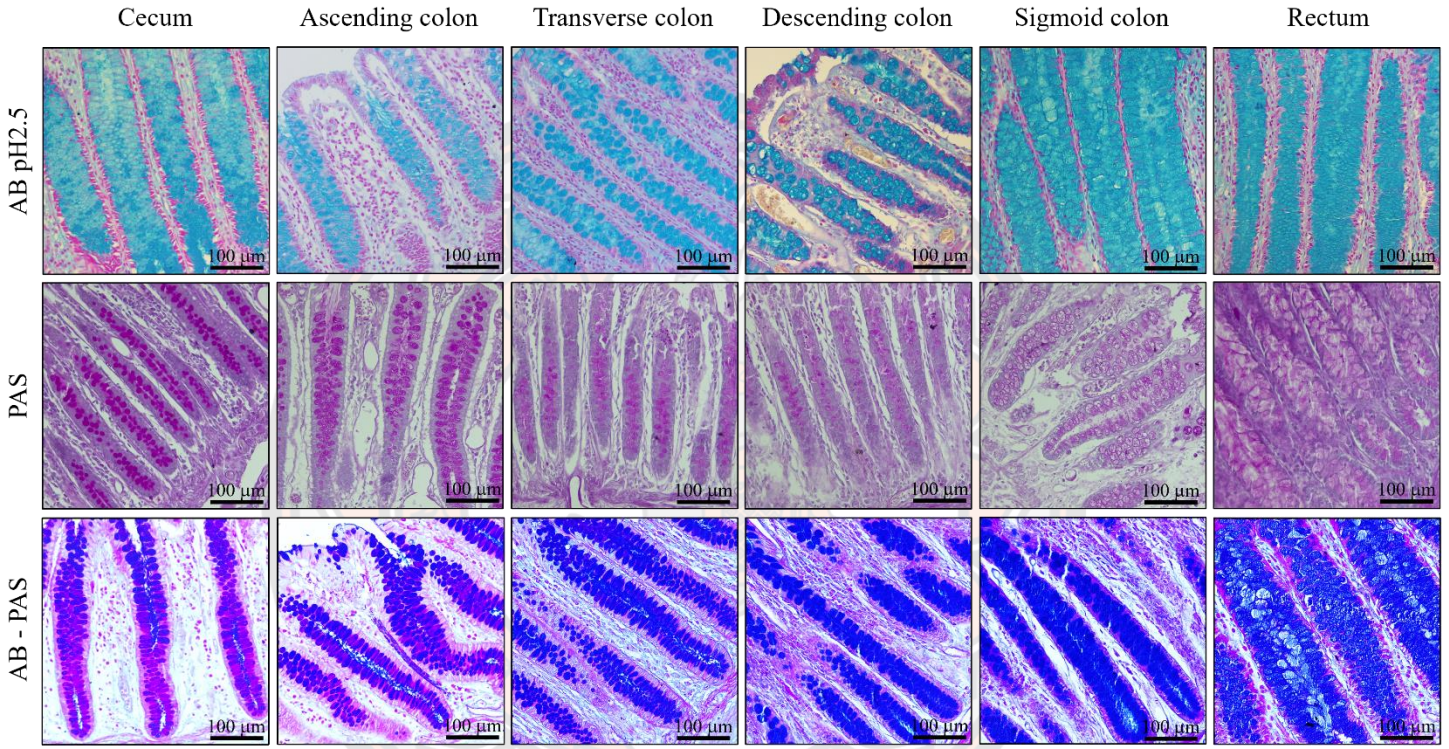

Figure 30 Acid and neutral mucins expression in differential parts in normal large intestine tissues

Acid and neutral mucins differential parts of normal large intestine tissues: Normal large intestine tissues were stained with Alcian blue (AB) pH2.5, Periodic acid Schiff (PAS), and Alcian blue combined with Periodic acid Schiff (AB/PAS) staining. All sections were investigated under a microscope connecting with ZISS ZEE software, the positive area staining was evaluated in the special stain color by the ImageJ Fiji program. Representative photographs are shown for acid mucin (top row), neutral mucin (middle row), and both acid and neutral mucins-stained tissues (lower row) of cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum in normal large intestine tissues, respectively, 200X

Table 1 Positive area of acid and neutral mucins in cecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum in normal large intestine.

Mucin / large intestine	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	Rectum	Overall p-value
Acid mucin **(Mean ± SD)**	$16.15 \pm 0.90^{2,3,4,5,6}$	$22.99 \pm 1.72^{1,3,4,5,6}$	$28.61 \pm 1.44^{1,2,4,5,6}$	$32.60 \pm 1.11^{1,2,3,5,6}$	$36.01 \pm 1.14^{1,2,3,4,6}$	$38.72 \pm 1.61^{1,2,3,4,5}$	**< 0.001**
Neutral mucin **(Mean ± SD)**	$10.74 \pm 3.53^{2,3,4,5,6}$	$6.69 \pm 1.71^{1,4,5,6}$	$6.35 \pm 2.31^{1,6,8,9}$	$3.87 \pm 1.40^{1,2,7,10}$	$3.85 \pm 1.09^{1,2,7,10}$	$1.70 \pm 0.51^{1,2,3,8,9}$	**< 0.001**

1 $p < 0.001$ vs. Cecum
2 $p < 0.001$ vs. Ascending colon
3 $p < 0.001$ vs. Transverse colon
4 $p < 0.001$ vs. Descending colon
5 $p < 0.001$ vs. Sigmoid colon
6 $p < 0.001$ vs. Rectum
7 $p < 0.05$ vs. Transverse colon
8 $p < 0.05$ vs. Descending colon
9 $p < 0.05$ vs. Sigmoid colon
10 $p < 0.05$ vs. Rectum

Table 2 Positive cells of mixed mucins in cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum in normal large intestine.

Positive cells of mixed mucins in various parts of normal large intestine						
	Cecum	Ascending colon	Transverse colon	Descending colon	Sigmoid colon	Rectum
Total cells	88.6	88	93.4	69.8	65.4	138.6
Mixed mucin	33.2	22.2	8.2	1	0.8	1.6
%Positive cells	38.34[2,3,4,5,6]	25.23[1,3,4,5,6]	8.78[1,2,4,5,6]	1.43[1,2,3]	1.22[1,2,3]	1.15[1,2,3]

1 $p < 0.001$ vs. Cecum
2 $p < 0.001$ vs. Ascending colon
3 $p < 0.001$ vs. Transverse colon
4 $p < 0.001$ vs. Descending colon
5 $p < 0.001$ vs. Sigmoid colon
6 $p < 0.001$ vs. Rectum
7 $p < 0.05$ vs. Transverse colon
8 $p < 0.05$ vs. Descending colon
9 $p < 0.05$ vs. Sigmoid colon
10 $p < 0.05$ vs. Rectum

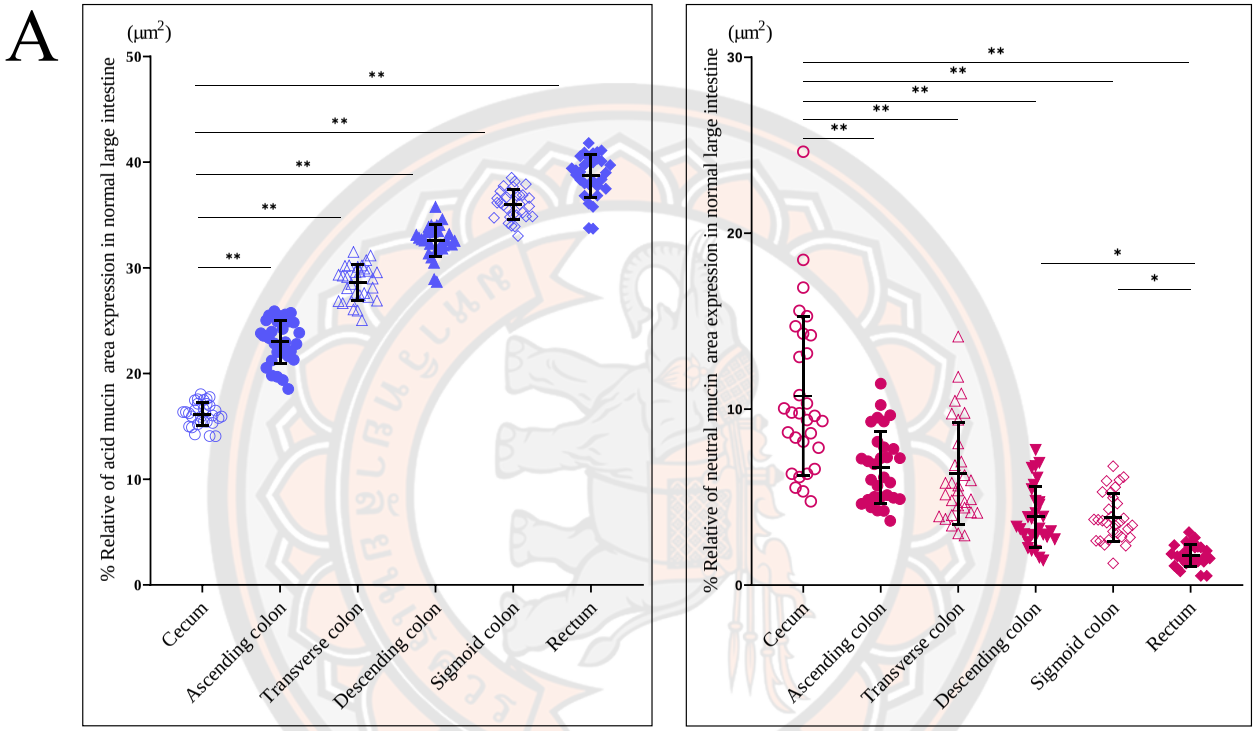

Figure 31 Acid and neutral mucins in differential parts in normal large intestine tissues

Acid and neutral mucins in differential parts of normal large intestine tissues: Normal large intestine tissues were stained with Alcian blue (AB) pH2.5, Periodic acid Schiff (PAS), and Alcian blue combined with Periodic acid Schiff (AB/PAS) staining. The positive area staining was evaluated in the special stain color by the ImageJ Fiji program. Acid mucin significantly increased whereas neutral mucin significantly decreased comparing another part and the cecum part (**p<0.001).

Acid, neutral and mixed mucins expression in non-mucinous and mucinous types in CRC tissues

Positive areas of acid and neutral mucins in non-mucinous and mucinous types of CRC were stained by AB pH 2.5, PAS, and AB-PAS, and were calculated by the ImageJ Fiji program. Acid mucin presented blue color and neutral mucin showed a magenta color. In the mucinous type, acid mucin significantly increased in the cancer area (39.05 ± 5.60) compared to the normal area (27.66 ± 3.92) at $^{**}p < 0.001$ (Figure 31A, B and Table 2). In the non-mucinous type, acid mucin was significantly decreased in cancer area (7.87 ± 1.79) compared to normal area (29.76 ± 4.59) at $^{**}p < 0.001$ (Figure 31C, D and Table 2). Moreover, acid mucin was significantly increased in mucinous type compared to non-mucinous type ($^{**}p < 0.001$). Neutral mucin in mucinous type was significantly decreased in cancer (4.23 ± 1.32) compared to normal area (10.72 ± 2.88) at $^{*}p < 0.001$ (Figure 32A, B and Table 2). In non-mucinous type, neutral mucin was significantly decreased in cancer area (2.01 ± 0.16) compared to normal area (10.41 ± 1.62) at $^{**}p < 0.001$ (Figure 32C, D and Table 2). Moreover, neutral mucin was significantly decreased in non-mucinous type compared to mucinous type ($^{**}p < 0.001$). Furthermore, high expression of acid mucin was found in mucinous type compared to non-mucinous type (Figure 33). Additionally, %positive cells of mixed mucins in non-mucinous type was significantly decreased in cancer area (4.39) compared to normal area (0.6). In mucinous type, mixed mucin was significantly decreased in cancer area (3.8) compared to normal area (0.18) (Table 4).

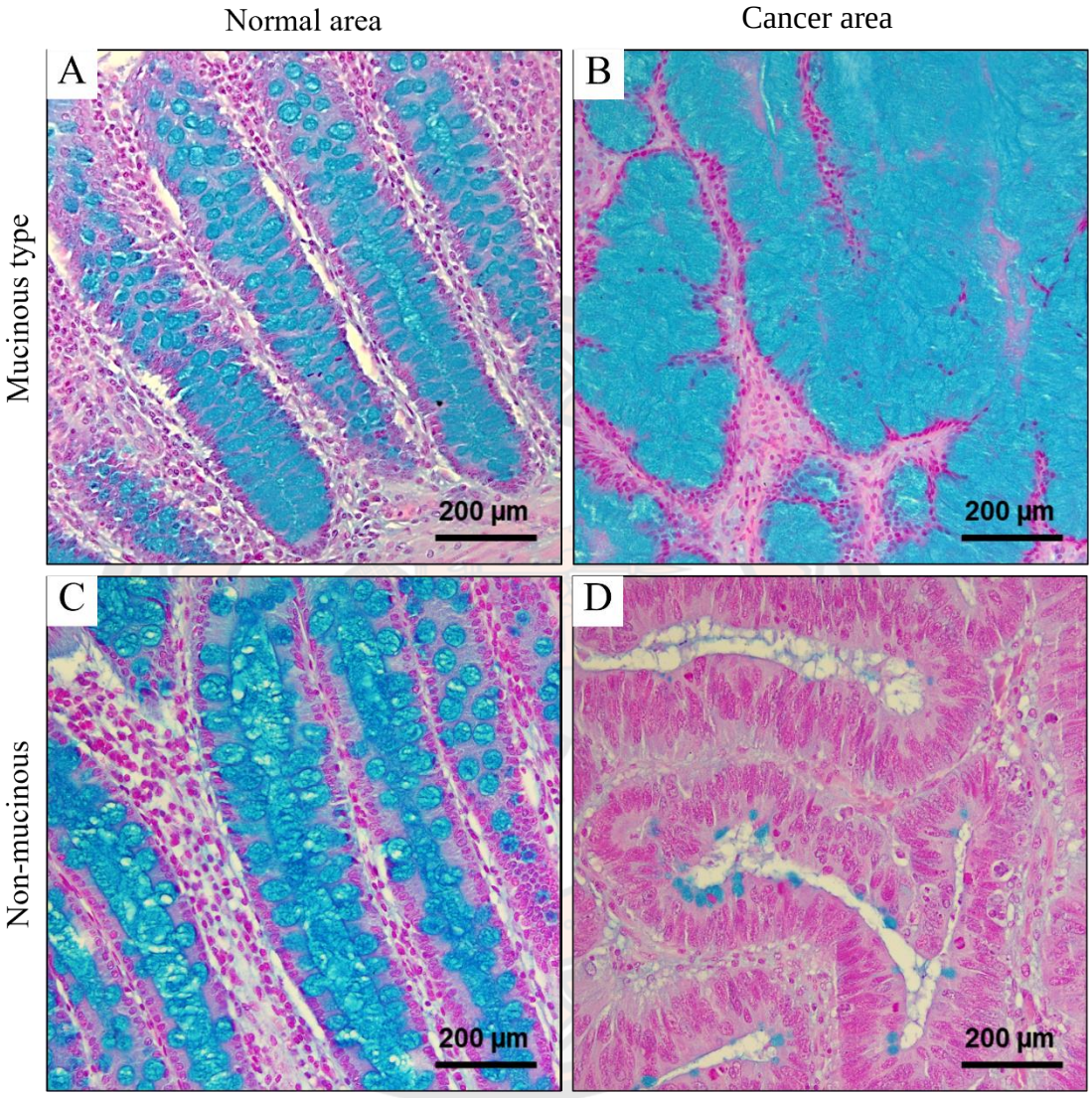

Figure 32 Acid mucin expression in normal and cancer areas in mucinous and non-mucinous types in CRC tissues

Acid mucin in normal and cancer areas in mucinous and non-mucinous types of colorectal cancer was stained with AB pH 2.5 staining. All sections were investigated under a microscope, the positive cell staining was evaluated in the blue stain color by the ImageJ Fiji program. Representative photographs show acid mucin in normal area (A) and cancer area (B) in mucinous type, normal area (C) and cancer area (D) in non-mucinous type of colorectal cancer, respectively (20X).

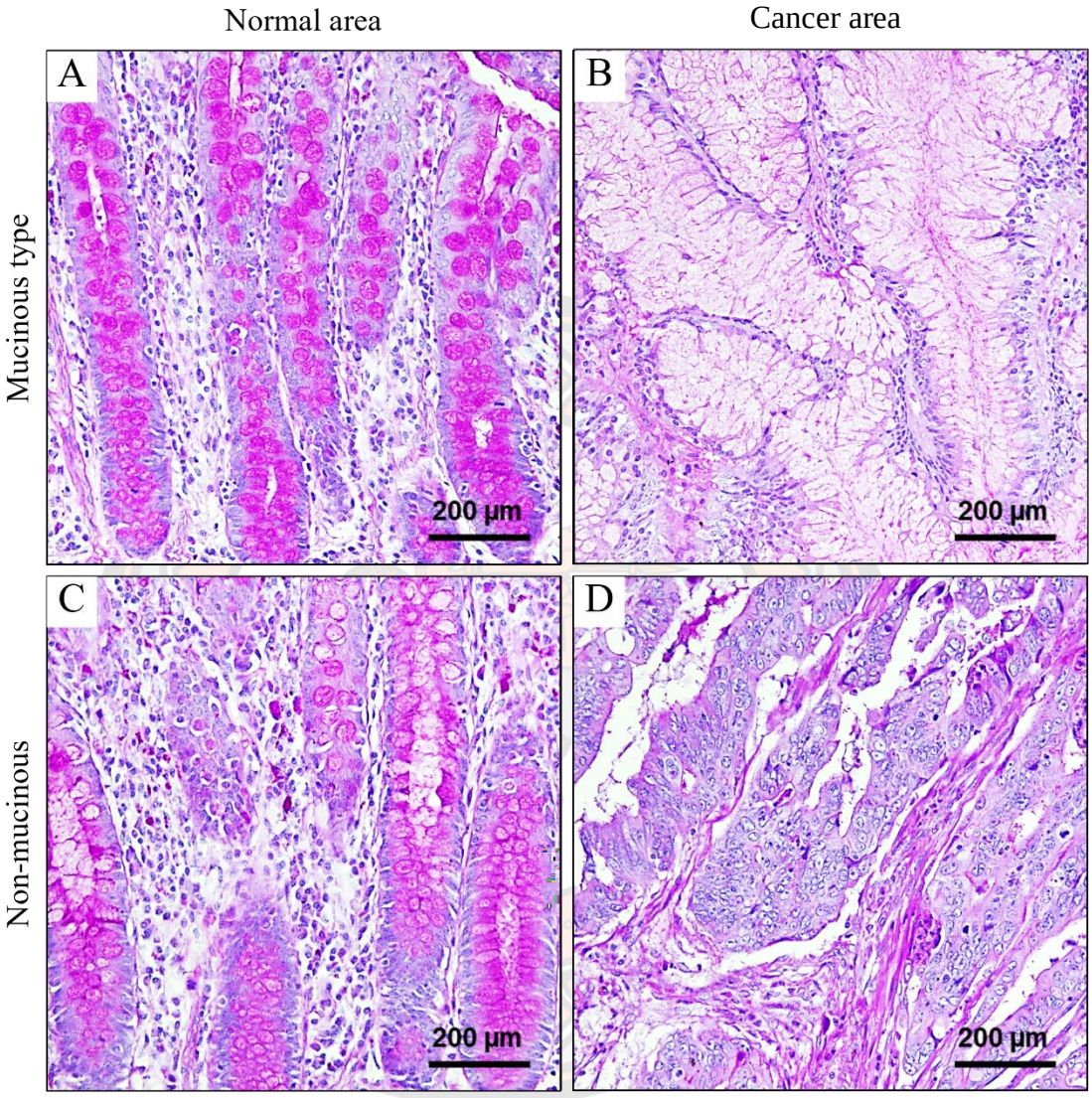

Figure 33 Neutral mucin expression in normal and cancer areas in mucinous and non-mucinous types in CRC tissues

Neutral mucin in normal and cancer areas in mucinous and non-mucinous types of colorectal cancer was stained with PAS staining. All sections were investigated under a microscope, the positive cell staining was evaluated in the magenta stain color by the ImageJ Fiji program. Representative photographs show neutral mucin in normal area (A) and cancer area (B) in mucinous type, normal area (C), and cancer area (D) in non-mucinous type of colorectal cancer, respectively (20X).

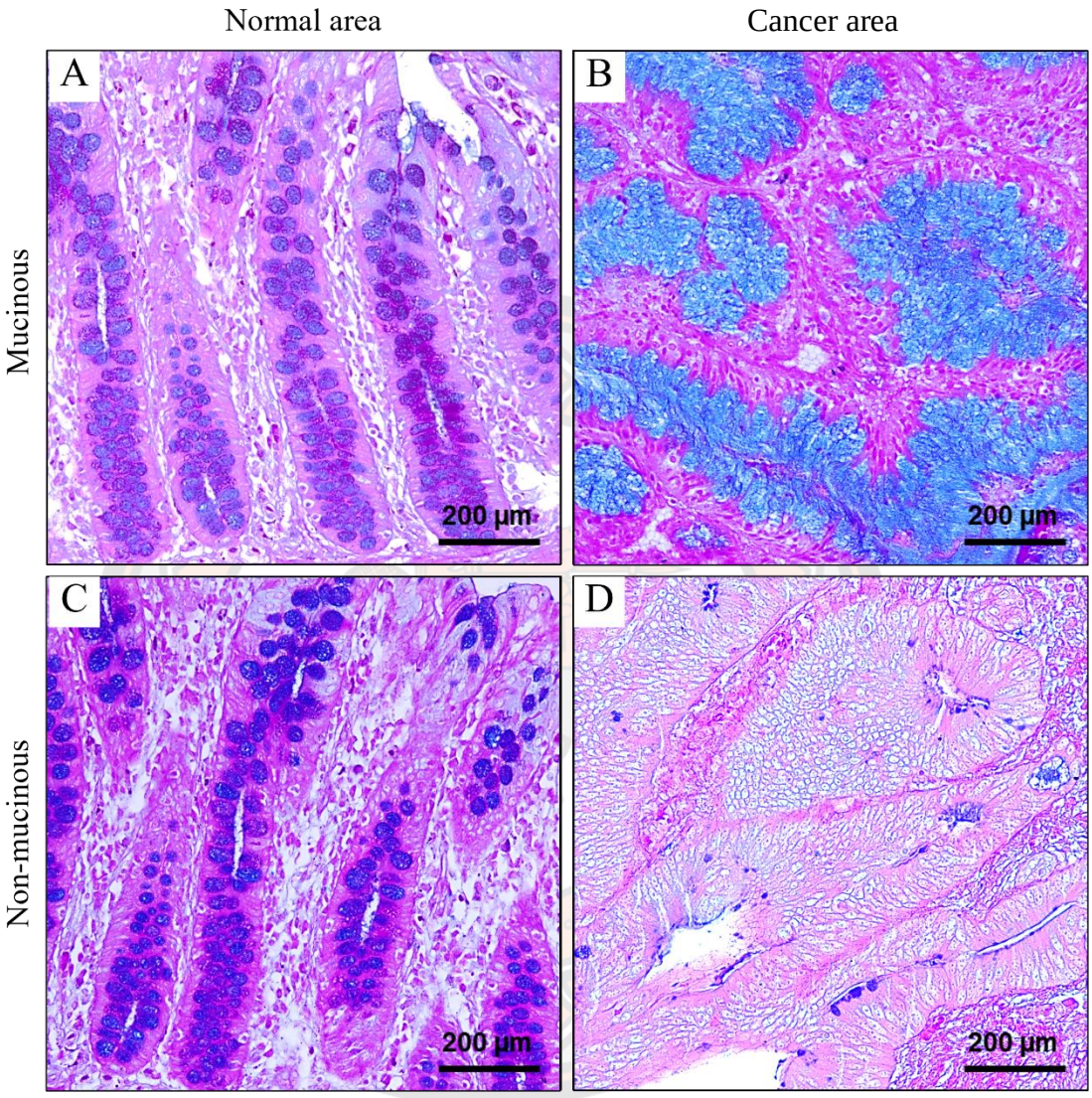

Figure 34 Acid and neutral mucins expression in normal and cancer areas in mucinous and non-mucinous types in CRC tissues

Acid and neutral mucins in normal and cancer areas in mucinous and non-mucinous types of colorectal cancer was stained with AB-PAS staining. All sections were investigated under a microscope, the positive cell staining was evaluated in the blue and magenta stain colors by the ImageJ Fiji program. Representative photographs show acid and neutral mucins in normal area (A) and cancer area (B) in mucinous type, normal area (C), and cancer area (D) in non-mucinous type of colorectal cancer, respectively (20X).

MUC2 protein expression in non-mucinous and mucinous types of CRC

MUC2 protein in mucinous and non-mucinous types of CRC tissues were stained by the IHC technique and positive areas were calculated by the ImageJ Fiji program. MUC2 protein was presented in brown color. In mucinous type, MUC2 protein was significantly increased in cancer area (37.91 ± 4.94) compared to normal area (26.52 ± 4.02 $^{**}p < 0.001$) (Figure 34A, B and Table 2). In non-mucinous type, MUC2 protein was significantly decreased in cancer area (6.51 ± 2.07) compared to normal area (27.60 ± 5.89 $^{**}p < 0.001$) (Figure 34C, D and Table 2). Moreover, MUC2 protein overexpressed in mucinous compared to non-mucinous types ($^{**}p < 0.001$).

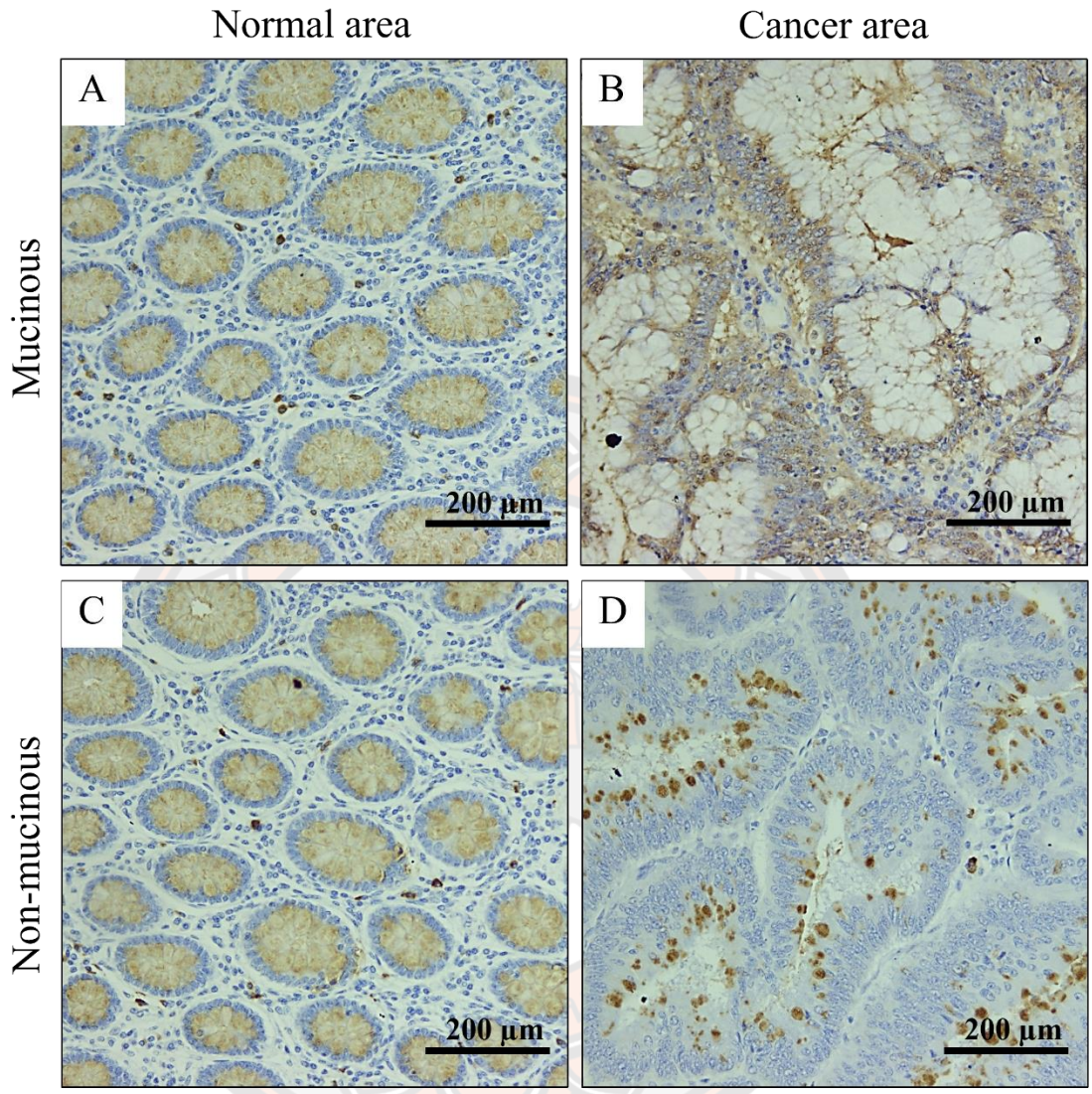

Figure 35 MUC2 protein expression in normal and cancer areas in mucinous and non-mucinous types in CRC tissues

MUC2 protein in normal and cancer areas in mucinous and non-mucinous types of CRC tissues were stained with IHC. All sections were investigated under a microscope. Positive cells staining was evaluated in brown stain colors by the ImageJ Fiji program. Representative photographs show MUC2 protein in normal area (A) and cancerous area (B) in mucinous type, normal area (C) and cancerous area (D) in non-mucinous type, respectively (20X).

Table 3 Positive areas of acid mucin, neutral mucins, and MUC2 protein in mucinous and non-mucinous types of CRC tissues

Mucins	Areas		Mucinous (n = 3)	Non-mucinous (n = 20)	P-value
Acid mucin	Normal area	(Mean ± SD) Median (Min, Max)	27.66 ± 3.92 28.15 (22.05, 39.67)	29.76 ± 4.59 30.40 (22.59, 39.25)	0.127
	Cancerous area	(Mean ± SD) Median (Min, Max)	39.05 ± 5.60 38.91 (29.35, 56.37)	7.87 ± 1.79 7.89 (4.24, 11.37)	> **0.001****
		P-value	> **0.001****	> **0.001****	
Neutral mucin	Normal area	(Mean ± SD) Median (Min, Max)	10.72 ± 2.88 10.19 (5.48, 17.04)	10.41 ± 1.62 10.53 (6.18, 13.65)	0.352
	Cancerous area	(Mean ± SD) Median (Min, Max)	4.23 ± 1.32 3.88 (2.45, 7.94)	2.01 ± 0.16 2.05 (1.57, 2.22)	> **0.001****
		P-value	>**0.001****	> **0.001****	
MUC2 protein	Normal area	(Mean ± SD) Median (Min, Max)	26.52 ± 4.02 25.71 (20.42, 39.67)	27.60 ± 5.89 26.93 (17.38, 41.09)	0.454
	Cancerous area	(Mean ± SD) Median (Min, Max)	37.91 ± 4.94 38.08 (28.44, 47.28)	6.51 ± 2.07 6.77 (2.14, 10.63)	> **0.001****
		P-value	> **0.001****	> **0.001****	

Table 4 Positive cells of mixed mucins protein in mucinous and non-mucinous types of CRC tissues

Positive cells of mixed mucins in non-mucinous and mucinous types of CRC				
	Non-mucinous type		Mucinous type	
	Normal area	Cancer area	Normal area	Cancer area
Total cells	159.6	200.6	184.4	223.2
Mixed mucin	7	1.2	7	0.4
%Positive cells	**4.39**	**0.6****	**3.8**	**0.18****

Correlation between acid and neutral mucins and MUC2 protein expression in mucinous and non-mucinous types of CRC tissues

Pearson correlation method was used to investigate correlation between acid or neutral mucins and MUC2 protein expression in CRC tissues. In mucinous type, MUC2 protein was correlated with acid mucin in normal (Correlation Coefficient; r = 0.947, $^{**}p < 0.001$) and cancer areas (r = 0.908, $^{**}p < 0.001$). However, expression of MUC2 protein was not corelated with neutral mucin in normal (r = 0.316, $p = 0.089$) and cancer areas (r = 0.229, $p = 0.223$) (Figure 35 and Table 3). In non-mucinous type, MUC2 protein was correlated with acid mucin in normal (r = 0.885, $^{**}p < 0.001$) and cancer areas (r = 0.884, $^{**}p < 0.001$). However, expression MUC2 protein was not corelated with neutral mucin in normal (r = 0.278, $p = 0.235$) and cancer areas (r = 0.070, $p = 0.768$) (Figure 35 and Table 4). Therefore, MUC2 protein expression was corelated with acid mucin expression in CRC tissues in both mucinous and non-mucinous types.

Table 5 Pearson correlation between acid and neutral mucins and MUC2 protein expression in normal and cancer areas in mucinous type

Pearson Correlation	Mucinous type					
	Normal area			Cancer area		
	Acid mucin	Neutral mucin	MUC2	Acid mucin	Neutral mucin	MUC2
Acid mucin	1	0.242 0.197	**0.947**** **0.000**	1	0.223 0.235	**0.908**** **0.000**
Neutral mucin	0.242 0.197	1	0.316 0.089	0.223 0.235	1	0.229 0.223
MUC2	**0.947**** **0.000**	0.316 0.089	1	**0.908**** **0.000**	0.229 0.223	1

Table 6 Pearson correlation between acid and neutral mucins and MUC2 protein expression in normal and cancer areas in non-mucinous type

	Non-mucinous type					
Pearson Correlation	Normal area			Cancer area		
	Acid mucin	Neutral mucin	MUC2	Acid mucin	Neutral mucin	MUC2
Acid mucin	1	0.280 0.232	**0.885**** **0.000**	1	0.320 0.169	**0.884**** **0.000**
Neutral mucin	0.280 0.232	1	0.278 0.235	0.320 0.169	1	0.070 0.768
MUC2	**0.885**** **0.000**	0.278 0.235	1	**0.884**** **0.000**	0.070 0.768	1

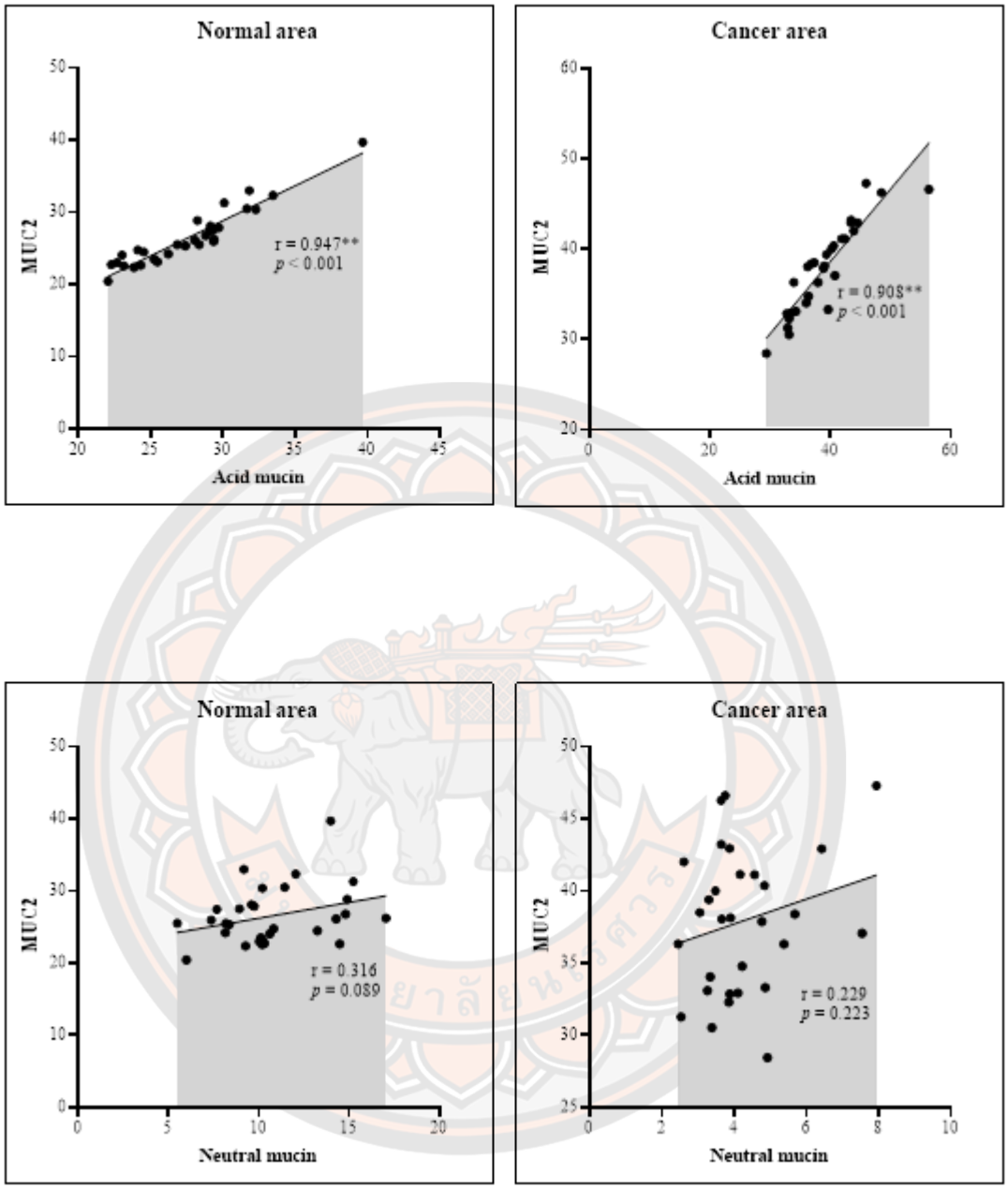

Figure 36 Pearson correlation between acid and neutral mucin and MUC2 protein expression in normal and cancer areas in mucinous type

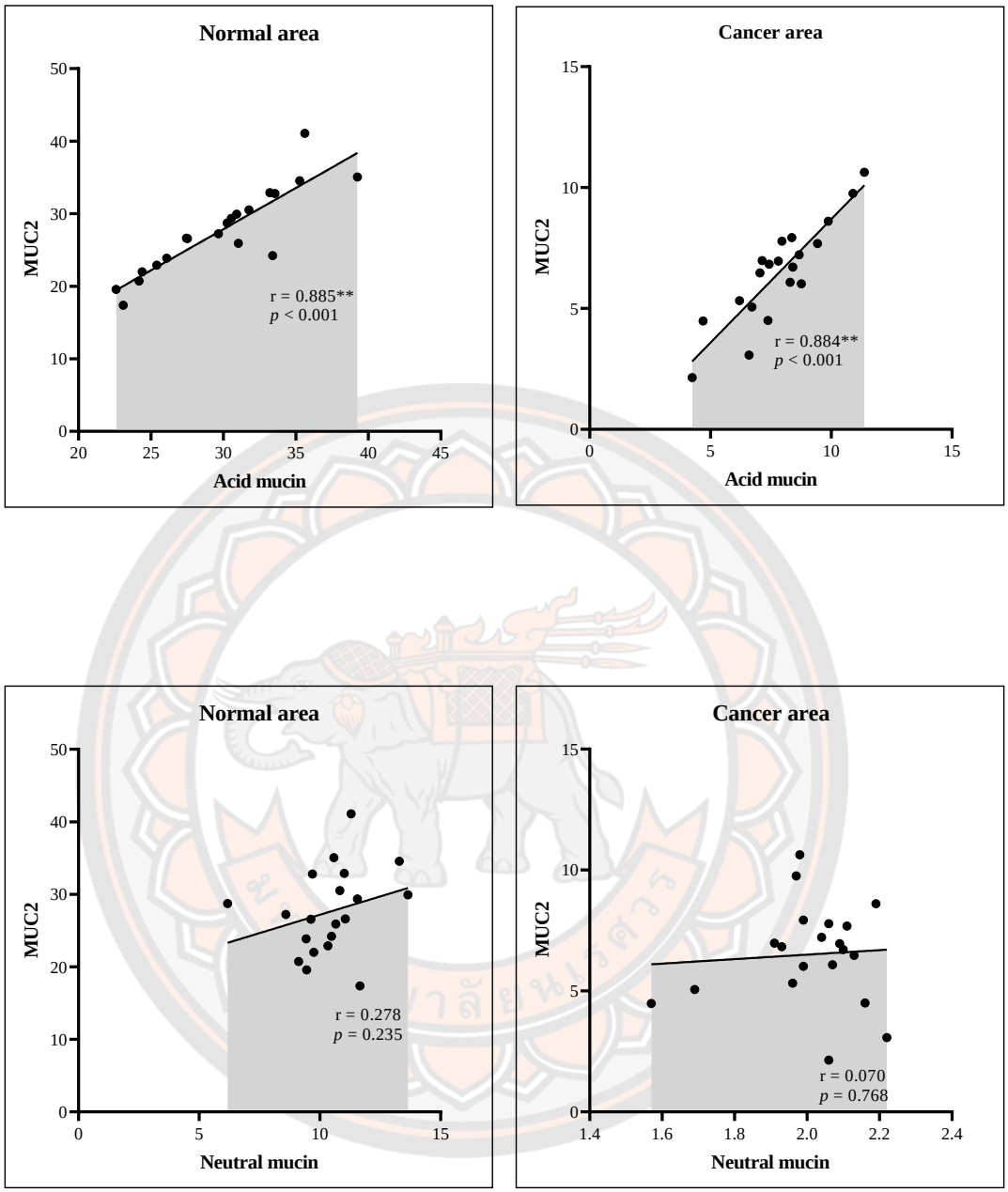

Figure 37 Pearson correlation between acid and neutral mucin and MUC2 protein expression in normal and cancer areas in non-mucinous type

Association of MUC2 protein expression status with clinicopathology in non-mucinous and mucinous types of CRC

The initial clinical association study showed that MUC2 protein were significantly correlation with gender in non-mucinous type of CRC (Table 7) $*p<0.05$. It was also unrelated to age, tumor greatest dimension and lymph node metastasis. In mucinous type were unrelated with gender, age, tumor greatest dimension and lymph node metastasis (Table 8).

Table 7 Association of MUC2 expression status with clinicopathology in non-mucinous type of CRC patients (total n=20)

Clinicopathological characteristics	n (%)	MUC2 expression		*P*-value
		Low MUC2 [n (%)]	High MUC2 [n (%)]	
Gender				**0.035***
Male	11 (55.00)	3 (15.00)	8 (40.00)	
Female	9 (45.00)	7 (35.00)	2 (10.00)	
Age				0.686
≤ 60 years old	6 (30.00)	3 (15.00)	3 (15.00)	
> 60 years old	14 (70.00)	7 (35.00)	7 (35.00)	
Tumor greatest dimension (cm.)				0.686
≥ 4.5 cm.	14 (70.00)	7 (35.00)	7 (35.00)	
< 4.5 cm.	6 (30.00)	3 (15.00)	3 (15.00)	
Lymph node metastasis				0.325
N1-N2	12 (60.00)	5 (25.00)	7 (35.00)	
NX-N0	8 (40.00)	5 (25.00)	3 (15.00)	

Table 8 Association of MUC2 expression status with clinicopathology in mucinous type of CRC patients (total n=3)

Clinicopathological characteristics	**n (%)**	**MUC2 expression**		***P*-value**
		Low MUC2 [n (%)]	**High MUC2 [n (%)]**	
Gender				0.667
Male	1 (33.33)	0 (0)	1 (33.33)	
Female	2 (66.67)	1 (33.33)	1 (33.33)	
Age				0.333
≤ 60 years old	1 (33.33)	1 (33.33)	0 (0)	
> 60 years old	2 (66.67)	0 (0)	2 (66.67)	
Tumor greatest dimension (cm.)				N/A
≥ 4.5 cm.	3 (100.00)	1 (33.33)	2 (66.67)	
< 4.5 cm.	0 (0.00)	0 (0.00)	0 (0.00)	
Lymph node metastasis				0.667
N1-N2	2 (66.67)	1 (33.33)	1 (33.33)	
NX-N0	1 (33.33)	0 (0.00)	1 (33.33)	

CHAPTER V
DISCUSSION AND CONCLUSION

AB, PAS, and AB-PAS staining were used to classify subtypes of mucins in the histological section in previous studies (Ali et al., 2012; Boonzaier et al., 2013; Mandal et al., 2013; Pélissier et al., 2010; Truter et al., 2017). Firstly, acid and neutral mucins were stained in differential parts of normal large intestine tissues (Figure 30). We found that acid mucin expression was significantly increased, but neutral mucin expression was significantly decreased when observed from the cecum to the rectal part (Table 1 and Figure 31). In differential parts of the normal large intestine were highly expression of acid mucin. In normal condition of the large intestine presents high bacterial quantity (Louis & Flint, 2009; Maier, Anderson, & Roy, 2015) , especially in the rectal part of the normal large intestine (Gajendran et al., 2019; Sun et al., 2019). Acid mucin functions is bacterial growth control and anti-cancer (Croix et al., 2011; Danquah et al., 2017; Truter et al., 2017). Therefore, increasing of acid mucin was associated with level of bacteria in differential parts of normal large intestine. Neutral mucin was found in the cecum part more than in other parts that were associated with pH level. In the normal condition of the gastrointestinal tract, the lowest pH presented in the stomach and increased in the small intestine; however, pH level dropped in the cecal part of the large intestine and increasing in rectal part (Fallingborg, 1999; Farmer, Mohammed, Dukes, Scott, & Hobson, 2014; Nugent, Kumar, Rampton, & Evans, 2001). Neutral mucin functions in adjusting pH balance and protection of the epithelial cell from damage agents (Truter et al., 2017). Therefore, decreasing of neutral mucin was associated with level of pH in differential parts of normal large intestine. We suggested that acid mucin was high in the rectal part more than in other parts for controlling normal flora and anti-pathogenic disease. In addition, neutral mucin was observed in the cecum part more than other parts because adjusting pH balance and protecting epithelial cells from damage agents, such as chemical agents and enzymes.

CRC was divided into mucinous and non-mucinous types. Acid and neutral mucins were stained in normal and cancer areas of mucinous and non-mucinous types of CRC tissues. In this study, we found that acid mucin was decreased in non-mucinous type but was increased in mucinous type. It was consistency with report by Danquah et al., 2017 and Kasprzak et al., 2019 (Danquah et al., 2017; Kasprzak et al., 2019). Danquah suggested that acid mucin functions were related to tumor growth inhibition and control cell division. Therefore, attenuation of acid mucin expression may activate the normal cells to CRC. Previous studies suggested that a common cause of non-mucinous types of CRC was started from oncogene and tumor suppressor gene mutations become to the tumor which inhibiting goblet cells division. Therefore, decreasing goblet cells causes acid and neutral mucins, and MUC2 protein degradation. However, Kasprzak et al. suggested that overexpression of acid mucin may oppose immune system response. Previous studies suggested that mucinous type of CRC was associated with inflammation in colon (Song et al., 2009, Kakar et al., 2004 and King-Yin et al., 2006). Inflammation can activate NF-kB via Tumor necrosis factor (TNF), Interleukin (IL) proinflammatory cytokines which NF-kB can induce goblet cells

division that cause of acid mucin increased in mucinous type of CRC. Moreover, they suggested that mucinous type can be resistant to immune cell and chemotherapies (Bhatia et al., 2019; Kakar, Aksoy, Burgart, & Smyrk, 2004; King-Yin Lam, Ong, & Ho, 2006; Song et al., 2009). Furthermore, overexpression of acid mucin in mucinous type was consistent with a previous study by with Kasprzak et al., 2019 study (Kasprzak et al., 2019). The result of this study was found that the amount increased, which may be reduced production because may be replaced by acid mucin production. Neutral mucin result in this study was found that was decreased in both mucinous and non-mucinous types. It was suggested by Jain et al that decreasing neutral mucin in both mucinous and non-mucinous types of CRC was caused by depletion of mucin production (Jain, Mondal, Sinha, Mukhopadhyay, & Chakraborty, 2014). Expression of MUC2 protein in this study was increased in mucinous type and was decreased in non-mucinous type that was related with previous studies (Luo et al., 2019, Hugen et al., 2014., Bu et al., 2010, Imai et al., 2013, Velcich et al., 2002, and O'Connell et al., 2021). They suggested that loss expression of MUC2 protein was associated with increased proliferation of epithelial cells for inflammation response. The proliferation of epithelial cells reduces goblet cells division and MUC2 protein production. MUC2 protein decreasing reduces mucus layers for epithelial cells protection. Thus, decreasing of mucus layers induces infection and inflammation which supporting tumor invasion and metastasis. The inflammation may appear paradoxical in MUC2 protein overexpression in mucinous type (Bu et al., 2010; Hugen et al., 2015; Imai et al., 2013; Luo et al., 2019; O'Connell et al., 2021; Velcich et al., 2002). However, Kufe's 2017 study suggested that overexpression of MUC2 protein in mucinous type may be modified by epigenetic mechanisms in cancer for anti-immune cells (Kufe, 2009). Moreover, O'Connell et al., 2021 and Bhatia et al., 2019 suggested that chronic inflammation is one cause of CRC and can induce MUC2 protein expression in mucinous in CRC. Wi et al reported that inflammation induces mucins production and reduces tumor suppressor gene function. Mucins were secreted for cover cancer cells which was supported cancer cells for invasion and metastasis (Wi, Cha, & Jung, 2021). Previous studies believed that mucins are one of the microenvironmental components of cancer. Roles mucins in mucinous cancer are a protection and invasion supporter of cancer cells (Luo et al., 2019; O'Connell et al., 2021). The clinicopathological association result in this study found that was found in male than female whereas mucinous type was tendency found in female than male was related with O'Connell et al., 2021. They suggested that non-mucinous type mostly found in male than female whereas mucinous type found in female than male.

Mucinous type in CRC studies was found in a few reports' correlation between MUC2 protein and mucins study. The result of this study has not been reported an association between mucins and MUC2 protein expression in mucinous type in CRC. Our study found that MUC2 protein was correlated with acid mucin in both mucinous and non-mucinous types in CRC tissues. Loss of MUC2 protein expression in non-mucinous type is well known in CRC. Thus, loss of goblet cells may cause of loss expression of acid and neutral mucins, and MUC2 protein. Therefore, in mucinous type,

we suggested that high goblet cells cause MUC2 protein and acid mucin high expression. However, neutral mucin in mucinous and non-mucinous types were not cleared in CRC. Role of acid mucin may protect cancer cells from pathogenic cells, chemical reagents, and immune cells. The result of the association between MUC2 protein and acid mucin in this study may use for first step a specific diagnosis, prognosis, and treatment research in mucinous type of CRC. However, the sample size in mucinous samples of CRC is imitated in this study because mucinous CRC is a rare case in Asians. However, this study provided basic knowledge of MUC2 protein and mucins expression in CRC.

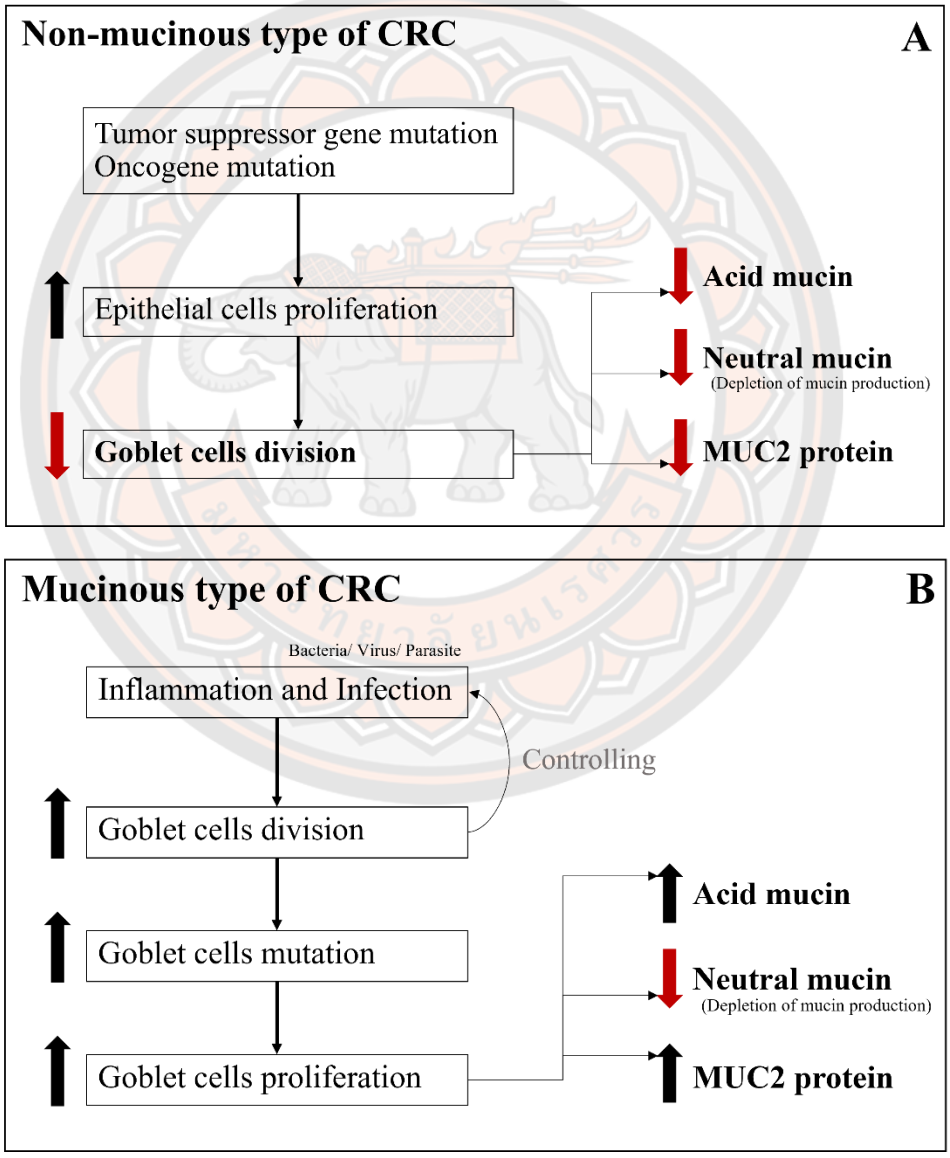

Figure 38 Summary acid and neutral mucins, and MUC2 protein in non-mucinous type (A) and mucinous type (B) of CRC

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APPENDIX

Sample collection

1. Normal large intestine tissues

We collected five parts of large intestine tissues of 30 of cadavers included cecum, ascending colon, transverse colon, descending colon, and sigmoid colon.

NO.	Sex	Age	Cause of death	Congenital disease	Smoke	Food consumption
1	1	76	Ischemic stroke	Diabetes mellitus	No	General
2	1	90	Old age disease	Hypertension	No	General
3	2	81	Cerebral hemorrhage	No	No	General
4	1	94	Old age disease	No	No	General
5	1	71	Heart attack	No	No	General
6	2	72	Heart attack	Diabetes mellitus	No	General
7	1	68	Heart valve regurgitation	Hypertension	No	General
8	2	77	Myocardial ischemia	Diabetes mellitus	No	General
9	1	65	Lung infection	Diabetes mellitus	No	General
10	2	69	Myocardial ischemia	Diabetes mellitus	No	General
11	1	79	Respiratory failure	No	No	General
12	1	68	Hypertension	Hypertension	No	General
13	2	74	Myocardial ischemia	Diabetes mellitus	No	General
14	1	76	Heart disease	Diabetes mellitus	No	General
15	2	72	Diabetes mellitus	Diabetes mellitus	No	General
16	1	73	Old age disease	No	No	General
17	2	96	Old age disease	No	No	General
18	1	65	Lung cancer	No	No	General
19	1	90	Lung infection	No	No	General
20	2	61	Stoke	No	No	General
21	1	78	Old age disease	No	No	General
22	2	70	Lung infection	Hypertension	No	General
23	1	77	Acute renal disease	renal disease, Diabetes mellitus	No	General
24	2	61	Diabetes mellitus	Diabetes mellitus	No	General
25	1	79	Ischemic stroke	No	No	General
26	1	86	Lung inflammation	Diabetes mellitus	No	General
27	1	73	Alzheimer's disease	No	No	General
28	2	78	Bloodstream infection	Hypertension	No	General
29	1	73	Herniated Disc	Diabetes mellitus	No	General
30	1	87	Myocardial ischemia	Hypertension	No	General

Table 9 This table shows all samples in normal large intestine tissues collection.

2. Colorectal cancer tissues

3 samples for mucinous and 20 samples for non-mucinous types paraffin blocks were received by Pathological Unit, Sawanpracharak hospital, Nakornsawan province.

Mucinous	Sex	Age	Area	Pathological stage
1	2	52	Ascending colon	Well differentiated adenocarcinoma
2	1	61	Sigmoid colon	Well differentiated adenocarcinoma
3	2	60	Rectosigmoid	Well differentiated adenocarcinoma
Non-mucinous	Sex	Age	Area	Pathological stage
1	1	64	Rectosigmoid colon	Well differentiated adenocarcinoma
2	1	66	Rectosigmoid colon	Well differentiated adenocarcinoma
3	2	83	Rectosigmoid colon	Well differentiated adenocarcinoma
4	2	79	Rectosigmoid colon	Well differentiated adenocarcinoma
5	1	70	Rectosigmoid colon	Well differentiated adenocarcinoma
6	1	56	Rectosigmoid colon	Well differentiated adenocarcinoma
7	2	65	Rectosigmoid colon	Well differentiated adenocarcinoma
8	1	62	Rectosigmoid colon	Well differentiated adenocarcinoma
9	2	75	Rectosigmoid colon	Well differentiated adenocarcinoma
10	2	57	Rectosigmoid colon	Well differentiated adenocarcinoma
11	2	57	Sigmoid colon	Well differentiated adenocarcinoma
12	1	70	Sigmoid colon	Well differentiated adenocarcinoma
13	1	88	Sigmoid colon	Well differentiated adenocarcinoma
14	1	55	Sigmoid colon	Well differentiated adenocarcinoma
15	2	75	Sigmoid colon	Well differentiated adenocarcinoma

16	1	64	Sigmoid colon	Well differentiated adenocarcinoma
17	1	75	Sigmoid colon	Well differentiated adenocarcinoma
18	1	30	Sigmoid colon	Well differentiated adenocarcinoma
19	1	50	Sigmoid colon	Well differentiated adenocarcinoma
20	2	64	Sigmoid colon	Well differentiated adenocarcinoma

Table 10 Detail of CRC sample paraffin blocks in mucinous and non-mucinous types

Tissue processing

Normal large intestine tissues were processed by tissue processor. Protocol followed program A processor of Department of Anatomy, Faculty of Medical Science, Naresuan University. As follows,

Step	Process	Times (hour)
1	70% ETOH	1 hour
2	80% ETOH	1 hour
3	90% ETOH	1 hour
4	90% ETOH	1 hour
5	95% ETOH	1 hour
6	95% ETOH	1 hour
7	100% ETOH	1 hour
8	100% ETOH	1 hour
9	Xylene	1 hour
10	Xylene	1 hour
11	Paraffin	1.5 hours
12	Paraffin	2 hours

Table 11 This table shows normal large intestine tissue processing protocol.

Tissue staining

1. Hematoxylin and Eosin (H&E) staining

Differential parts of normal large intestine tissues were sectioned at 3 µm and were stained by H&E for morphological structure observation. H&E staining protocol was followed by Faculty of Anatomy, Faculty of Medical Science, Naresuan University.

H&E protocol

Steps	Processes	Reagents	Times (minutes)
1	Deparaffinization	Xylene	5
2		Xylene	5
3	Rehydration	Absolute alcohol	3
4		Absolute alcohol	3
5		95% alcohol	3
6		95% alcohol	3
7		Distilled water	5
8	Staining	Hematoxylin	5
9		Tap water	5
10		1% lithium	10 dips
11		95% alcohol	10 dips
12		Eosin	16 second
13	Dehydration	Distilled water	5
14		95% alcohol	3
15		95% alcohol	3
16		Absolute alcohol	3
17		Absolute alcohol	3
18	Clearing	Xylene	5
19		Xylene	5
20	Mounting	Pre-mount	

H&E reagent preparation

1. 1% Lithium carbonate----------300 ml (w/v)
• Lithium carbonate …….3 g. • Distilled water ……. 300 ml

Table 12 These tables show H&E staining protocol and H&E reagent preparation.

2. Periodic acid Schiff (PAS) staining

Normal large intestine and CRC slides were stained by PAS for neutral mucin detection. PAS protocol was followed by US Biological, 2015.

Steps	Processes	Reagents	Times (minutes)
1	Deparaffinization	Xylene	5
2		Xylene	5
3	Rehydration	Absolute alcohol	3
4		Absolute alcohol	3
5		95% alcohol	3
6		95% alcohol	3
7		Distilled water	5
8	Staining	1% Periodic acid	5
9		Distilled water	10 dips
10		Distilled water	10 dips
11		Distilled water	10 dips
12		Schiff reagent	20
13		Tap water	5
14		Hematoxylin	1
15		Tap water	5
16	Dehydration	Distilled water	5
17		95% alcohol	3
18		95% alcohol	3
19		Absolute alcohol	3
20		Absolute alcohol	3
21	Clearing	Xylene	5
22		Xylene	5
23	Mounting	Pre-mount	

PAS reagents preparation

1. 1% Periodic acid ---------- 300 ml (w/v)
• Periodic acid ……. 3 g. • Distilled water ……. 300 ml
2. Schiff reagent ---------- 1000 ml (w/v)
• Dissolve 5 g. of basic fuchsin in 900 ml of boiling distilled water. • Cool to approximately 50 degrees Celsius and slowly add 100 ml of 1N HCl. • Cool to approximately 25 degrees Celsius and dissolve 10 g. of $K_2S_2O_5$. • Shake for 3 minutes and incubate in the dark at room temperature for 1 week. • Add 5 g of fine activated charcoal and shake for 3 minutes. • Filter solution (should be clear) • Store at 4 degrees Celsius in foil covered bottle.

Table 13 These tables show PAS staining protocol and PAS reagents preparation

3. Alcian blue (AB) pH2.5 staining

Normal large intestine and CRC slides were stained by AB pH2.5 for acid mucin detection. AB protocol was followed by (Kasprzak et al., 2019)

Steps	Processes	Reagents	Times (minutes)
1	Deparaffinization	Xylene	5
2		Xylene	5
3	Rehydration	Absolute alcohol	3
4		Absolute alcohol	3
5		95% alcohol	3
6		95% alcohol	3
7		Distilled water	5
8	Staining	Alcian blue	30
9		Tap water	5
10		Distilled water	10 dips
11		Nuclear fast red	7
12		Tap water	1
13	Dehydration	Distilled water	5
14		95% alcohol	3
15		95% alcohol	3
16		Absolute alcohol	3
17		Absolute alcohol	3
18	Clearing	Xylene	5
19		Xylene	5
20	Mounting	Pre-mount	

<u>AB pH2.5 reagents preparation</u>

1. 1%Alcian blue pH2.5 ---------- 300 ml (w/v)
• Alcian blue ……. 3 g. • 3% acetic acid ……. 300 ml
2. 3% acetic acid ---------- 300 ml (v/v)
• Glacial acetic acid …… 9 ml • Distilled water ……. 291 ml * Mix well and adjust pH to 2.5 using acetic acid
3. 0.1% Nuclear fast red ---------- 300 ml (w/v)
• Nuclear fast red ……. 0.3 g • Aluminum sulfate ……. 15 g • Distilled water ……. 300 ml

Table 14 These tables show AB pH2.5 staining protocol and AB pH 2.5 reagents preparation.

4. Alcian blue pH2.5 combined Periodic acid Schiff (AB-PAS) staining

Normal large intestine and CRC slides were stained by AB-PAS for coexistence of acid and neutral mucins in goblet cells. AB-PAS protocol was followed by Mowry RW., 1956 (IHC world).

Steps	Processes	Reagents	Times (minutes)
1	Deparaffinization	Xylene	5
2		Xylene	5
3	Rehydration	Absolute alcohol	3
4		Absolute alcohol	3
5		95% alcohol	3
6		95% alcohol	3
7		Distilled water	5
8	Staining	Alcian bule pH2.5	15
9		Tap water	5
10		Distilled water	10 dips
11		Periodic acid	5
12		Distilled water	10 dips
13		Schiff reagent	15
14		Tap water	5
15		Hematoxylin	1
16		Tap water	5
17	Dehydration	Distilled water	5
18		95% alcohol	3
19		95% alcohol	3
20		Absolute alcohol	3
21		Absolute alcohol	3
22	Clearing	Xylene	5
23		Xylene	5
24	Mounting	Pre-mount	

AB-PAS reagents preparation

1. 1%Alcian blue pH2.5 ---------- 300 ml (w/v)
2. 1% Periodic acid ---------- 300 ml (v/v)
3. Schiff reagent ---------- 300 ml (w/v)

Table 15 These tables show AB-PAS staining protocol and AB-PAS reagents preparation.

5. Immunohistochemistry (IHC)

MUC2 protein expression in CRC slides wea detected by IHC technique. IHC technique was referenced by Liu L. et al., 2020.

Steps	Processes	Reagents	Times (minutes)
1	Deparaffinization	Xylene	
2		Xylene	
3	Rehydration	Absolute alcohol	
4		Absolute alcohol	
8		95% alcohol	
9		95% alcohol	
10		Distilled water	
11	Antigen retrieval	Target retrieval solution pH 9 or pH 6	40
12		Cooldown	
13	Endogenous peroxidase blocking	Hydrogen peroxidase blocking	
14		PBS	
15		PBS	
16		Protein blocking	
17	Primary antibody	Primary antibody at room temperature	
18		Primary antibody at 4 degrees Celsius	
19		Primary antibody at room temperature	
20		PBS	
21		PBS	
22	Secondary antibody	Biotinylated Goat anti-polyvalent	
23		PBS	
24		PBS	
25		PBS	
26		Streptavidin peroxidase	
27		PBS	
28		PBS	
29		PBS	
30	DAB detection	DAB detection solution	
31		PBS	
32		Distilled water	
33	Counter staining	Hematoxylin	

34		Tap water	
35	Dehydration	Distilled water	
36		95% alcohol	
37		95% alcohol	
38		Absolute alcohol	
39		Absolute alcohol	
40	Clearing	Xylene	
41		Xylene	
42	Mounting	Pre-mount	

IHC reagents preparation

1. MUC1 antibody 1:500 µl ---------- 1000 µl (v/v)
• PBS……. 998 µl • MUC1 antibody …… 2 µl
2. MUC2 antibody 1:1000 µl ---------- 1000 µl (v/v)
• PBS ……... 999 µl • MUC2 antibody …… 1 µl
3. MUC13 antibody 1: 500 µl ---------- 1000 µl
• PBS ……... 999 µl • MUC13 antibody …….. 1 µl
4. 10X phosphate buffer saline (PBS) pH 7.4 ---------- 1000 ml (w/v)
• Disodium hydrogen phosphate ($Na_2HPO_4(2H_2O)$) …… 18.55 g • Potassium dihydrogen phosphate (KH_2PO_4) ……. 4.3 g • Sodium chloride (NaCl) ……. 77 g • Distilled water …… 1000 ml *pH is neutralized by NaOH and HCL.
5. DAB detection ---------- 1000 µl (v/v)
• DAB substrate …… 1000 µl • DAB chromogen ……... 20 ul

Table 16 These tables show IHC technique protocol and IHC reagents preparation.

Image analysis

1. How to use ZEISS ZEN program

We used ZEN program for normal large intestine and CRC photography.

Steps used for the ZEISS ZEN program

1. Open ZEISS ZEN program in desktop
2. Click **live** for observed picture from microscopic.
3. Photos adjust such as light background or focus adjustment after that click **snap** for taking a photo.
4. Click ruler for scale bar assigning.
5. Save picture on your document.

2. How to use ImageJ Fiji program

We used ImageJ Fiji program for mucins and mucin proteins calculation.

Steps for used ImageJ Fiji program

1. Open ImageJ Fiji program
2. Open picture file for mucins or mucin proteins
3. Set scale bar at staining picture, as follows.
    - Click **straight line** on the material tap.
    - Draw at **scale bar** at the picture.
    - Click **analyzed** on the material tap.
    - Chang **unit of length** from pixel to µm
    - Click **OK**
4. Color separation of picture, as follows
    - Click **image** on the material tap.
    - Click **color** and **colour deconvolution**, respectively
    - Choose special stain for picture such as PAS, AB or DAB
    - Choose picture for mucins or mucin proteins expression
5. Threshold adjustment
    - Click **image** on the material tap
    - Click **adjust and threshold**, respectively
    - Setting adjusts the threshold for each special staining
6. Mucins and mucin proteins analysis
    - Click analyze on the material tap and choose analyze particles

- Setting for analyze: ☑ Display result, ☑ clear results, ☑ summarize and ☑ exclude on edges
- Click OK
- Choose %area for calculation

Raw data of mucin

1. Acid mucin in normal large intestine

Data of acid mucins areas were collected and calculated by Microsoft excel and ImageJ Fiji programs. As follows,

1.1 Acid mucins data in cecum part

%Acid mucin in cecum part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	15.27	17.23	17.78	16.60	14.17	10.48	18.68	16.17	13.55	14.47	15.44
2	12.59	18.71	19.13	15.24	12.12	19.14	17.43	17.43	17.65	12.93	16.24
3	15.23	19.87	18.71	16.57	14.08	15.33	14.11	18.31	10.44	16.84	15.95
4	18.71	13.49	19.01	10.25	14.11	19.01	20.95	15.23	12.38	11.62	15.47
5	19.48	20.12	20.15	12.28	30.48	19.81	15.62	13.06	17.70	11.86	18.05
6	19.39	11.43	15.79	15.66	15.91	13.50	12.61	15.60	11.95	17.21	14.90
7	16.64	15.97	17.90	17.49	16.74	17.08	18.75	11.57	17.25	15.62	16.50
8	10.03	13.20	18.58	18.93	10.48	11.04	17.80	16.97	16.86	18.24	15.21
9	15.27	15.35	17.62	15.62	12.70	12.19	10.46	13.54	19.15	18.01	14.99
10	19.79	17.43	10.89	19.66	13.70	16.11	16.39	17.11	16.80	10.04	15.79
11	15.47	19.59	16.73	18.89	17.83	19.42	19.81	16.87	17.77	15.48	17.78
12	15.69	15.66	15.75	16.68	13.79	12.31	12.03	16.67	16.28	18.45	15.33
13	13.54	13.70	13.29	18.86	15.18	10.96	11.56	10.53	17.83	15.34	14.08
14	18.73	14.71	14.09	16.05	19.53	16.29	14.29	19.44	15.90	14.54	16.36
15	15.80	16.71	18.21	10.18	14.08	12.59	17.32	10.06	10.79	14.94	14.07
16	13.51	25.81	19.53	17.77	13.03	15.80	15.79	14.30	15.98	12.09	16.36
17	19.48	14.90	12.98	22.88	10.64	13.48	17.51	13.06	11.30	18.29	15.45
18	12.10	10.91	21.17	15.89	18.54	16.76	17.24	16.42	17.95	12.00	15.90
19	12.86	19.14	17.03	18.29	19.18	17.44	17.69	17.66	17.39	18.25	17.49
20	18.87	16.53	17.39	18.08	15.97	15.03	17.54	15.87	19.67	16.45	17.14
21	18.68	16.22	17.26	16.51	17.28	16.24	16.90	17.84	14.14	18.55	16.96
22	17.55	17.51	17.78	11.95	17.85	11.55	18.97	12.90	16.52	16.76	15.93
23	18.20	17.15	18.05	18.59	17.20	17.82	16.56	17.44	18.16	16.44	17.56
24	18.30	17.80	16.48	17.28	15.37	16.43	17.94	18.92	17.11	19.08	17.47
25	18.55	18.26	18.52	16.64	16.84	18.06	16.54	17.87	17.26	17.37	17.59
26	16.08	11.38	12.98	15.93	19.30	18.05	17.87	17.58	17.53	18.46	16.52
27	16.03	18.15	10.22	19.62	19.17	15.97	17.99	19.65	19.39	15.13	17.13
28	17.10	15.98	15.51	16.16	16.47	16.16	17.19	18.04	16.65	17.00	16.63
29	13.16	14.40	17.41	14.29	10.15	10.46	26.38	19.94	16.29	17.85	16.03
30	18.72	12.15	10.95	15.82	18.41	11.97	11.11	12.25	18.58	12.43	14.24
% Mean of acid mucin in cecum part											**16.15**
										SD	0.90

Table 17 This table shows raw data of acid mucins in cecum part of normal large intestine.

1.2 Acid mucins data in ascending colon part

%Acid mucin in ascending colon part of normal large intestine											
NO.	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00	Mean of %acid mucin in sample
1	19.86	20.14	27.52	19.14	18.79	16.68	25.54	20.30	25.44	26.91	22.03
2	18.40	20.72	27.92	23.62	26.36	29.44	28.97	31.16	26.56	22.63	25.58
3	24.93	23.76	17.24	16.59	20.16	15.62	17.48	18.54	19.49	20.01	19.38
4	23.94	18.89	23.86	23.21	14.86	20.65	21.66	15.20	25.87	17.22	20.54
5	20.48	23.32	18.74	26.15	20.97	19.33	24.89	24.50	22.48	28.74	22.96
6	23.57	21.03	18.44	22.72	19.46	23.67	21.23	21.23	20.33	21.35	21.30
7	21.77	15.31	21.30	22.55	19.67	23.42	26.68	28.96	19.14	17.54	21.63
8	24.79	15.65	15.66	26.68	13.92	22.69	20.25	25.38	24.47	22.82	21.23
9	19.19	26.24	23.33	20.27	29.57	27.41	25.52	21.28	23.85	21.82	23.85
10	25.99	28.49	23.15	20.32	18.49	23.68	21.62	17.62	21.56	20.31	22.12
11	19.68	29.83	27.08	12.59	21.79	22.00	23.38	26.49	20.01	25.60	22.84
12	30.65	19.87	14.66	14.22	22.33	14.13	19.83	17.06	20.43	23.74	19.69
13	21.86	20.86	16.13	12.68	20.90	14.98	12.41	21.25	23.24	21.14	18.54
14	14.95	25.62	15.15	17.84	18.22	22.58	17.51	23.70	17.64	24.76	19.80
15	18.56	30.16	24.73	23.94	29.12	21.59	20.32	13.04	20.73	20.19	22.24
16	26.37	17.79	21.04	18.96	24.60	24.78	25.51	28.30	25.75	22.64	23.57
17	23.88	19.48	23.35	27.31	18.80	28.88	32.18	27.63	28.36	24.59	25.45
18	25.84	23.46	23.38	23.93	28.06	25.61	24.09	19.44	20.66	23.60	23.81
19	23.57	24.49	21.27	22.64	20.48	19.27	19.03	28.50	24.26	16.21	21.97
20	26.40	29.43	19.83	23.49	29.19	19.77	29.32	18.29	20.83	23.17	23.97
21	30.06	25.08	25.91	25.89	25.29	24.24	23.03	29.93	24.84	23.09	25.74
22	22.56	30.50	28.25	26.03	26.67	26.48	22.05	22.09	23.72	21.02	24.94
23	20.32	22.87	26.57	28.44	26.56	22.82	22.02	27.77	25.36	26.36	24.91
24	23.16	25.17	20.71	27.15	27.71	25.88	27.12	25.17	23.70	24.68	25.04
25	28.88	20.17	26.50	27.24	26.33	20.54	26.77	29.85	23.01	25.69	25.50
26	28.40	24.63	22.82	26.38	15.15	15.28	28.85	22.15	28.86	21.13	23.36
27	22.01	23.63	24.95	19.49	21.61	25.77	16.75	27.30	21.65	24.77	22.79
28	23.56	27.26	21.78	27.80	20.13	29.69	21.33	25.65	21.64	29.39	24.82
29	26.93	27.69	25.15	25.62	25.42	25.82	28.10	22.60	24.97	26.60	25.89
30	22.89	24.46	22.29	24.29	27.38	22.71	27.76	22.01	23.38	25.04	24.22
% Mean of acid mucin in ascending colon part											**22.99**
										SD	1.66

Table 18 This table shows raw data of acid mucins in ascending colon part of normal large intestine.

1.3 Acid mucins data in transverse colon part

%Acid mucin in transverse colon part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	20.13	26.66	25.19	25.25	29.82	20.85	28.16	24.98	21.29	28.20	25.05
2	26.14	32.37	27.41	28.25	37.97	33.61	25.12	41.39	20.71	28.84	30.18
3	22.45	29.82	32.08	34.02	28.67	28.79	33.96	32.67	31.03	28.96	30.24
4	28.69	29.50	32.00	29.98	25.23	27.15	32.16	30.06	27.71	30.15	29.26
5	26.40	39.25	27.33	29.88	29.12	26.71	32.16	32.15	22.74	25.59	29.13
6	23.13	34.98	27.48	34.47	32.31	38.23	32.88	26.64	24.55	24.58	29.93
7	28.62	23.42	24.69	26.58	27.04	31.99	25.36	31.31	28.70	24.84	27.25
8	29.16	31.67	25.47	23.85	31.81	28.42	30.86	27.69	24.63	22.67	27.62
9	23.48	30.23	25.32	23.11	25.55	33.64	28.45	23.13	21.59	24.96	25.95
10	28.28	26.43	26.24	27.96	22.32	25.51	30.89	27.02	27.03	26.81	26.85
11	24.48	30.51	28.57	29.67	24.68	22.07	25.80	31.53	21.97	27.23	26.65
12	26.50	30.75	24.15	29.12	29.22	28.62	26.55	31.55	23.57	23.93	27.40
13	24.50	26.75	24.19	27.51	30.04	32.83	30.90	29.65	29.04	29.39	28.48
14	23.41	24.75	23.09	29.94	30.10	25.00	30.94	29.36	26.69	32.11	27.54
15	23.07	25.59	28.32	30.71	23.85	21.94	32.80	22.93	28.38	31.00	26.86
16	28.31	26.83	27.40	32.69	33.62	34.35	27.55	28.51	25.42	31.34	29.60
17	21.20	23.46	25.35	22.57	24.92	33.02	30.55	27.32	26.30	26.01	26.07
18	26.28	25.06	31.42	33.92	30.87	26.15	29.43	31.49	27.27	33.77	29.57
19	29.73	27.72	33.95	30.81	28.55	27.51	30.80	28.87	30.37	28.19	29.65
20	26.86	30.31	30.87	26.20	33.40	32.73	31.39	33.34	32.32	29.92	30.73
21	28.42	32.63	24.18	28.19	25.45	25.08	23.63	29.49	30.68	32.95	28.07
22	34.61	26.09	25.37	22.21	31.11	29.60	31.96	25.95	30.35	23.91	28.12
23	32.70	33.58	25.27	31.98	30.62	32.81	31.86	33.07	33.62	29.65	31.51
24	28.50	30.39	30.02	31.92	31.74	31.54	28.86	21.55	29.98	32.67	29.72
25	24.60	30.57	30.70	32.97	30.47	30.24	31.99	30.69	31.48	28.50	30.22
26	30.43	24.07	30.90	32.79	34.46	28.39	28.10	29.79	30.80	31.29	30.10
27	29.29	32.01	32.78	31.11	24.39	23.13	32.79	26.67	32.98	28.31	29.35
28	26.10	32.63	28.85	33.01	30.51	28.99	25.26	28.86	28.54	27.08	28.98
29	30.98	29.12	30.95	33.05	31.41	30.24	30.24	32.38	31.69	31.68	31.17
30	29.95	28.53	31.65	28.89	32.62	29.76	25.03	28.52	21.20	18.55	27.47
% Mean of acid mucin in transverse colon part											**28.62**
										SD	1.38

Table 19 This table shows raw data of acid mucins in transverse colon part of normal large intestine.

1.4 Acid mucins data in descending colon part

%Acid mucin in descending colon part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	34.53	31.93	40.73	31.28	31.23	29.17	32.57	37.49	34.64	36.91	34.05
2	31.06	35.55	33.50	31.67	32.21	34.01	32.88	31.77	39.72	31.69	33.41
3	29.65	36.62	23.85	39.58	26.95	25.16	40.31	34.42	34.51	40.01	33.11
4	29.20	29.67	28.94	35.36	34.79	33.17	34.11	47.24	27.23	32.30	33.20
5	30.24	32.21	38.86	32.71	29.60	32.51	36.75	36.33	24.88	32.78	32.69
6	30.66	34.67	35.53	32.72	33.49	27.35	26.51	35.52	34.22	31.10	32.18
7	33.12	31.18	34.52	31.23	29.21	26.43	24.59	31.21	31.76	31.44	30.47
8	33.91	35.12	33.02	31.21	29.54	25.66	37.68	28.73	30.18	32.33	31.74
9	38.72	37.73	34.70	32.71	33.46	28.40	36.89	39.94	39.57	35.48	35.76
10	34.42	28.13	25.19	35.60	36.35	31.17	36.07	39.12	34.74	33.92	33.47
11	32.95	27.00	34.20	29.51	32.04	34.18	32.49	31.79	36.03	35.48	32.57
12	31.90	28.82	40.34	37.02	35.53	37.84	33.80	31.59	33.15	35.95	34.59
13	33.68	31.98	33.95	28.98	32.26	34.82	35.51	35.34	29.72	32.15	32.84
14	37.88	33.47	36.08	27.19	35.49	39.86	32.74	32.80	30.75	27.44	33.37
15	31.14	30.23	28.10	34.76	40.70	31.11	35.09	27.31	34.55	25.13	31.81
16	29.00	33.76	32.25	28.63	30.65	34.56	27.02	35.01	32.83	35.16	31.89
17	35.95	30.55	33.46	32.41	27.29	36.97	32.10	36.71	23.02	34.24	32.27
18	37.63	31.78	30.03	36.75	29.96	32.64	26.92	31.13	29.82	23.00	30.97
19	27.88	33.42	35.67	30.62	33.88	33.15	35.53	30.81	38.39	35.70	33.50
20	33.11	39.04	29.57	30.13	37.54	32.69	28.07	32.48	32.44	30.46	32.55
21	36.99	34.78	35.65	26.41	35.75	35.00	35.88	35.42	36.83	25.97	33.87
22	30.09	36.59	33.36	29.40	35.12	32.84	33.40	31.90	33.54	41.83	33.81
23	44.04	28.61	29.99	33.35	31.79	32.96	33.65	29.20	31.47	31.15	32.62
24	28.34	38.98	29.72	34.09	37.63	30.90	44.76	36.81	31.43	27.53	34.02
25	27.26	34.43	28.82	33.81	35.73	37.97	38.40	35.71	32.52	35.67	34.03
26	30.43	30.67	34.97	31.50	31.96	31.81	27.96	39.17	31.50	31.12	32.11
27	31.07	25.33	32.65	32.55	30.36	26.72	20.60	24.17	33.09	32.76	28.93
28	29.95	34.86	32.77	34.80	35.91	32.78	32.02	29.24	31.03	28.69	32.20
29	28.57	33.43	31.99	35.07	30.68	28.29	33.04	28.13	30.39	33.92	31.35
30	32.58	30.21	27.18	24.27	30.69	27.74	29.01	25.00	31.00	29.03	28.67
% Mean of acid mucin in descending colon part											**32.60**
										SD	1.08

Table 20 This table shows raw data of acid mucins in descending colon part of normal large intestine.

1.5 Acid mucins data in sigmoid colon part

%Acid mucin in sigmoid colon part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	37.85	31.09	36.49	32.83	38.44	43.52	30.93	39.64	35.42	35.42	36.16
2	39.10	32.68	31.36	34.28	31.62	36.60	38.58	31.54	35.65	30.64	34.21
3	31.31	37.54	30.69	36.24	35.77	30.51	35.26	36.02	30.49	35.33	33.91
4	30.99	34.26	33.45	32.97	38.40	30.18	38.64	36.23	39.46	32.67	34.73
5	31.79	31.13	34.61	37.27	30.56	39.75	39.09	37.60	30.76	35.40	34.80
6	35.24	37.71	36.23	37.21	30.14	33.82	37.74	41.32	34.50	35.18	35.91
7	39.25	30.47	40.82	39.49	32.49	36.09	33.44	35.70	33.62	36.71	35.81
8	33.03	35.73	38.23	39.74	32.44	41.08	37.26	31.19	48.12	44.85	38.17
9	31.02	30.51	46.93	41.21	39.99	30.43	39.18	32.64	41.18	35.72	36.88
10	48.57	30.34	31.97	37.54	32.30	32.25	42.79	42.17	32.27	35.55	36.57
11	37.04	34.35	35.31	39.47	30.71	31.24	35.69	39.51	30.90	34.35	34.86
12	34.72	37.58	30.76	34.38	39.51	35.15	39.38	38.81	31.58	30.43	35.23
13	39.25	43.37	35.92	32.70	34.53	32.51	46.02	34.33	34.42	43.54	37.66
14	39.38	31.69	32.67	31.47	34.27	31.50	30.92	38.53	34.72	34.77	33.99
15	37.39	46.18	38.42	38.99	31.11	38.64	36.26	33.79	35.14	30.11	36.60
16	31.76	36.63	39.25	35.26	35.34	31.08	31.58	37.27	31.80	38.65	34.86
17	35.20	40.32	30.86	36.08	38.45	35.60	37.89	32.61	37.33	32.09	35.64
18	31.35	31.49	32.64	32.44	32.12	31.73	39.77	32.03	34.70	31.83	33.01
19	30.27	45.51	42.95	34.35	32.41	34.82	32.42	30.90	30.60	35.69	34.99
20	37.42	34.27	34.01	38.00	34.11	36.46	34.48	33.35	36.78	31.41	35.03
21	36.19	39.88	31.92	39.68	36.30	35.50	35.33	32.56	31.30	41.53	36.02
22	41.55	34.67	36.32	33.04	36.25	33.04	36.25	36.01	39.14	40.54	36.68
23	41.30	40.47	35.37	42.59	35.86	35.71	42.24	35.03	34.04	36.56	37.92
24	35.52	30.44	40.88	41.88	42.03	30.44	33.27	35.86	42.03	38.98	37.13
25	39.23	37.38	41.30	40.47	35.37	42.59	38.98	39.23	37.38	33.27	38.52
26	40.88	41.21	35.71	42.24	35.03	35.39	34.04	36.56	35.52	32.02	36.86
27	33.27	36.96	40.32	40.64	33.70	32.62	42.99	40.09	39.15	32.66	37.24
28	38.80	40.81	37.05	32.45	40.86	38.20	41.85	35.59	42.49	29.40	37.75
29	32.49	35.73	37.24	34.97	37.30	34.79	36.72	41.90	36.74	33.71	36.16
30	35.57	41.18	32.58	39.53	36.12	36.38	34.84	34.32	42.22	36.03	36.88
% Mean of acid mucin in sigmoid colon part											**36.01**
										SD	1.10

Table 21 This table shows raw data of acid mucins in sigmoid colon part of normal large intestine.

1.6 Acid mucins data in sigmoid colon part

%Acid mucin in rectum part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	46.91	45.20	36.82	35.43	40.67	30.91	32.73	38.83	37.63	32.33	37.75
2	34.81	31.71	35.08	30.07	40.48	36.79	38.72	37.17	37.46	45.92	36.82
3	33.75	37.87	37.79	37.64	32.69	33.24	26.44	41.18	40.03	37.23	35.78
4	41.76	41.46	37.23	39.82	39.07	35.57	39.31	36.58	31.44	32.53	37.48
5	41.55	40.87	37.53	40.69	33.21	40.69	37.72	41.18	44.31	42.10	39.98
6	36.04	37.88	33.29	43.36	42.79	44.40	37.62	39.19	36.90	45.71	39.72
7	42.10	44.74	41.81	33.84	35.72	34.77	31.12	41.79	44.96	35.57	38.64
8	35.92	35.67	41.62	40.79	44.38	40.92	44.71	43.72	40.45	41.25	40.94
9	42.26	41.24	33.19	41.85	42.95	39.00	41.49	41.28	42.08	42.91	40.83
10	41.39	41.71	42.47	41.92	39.84	35.41	42.15	42.80	40.85	40.54	40.91
11	42.08	42.91	41.39	41.71	42.47	41.16	40.63	42.33	41.71	41.70	41.81
12	43.07	40.04	40.03	44.96	43.22	31.92	39.00	41.49	40.54	41.28	40.56
13	41.41	38.01	42.29	37.51	41.75	37.24	37.73	33.69	38.56	31.70	37.99
14	42.50	37.97	40.82	34.20	31.52	36.33	39.50	38.05	40.32	42.35	38.36
15	40.09	40.60	42.75	41.01	31.82	31.18	35.81	41.46	42.18	45.40	39.23
16	41.28	45.41	36.69	41.47	37.96	36.18	36.37	44.54	39.64	34.54	39.41
17	40.49	41.40	40.24	40.59	37.64	37.57	68.01	31.42	31.80	41.86	41.10
18	41.86	41.96	41.55	44.23	41.37	30.53	42.20	42.27	42.25	35.00	40.32
19	41.45	41.34	40.86	44.99	41.91	38.01	32.42	39.72	40.84	39.34	40.09
20	33.18	35.80	41.78	38.20	42.58	36.34	36.72	39.48	37.00	40.73	38.18
21	38.87	37.67	41.21	37.93	41.55	38.17	38.89	39.95	33.95	35.12	38.33
22	39.55	38.93	40.24	40.59	37.38	32.10	31.01	36.77	36.42	35.32	36.83
23	36.74	41.15	44.05	40.97	42.68	41.57	44.43	37.97	39.24	35.73	40.45
24	39.32	39.30	40.68	45.39	39.77	33.25	31.91	40.57	39.10	38.61	38.79
25	33.76	41.14	38.97	31.27	37.99	31.14	31.03	32.70	41.41	41.41	36.08
26	37.51	38.59	38.72	36.79	39.31	39.97	40.93	38.79	38.41	40.96	39.00
27	44.07	31.08	36.76	41.31	38.65	37.98	43.53	41.58	39.20	43.18	39.73
28	36.93	36.84	35.01	34.75	31.13	41.83	47.42	37.20	38.30	39.66	37.91
29	36.50	39.13	31.15	30.83	31.07	31.47	36.52	31.45	37.48	31.37	33.70
30	30.83	31.20	30.59	33.43	33.42	39.16	39.20	34.77	32.00	33.01	33.76
% Mean of acid mucin in rectum part											**38.68**
										SD	1.55

Table 22 This table shows raw data of acid mucins in rectum part of normal large intestine.

2. Neutral mucin in normal large intestine

Data of Neutral mucins areas were collected and calculated by Microsoft excel and ImageJ Fiji programs. As follows,

2.1 Neutral mucins data in cecum part

%Neutral mucin in cecum part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	6.56	9.73	7.60	4.54	10.57	6.56	3.36	14.75	7.79	15.37	8.68
2	19.87	11.11	10.12	11.65	13.87	17.45	19.91	14.31	6.05	7.39	13.17
3	11.70	12.38	10.29	17.42	16.96	11.97	12.66	8.13	15.82	12.35	12.97
7	6.32	10.91	10.64	5.71	11.25	8.67	16.71	19.89	3.37	4.19	9.77
5	4.17	4.53	5.62	5.90	5.16	6.60	7.03	7.02	10.92	4.44	6.14
6	9.95	9.85	4.27	6.53	5.52	7.53	10.72	14.90	16.03	22.68	10.80
7	12.32	15.95	11.45	6.03	12.97	4.77	3.54	4.87	4.21	7.78	8.39
8	5.70	9.37	10.27	5.95	3.89	6.12	7.01	5.42	7.06	5.21	6.60
9	10.29	7.73	8.00	9.32	17.23	4.56	16.11	4.67	15.00	1.04	9.39
10	1.01	1.38	1.81	6.75	8.45	10.76	9.63	14.54	13.20	10.59	7.81
11	3.50	4.25	5.79	10.08	3.93	7.63	3.41	3.64	10.75	2.43	5.54
12	7.24	4.19	5.75	2.08	4.77	9.29	6.21	3.74	4.86	5.21	5.33
13	9.57	9.52	5.90	16.87	6.33	6.39	10.68	10.11	10.18	7.73	9.33
14	6.16	12.95	6.09	7.49	10.61	5.94	5.67	11.98	20.85	12.70	10.05
15	6.69	6.28	11.48	16.99	4.65	8.25	4.71	9.96	8.98	8.28	8.63
16	11.39	26.44	33.56	24.27	20.22	11.31	18.34	12.08	11.99	15.29	18.49
17	14.36	4.79	5.97	18.09	27.20	26.38	17.94	8.06	20.16	9.93	15.29
18	21.19	19.11	18.07	26.39	26.28	21.31	16.92	15.21	3.31	1.31	16.91
19	5.34	9.59	7.19	9.52	6.05	11.22	11.39	6.14	5.40	9.76	8.16
20	6.85	8.05	5.84	6.24	10.82	32.05	4.49	2.22	6.73	14.82	9.81
21	7.02	2.19	6.54	6.42	2.69	4.40	7.05	8.19	10.46	8.29	6.33
22	5.71	11.55	8.69	12.04	2.64	2.68	3.66	5.95	6.94	3.40	6.33
23	3.80	3.88	5.53	2.83	3.90	2.90	7.41	3.33	5.02	8.98	4.76
24	6.35	27.05	24.24	17.66	16.62	17.83	11.14	12.30	6.27	7.67	14.71
25	10.05	13.12	8.82	6.01	12.44	9.99	31.99	24.29	19.96	19.27	15.59
26	33.73	23.94	16.84	11.61	31.51	14.01	31.27	28.52	26.01	28.94	24.64
27	10.73	9.89	12.58	23.98	12.63	13.20	9.66	14.95	27.07	8.30	14.30
28	9.34	6.49	6.14	24.97	13.36	9.73	5.75	9.63	13.03	4.84	10.33
29	11.88	6.73	4.53	12.35	10.99	21.40	15.54	16.66	25.71	16.28	14.20
30	11.00	22.90	4.81	8.51	4.77	6.73	8.10	16.98	4.46	7.93	9.62
%Mean of neutral mucin in cecum part											**10.73**
										SD	3.53

Table 23 This table shows raw data of neutral mucins in cecum part of normal large intestine.

2.2 Neutral mucins data in ascending colon part

%Neutral mucin in ascending colon part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	11.17	5.18	5.32	6.77	12.02	10.86	11.83	10.00	10.96	11.08	9.52
2	4.91	2.83	9.28	8.95	3.87	7.92	11.81	4.53	3.93	10.72	6.87
3	2.87	2.61	4.29	7.18	3.04	3.69	1.06	5.41	10.86	1.44	4.24
7	11.46	2.78	2.55	11.99	11.69	11.58	7.36	12.91	4.45	4.75	8.15
5	3.65	10.32	3.63	7.59	3.86	3.20	3.42	11.36	2.63	11.63	6.13
6	11.63	4.08	3.37	1.61	1.18	4.01	3.28	11.64	5.00	4.44	5.02
7	3.68	3.33	3.28	3.43	4.08	3.98	3.67	4.71	9.67	9.11	4.89
8	4.95	3.64	11.66	11.12	11.42	11.44	3.91	11.69	3.57	4.05	7.74
9	10.84	1.24	11.45	11.44	3.49	8.23	11.44	11.52	11.80	11.45	9.29
10	11.17	4.24	9.16	2.54	4.21	4.08	3.79	3.92	1.26	12.12	5.65
11	11.03	6.57	4.61	1.96	4.52	2.92	4.52	2.92	4.29	6.36	4.97
12	11.56	0.84	0.99	2.60	7.27	4.36	2.62	2.03	1.89	2.34	3.65
13	6.60	8.95	12.56	8.24	6.74	4.43	4.98	5.08	5.77	9.23	7.26
14	2.18	9.74	7.18	10.16	14.62	3.54	2.25	6.96	7.10	8.46	7.22
15	3.12	4.62	16.53	7.45	2.54	8.20	9.49	2.93	6.71	10.66	7.22
16	5.84	12.04	6.19	7.98	5.09	6.37	4.78	10.08	4.04	7.99	7.04
17	4.84	7.16	4.86	4.63	2.64	3.37	4.05	4.15	4.21	8.59	4.85
18	6.51	5.85	10.02	5.16	5.61	5.66	4.12	4.56	8.42	3.95	5.99
19	4.27	5.53	4.88	4.56	4.95	5.42	2.48	5.00	5.03	4.12	4.62
20	2.23	5.88	9.09	5.60	9.70	7.34	18.44	5.03	4.56	4.09	7.19
21	3.71	3.20	2.92	5.99	3.37	3.29	2.82	11.38	3.06	2.41	4.22
22	2.75	5.60	2.73	3.76	4.86	4.62	8.61	2.66	2.88	11.50	5.00
23	2.45	2.86	3.40	17.32	5.13	4.19	3.75	13.29	2.94	2.95	5.83
24	1.71	5.72	10.14	18.83	14.41	16.79	11.93	12.41	13.98	8.56	11.45
25	12.16	1.71	3.63	6.34	4.43	4.17	3.81	1.26	3.00	3.66	4.42
26	2.54	7.96	12.35	13.12	4.33	6.56	5.70	5.39	16.49	18.55	9.30
27	18.09	10.85	19.49	4.19	8.20	4.53	9.67	11.12	4.67	5.77	9.66
28	9.12	4.34	5.26	10.16	11.33	5.24	12.32	5.14	7.69	7.84	7.84
29	8.24	4.62	4.32	6.04	7.91	3.54	3.54	4.81	3.81	4.35	5.12
30	4.75	4.85	13.58	4.66	7.25	16.13	19.00	12.03	5.16	15.00	10.24
%Mean of neutral mucin in ascending colon part											**6.69**
										SD	1.71

Table 24 This table shows raw data of neutral mucins in ascending colon part of normal large intestine.

2.3 Neutral mucins data in transverse colon part

%Neutral mucin in transverse colon part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	10.74	3.83	9.67	4.02	5.54	5.05	4.31	8.24	3.54	4.62	5.96
2	4.73	3.49	4.68	1.08	2.96	3.67	5.00	5.08	4.71	4.42	3.98
3	1.84	4.45	5.20	5.35	3.64	9.87	4.63	4.20	5.26	4.39	4.88
4	5.14	5.69	4.93	5.61	1.38	4.58	6.15	4.88	4.82	5.00	4.82
5	5.02	4.96	1.80	1.82	4.97	5.17	5.19	4.63	2.08	3.50	3.91
6	2.31	2.92	3.87	4.81	5.05	8.00	3.61	3.49	3.56	3.44	4.11
7	3.69	4.14	1.30	5.47	4.49	5.08	5.23	5.06	4.82	4.83	4.41
8	5.59	5.04	5.93	6.71	3.78	4.61	3.07	9.19	4.79	5.14	5.38
9	4.42	4.86	4.58	3.89	6.25	5.32	5.30	5.67	5.27	5.94	5.15
10	10.57	12.30	4.39	2.07	4.47	2.56	5.60	5.00	8.39	2.73	5.81
11	3.18	3.05	7.09	3.46	2.29	3.11	5.72	2.88	2.65	4.15	3.76
12	2.82	5.03	4.04	5.22	3.35	2.74	2.65	0.46	0.96	2.10	2.94
13	0.45	0.76	1.38	6.36	6.88	6.36	4.38	5.73	6.94	6.05	4.53
14	3.14	6.99	9.85	10.23	13.43	8.96	10.01	16.49	9.38	20.42	10.89
15	22.12	20.38	4.71	2.10	3.70	2.71	4.77	2.11	3.04	4.58	7.02
16	2.67	2.93	15.46	13.48	13.59	9.58	10.96	11.04	9.84	4.45	9.40
17	7.94	4.31	4.64	3.01	3.99	4.79	4.73	2.69	1.64	3.65	4.14
18	4.26	3.37	21.05	11.54	17.34	10.10	18.95	13.90	19.67	21.09	14.13
19	23.01	12.72	14.21	8.10	10.20	10.02	6.24	3.50	2.91	6.57	9.75
20	2.03	14.30	6.84	6.10	9.85	5.75	2.07	6.52	6.91	7.83	6.82
21	4.21	4.42	8.96	8.98	8.61	8.66	8.61	8.66	9.47	10.00	8.06
22	9.88	10.98	3.26	4.34	8.54	5.35	4.06	5.15	5.42	5.53	6.25
23	4.51	5.56	6.95	4.92	8.44	17.46	10.33	17.12	11.95	10.68	9.79
24	9.00	6.21	5.37	6.88	2.74	3.93	5.16	9.92	3.13	4.27	5.66
25	5.78	1.49	6.08	2.89	1.36	2.69	4.19	0.97	1.34	1.45	2.82
26	12.17	13.86	10.04	17.83	14.81	6.52	6.73	17.80	9.22	9.39	11.84
27	7.76	4.22	3.18	9.27	6.02	6.72	6.46	6.77	4.04	3.65	5.81
28	3.59	4.14	4.16	4.99	5.67	3.07	7.29	5.07	5.77	3.45	4.72
29	7.44	18.45	10.11	14.59	20.78	12.99	8.24	3.54	4.62	4.01	10.48
30	4.01	4.68	2.66	2.96	2.94	3.67	5.78	1.00	2.11	4.01	3.38
%Mean of neutral mucin in transverse colon part											**6.35**
										SD	2.31

Table 25 This table shows raw data of neutral mucins in transverse colon part of normal large intestine.

2.4 Neutral mucins data in descending colon part

%Neutral mucin in descending colon part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	0.77	2.21	2.88	2.61	2.43	2.61	1.60	2.28	1.52	2.21	2.11
2	8.24	6.53	1.23	1.17	1.06	1.07	1.06	1.37	1.51	1.30	2.45
3	1.98	2.50	2.08	1.43	1.62	1.02	1.59	2.24	2.73	2.51	1.97
4	1.57	2.59	1.73	2.23	2.82	1.92	9.56	1.09	1.01	1.57	2.61
5	2.60	1.33	1.28	1.52	1.48	1.13	1.35	1.14	1.29	1.00	1.41
6	2.56	1.76	1.19	1.27	1.87	0.90	1.47	1.95	1.76	1.00	1.57
7	0.91	3.63	4.67	4.57	4.53	1.27	2.14	3.47	1.64	3.92	3.07
8	4.10	4.82	2.33	1.60	1.71	2.22	1.50	1.70	1.72	5.80	2.75
9	1.38	1.39	1.67	4.59	3.70	4.25	5.14	4.54	1.08	1.03	2.88
10	2.48	2.65	1.64	1.18	1.10	1.29	7.24	1.24	6.33	3.59	2.87
11	13.66	4.15	3.92	3.47	2.64	3.32	3.53	4.08	3.29	3.74	4.58
12	7.83	11.37	4.08	3.11	10.47	10.49	3.59	4.32	3.06	2.90	6.12
13	9.65	3.95	4.55	10.63	3.41	3.32	12.79	6.27	11.98	10.35	7.69
14	9.76	12.17	4.43	10.11	10.07	3.57	4.52	4.47	4.21	4.40	6.77
15	1.97	3.26	4.72	3.97	2.87	3.29	1.19	4.58	3.03	3.00	3.19
16	5.12	7.50	10.67	8.91	5.78	10.55	7.09	3.96	5.53	4.52	6.96
17	11.29	4.90	5.44	10.13	4.46	5.33	5.77	1.91	3.82	4.04	5.71
18	3.79	3.25	4.67	2.14	4.59	3.57	4.45	4.69	3.65	3.11	3.79
19	6.38	10.07	5.54	5.33	7.78	6.73	10.30	7.69	3.93	3.07	6.68
20	9.63	3.92	4.95	4.29	6.33	12.22	4.18	6.75	1.11	1.22	5.46
21	4.14	6.84	3.38	1.39	1.39	1.63	2.15	5.19	4.48	1.61	3.22
22	2.50	1.56	2.90	1.66	1.33	1.34	1.14	2.43	2.94	1.70	1.95
23	1.95	1.78	1.20	2.32	2.23	5.03	3.85	7.33	3.00	4.57	3.33
24	2.89	2.06	3.01	1.73	3.15	1.79	2.27	4.57	3.54	2.93	2.79
25	6.98	6.94	5.60	2.96	3.46	3.24	2.39	2.93	5.41	1.00	4.09
26	3.27	3.64	5.27	6.91	5.41	2.70	2.43	4.31	7.04	7.05	4.80
27	2.16	6.19	3.40	2.75	1.69	3.01	7.32	8.17	4.37	8.15	4.72
28	2.58	2.14	3.30	4.39	2.54	1.51	3.43	11.61	2.77	4.46	3.87
29	3.62	3.06	2.69	2.50	2.33	1.73	1.82	1.80	2.30	7.16	2.90
30	4.12	2.13	2.51	7.04	7.05	6.19	2.16	3.40	2.75	1.69	3.90
% Mean of neutral mucin in descending colon part											**3.87**
										SD	1.40

Table 26 This table shows raw data of neutral mucins in descending colon part of normal large intestine.

2.5 Neutral mucins data in sigmoid colon part

%Neutral mucin in sigmoid colon part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	2.40	0.81	11.23	0.45	5.11	2.03	0.76	7.12	1.93	0.18	3.20
2	2.27	4.11	5.44	1.13	0.60	5.25	4.51	1.65	0.59	0.21	2.57
3	3.79	4.75	1.68	0.60	0.22	5.14	1.91	0.71	0.27	10.57	2.96
4	0.84	0.69	0.99	0.38	0.98	1.76	0.86	1.45	2.00	2.45	1.24
5	1.70	0.58	6.10	5.17	2.39	4.83	5.23	7.21	2.95	0.95	3.71
6	6.23	4.89	1.97	0.81	0.32	4.78	5.43	2.10	0.73	0.22	2.75
7	6.18	5.57	2.51	1.18	5.78	2.49	1.16	0.60	3.76	4.81	3.40
8	1.86	0.72	0.28	6.44	10.99	4.86	1.73	5.43	4.37	1.32	3.80
9	11.37	7.19	4.36	8.78	3.38	1.12	10.49	6.53	5.99	2.15	6.14
10	0.73	0.26	4.52	5.19	1.88	0.63	0.20	4.19	2.47	2.36	2.24
11	0.80	0.25	10.65	7.06	4.56	2.51	6.09	2.23	0.76	0.86	3.57
12	6.88	6.66	2.52	0.88	0.30	6.89	8.50	3.31	1.09	0.31	3.73
13	6.35	17.45	8.17	3.26	1.05	3.98	8.42	3.33	1.05	6.61	5.97
14	7.55	2.85	0.88	0.22	6.13	10.27	4.51	1.64	0.52	4.15	3.87
15	7.60	3.05	0.37	4.00	8.36	3.53	1.29	0.40	3.30	4.72	3.66
16	6.43	9.72	3.66	0.98	10.32	6.43	3.83	9.72	3.66	0.98	5.57
17	7.04	2.36	0.60	3.49	1.68	1.19	5.07	1.81	1.38	2.53	2.71
18	1.59	3.04	4.20	2.94	2.46	3.95	3.64	2.57	4.70	4.95	3.40
19	3.69	1.14	1.34	2.78	1.91	2.52	1.67	0.40	4.66	2.77	2.29
20	1.52	4.09	1.09	0.24	1.98	7.61	2.99	1.00	9.19	5.83	3.55
21	3.60	11.38	5.06	2.01	14.85	9.53	5.78	5.36	7.19	2.86	6.76
22	4.13	9.14	5.53	7.68	3.43	1.62	7.46	4.58	1.55	4.57	4.97
23	1.45	2.13	7.64	4.21	6.66	2.50	2.55	1.72	0.39	1.25	3.05
24	7.68	3.19	1.21	8.36	7.72	2.98	0.98	2.36	8.12	3.76	4.64
25	1.24	7.98	5.11	10.78	5.08	2.17	4.43	5.84	2.23	7.90	5.28
26	2.11	2.51	3.83	1.42	2.70	1.52	4.88	2.45	1.07	2.68	2.52
27	2.13	6.95	2.89	1.14	8.05	9.09	3.86	9.61	10.82	4.65	5.92
28	1.77	10.26	6.17	3.77	1.58	9.08	5.55	4.68	6.88	3.24	5.30
29	1.60	4.47	6.62	2.85	1.23	7.92	3.28	5.45	1.54	8.12	4.31
30	1.29	1.62	1.02	3.53	1.13	3.43	4.31	3.04	1.52	4.11	2.50
%Mean of neutral mucin in sigmoid colon part											**3.85**

Table 27 This table shows raw data of neutral mucins in sigmoid colon part of normal large intestine.

2.6 Neutral mucins data in rectum part

%Neutral mucin in rectum part of normal large intestine											
NO.	1	2	3	4	5	6	7	8	9	10	Mean of %acid mucin in sample
1	2.12	1.08	0.10	0.16	0.13	0.07	0.16	0.58	0.58	0.32	0.53
2	2.14	1.64	0.38	1.77	0.92	1.01	0.43	0.30	0.50	0.49	0.96
3	3.99	2.57	0.13	1.20	0.24	0.72	1.03	1.49	1.14	0.84	1.34
4	1.57	3.32	1.28	1.37	1.63	2.15	2.28	1.23	0.33	1.17	1.63
5	1.30	1.53	0.43	0.38	0.41	0.05	0.30	0.30	0.30	0.33	0.53
6	1.11	1.36	0.08	0.15	4.30	1.15	3.06	0.99	2.67	7.83	2.27
7	1.34	1.87	1.66	1.81	0.55	1.35	0.51	0.33	0.98	0.58	1.10
8	1.78	0.66	1.27	0.77	0.26	0.30	0.17	0.23	0.13	2.21	0.78
9	4.51	0.66	1.08	3.08	0.99	0.96	3.55	2.26	1.41	1.06	1.95
10	1.91	0.79	1.41	0.12	0.12	2.47	1.79	0.95	5.20	1.00	1.58
11	1.96	1.93	1.97	1.45	2.47	1.17	0.96	1.51	1.10	0.70	1.52
12	1.42	1.64	1.48	1.39	0.83	2.18	0.95	0.61	1.40	1.44	1.33
13	1.71	0.78	1.81	0.38	0.90	0.83	1.94	2.34	0.82	0.76	1.23
14	4.25	0.37	2.25	0.25	1.08	0.41	0.42	1.17	1.68	1.57	1.34
15	1.58	2.18	3.53	1.02	0.38	1.57	1.54	0.52	0.54	1.41	1.43
16	1.14	0.55	1.93	1.29	0.56	1.03	5.56	4.34	1.12	3.93	2.14
17	1.83	5.09	2.64	0.98	6.18	2.38	1.78	1.78	3.27	4.15	3.01
18	1.74	1.14	4.52	3.90	3.91	2.42	1.52	4.37	1.02	0.33	2.49
19	3.28	1.03	1.88	1.19	2.63	0.93	3.60	3.19	2.89	1.59	2.22
20	1.03	0.91	1.04	0.45	0.63	4.47	2.18	1.02	5.44	6.23	2.34
21	1.16	0.85	0.89	1.17	0.39	0.41	0.87	1.22	4.56	2.04	1.36
22	2.84	0.89	0.81	0.62	0.63	1.48	1.54	0.85	4.45	1.51	1.56
23	3.60	0.97	0.71	0.89	0.86	2.15	1.32	0.96	1.06	1.13	1.36
24	2.76	1.60	2.16	1.21	1.52	4.13	1.18	1.17	1.85	2.03	1.96
25	1.48	1.28	1.76	0.65	0.65	1.48	3.78	4.70	1.15	0.82	1.77
26	1.84	2.73	1.05	0.92	2.05	1.18	3.31	1.25	1.19	0.87	1.64
27	2.72	0.98	1.05	1.03	1.59	3.56	4.14	1.98	3.78	1.02	2.18
28	1.39	3.52	1.41	2.14	4.04	2.69	3.04	2.50	0.71	3.27	2.47
29	2.26	1.99	1.13	4.28	1.63	3.49	1.17	1.02	1.83	2.67	2.15
30	1.92	3.39	5.76	3.96	2.40	2.77	0.57	0.63	0.45	5.11	2.70
%Mean of neutral mucin in rectum part											**1.70**
										SD	0.51

Table 28 This table shows raw data of neutral mucins in rectum part of normal large intestine.

3. Acid and neutral mucins and MUC2 protein in mucinous types

NO.	Mucinous					
	Normal area			Cancer area		
	Acid	Neutral	MUC2	Acid	Neutral	MUC2
1	29.332	8.927	27.496	39.317	3.299	39.384
2	24.316	14.473	22.629	38.824	4.766	37.86
3	29.092	7.667	27.418	43.378	3.873	42.932
4	22.678	10.034	22.977	43.844	2.608	42.007
5	23.142	10.18	22.556	40.735	7.541	37.057
6	22.29	10.309	22.715	56.372	3.749	46.6
7	27.409	8.354	25.327	29.345	4.923	28.436
8	29.38	17.038	26.201	37.908	5.38	36.298
9	28.826	14.794	26.768	36.34	4.223	34.769
10	22.049	5.989	20.418	33.114	3.382	30.522
11	28.367	5.483	25.482	32.719	3.877	32.849
12	39.67	13.973	39.669	33.494	4.102	32.914
13	30.089	15.223	31.283	40.538	4.836	40.36
14	33.468	12.033	32.306	37.333	3.05	38.488
15	24.117	10.811	24.741	32.897	2.526	31.258
16	28.243	14.887	28.838	34.24	3.262	33.086
17	23.846	9.265	22.347	35.955	3.338	34.028
18	23.026	10.607	24.03	42.393	4.163	41.132
19	28.057	14.271	26.109	40.134	3.482	39.989
20	26.851	8.172	25.487	43.416	3.644	43.211
21	31.649	11.423	30.478	38.999	3.903	38.121
22	31.834	9.167	32.978	39.617	4.862	33.298
23	29.714	9.746	27.837	36.842	5.679	38.377
24	24.534	13.244	24.466	41.843	4.563	41.114
25	25.466	10.205	23.097	33.109	3.853	32.285
26	26.22	8.142	24.199	36.187	3.655	38.042
27	29.365	7.363	25.939	48.43	3.639	46.258
28	25.25	10.105	23.468	45.904	7.938	47.28
29	32.274	10.197	30.36	44.528	6.422	42.909
30	29.136	9.591	28.068	33.905	2.45	36.308
Mean	27.65633	10.72243	26.5229	39.05533	4.232933	37.90573
SD	3.923648	2.882719	4.022404	5.598127	1.317441	4.941142
Median	28.15	10.1885	25.713	38.9115	3.875	38.0815
Min	22.049	5.483	20.418	29.345	2.45	28.436
Max	39.67	17.038	39.669	56.372	7.938	47.28

Table 29 This table shows acid and neutral mucins and MUC2 protein expression mucinpus type in CRC

4. Acid and neutral mucins and MUC2 protein expression in non-mucinous type

NO.	Non-mucinous					
	Normal area			Cancer area		
	Acid	Neutral	MUC2	Acid	Neutral	MUC2
1	30.26	6.18	28.72	4.69	1.57	4.48
2	26.08	9.43	23.86	4.24	2.06	2.14
3	24.18	9.12	20.73	6.6	2.22	3.07
4	31.03	10.66	25.91	6.72	1.69	5.06
5	29.65	8.59	27.23	7.14	1.91	6.98
6	27.45	11.05	26.62	8.3	2.07	6.08
7	31.76	10.83	30.52	11.37	1.98	10.63
8	33.56	9.69	32.79	9.88	2.19	8.61
9	27.52	9.63	26.57	9.43	2.11	7.68
10	24.39	9.75	22	7.81	2.09	6.96
11	22.59	9.45	19.58	8.67	2.04	7.22
12	25.4	10.33	22.9	7.38	2.16	4.5
13	23.09	11.66	17.38	7.05	2.13	6.47
14	30.54	11.55	29.36	8.37	1.99	7.93
15	33.39	10.48	24.22	7.96	2.06	7.78
16	33.21	11.01	32.9	8.41	2.1	6.71
17	35.26	13.29	34.55	6.2	1.96	5.32
18	39.25	10.58	35.05	8.76	1.99	6.02
19	30.91	13.65	29.93	7.43	1.93	6.83
20	35.62	11.29	41.09	10.9	1.97	9.76
Mean	29.757	10.411	27.5955	7.8655	2.011	6.5115
SD	4.592623	1.61882	5.889152	1.787782	0.156538	2.0654
Median	30.4	10.53	26.925	7.885	2.05	6.77
Min	22.59	6.18	17.38	4.24	1.57	2.14
Max	39.25	13.65	41.09	11.37	2.22	10.63

Table 30 This table shows acid and neutral mucins and MUC2 protein expression in non-mucinous type in CRC

Mix mucins in various parts of normal large intestine				
NO.	Cecum		Ascending colon	
	Total cell	Mix mucin	Total cell	Mix mucin
1	95	35	68	14
2	52	12	63	18
3	108	46	66	19
4	88	41	139	29
5	90	32	104	31
	86.6	33.2	88	22.2
		38.33718		25.22727273

NO.	Transverse colon		Descending colon	
	Total cell	Mix mucin	Total cell	Mix mucin
1	110	8	79	1
2	111	8	92	2
3	100	12	68	1
4	49	6	48	1
5	97	7	62	0
	93.4	8.2	69.8	1
		8.779443		1.432664756

NO.	Sigmoid colon		Rectum	
	Total cell	Mix mucin	Total cell	Mix mucin
1	51	0	133	4
2	58	1	206	2
3	78	1	118	0
4	88	1	150	1
5	52	1	86	1
	65.4	0.8	138.6	1.6
		1.223242		1.154401154

Table 31 This table shows raw data of mix mucins in cecum to rectum parts of normal large intestine.

Mix mucins in mucinous and non-mucinous types of CRC								
	Normal area		Cancer area		Normal area		Cancer area	
NO.	Non-mucinous type				Mucinous type			
	Total cell	Mix mucin	Total cell	Mix mucin	Total cell	Mix mucin	Total cell	Mix mucin
1	168	8	220	5	208	8	270	0
2	151	8	142	0	134	7	241	1
3	176	9	167	0	192	7	254	1
4	160	8	240	1	178	5	198	0
5	143	2	234	0	210	8	153	0
	159.6	7	200.6	1.2	184.4	7	223.2	0.4
		4.385965		0.598205		3.796095		0.179211

Table 32 This table shows raw data of mix mucins in non-mucinous and mucinous types of CRC.