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An increase in inflammatory cells related to the increase incidence of colitis and colorectal cancer

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ABSTRACT

Background: Inflammation plays an important role in colorectal cancer formation, where colitis inflammatory cells can trigger the production of free radicals that interact with genes in carcinogenesis. Meanwhile, the colonic mucosal epithelium contains goblet cells that function to prevent inflammation. This study aims to examine the correlation between the number of goblet and inflammatory cells in the colon of patients with colorectal cancer, colitis, and healthy colon and their relationship with the incidence of colorectal cancer and colitis.

Methods: Histopathological samples consisting of 30 healthy colons, 30 colons with colitis, and 31 colons with colorectal cancer were examined to obtain the average cell number in 10 or the entire field of view using a light microscope with 400 magnification. Using the Pearson correlation test and Multivariate Multinomial Logistic Regression, these samples were analyzed with SPSS version 26 for Windows.

Results: Consequently, the results showed a weak but insignificant negative correlation between the number of goblet and inflammatory cells in the colon with colorectal cancer (r^2=0.055; p=0.200), colitis (r^2 = 0.002, p = 0.833), and healthy colon (r^2 = 0.110, p = 0.073). The number of inflammatory cells is significantly associated with the incidence of colorectal cancer (OR=1.326; 95%CI=1.155-1.521; p=0.000) and colitis (OR=1.374; 95%CI=1.192-1.583; p=0.000) compared to normal colon.

Conclusion: An increase in inflammatory cells is significantly associated with the incidence of colorectal cancer and colitis.

Keywords: Goblet Cells, Inflammatory Cells, Colorectal Cancer.
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INTRODUCTION

Colitis related to colon cancer is also known as *colitis-associated cancer* (CAC).[1] Mucus as a physical barrier protects the intestinal wall from digestive enzymes and bacteria attachment to the epithelium and prevents inflammation.[2] The main component of mucus produced by goblet cells is mucin (MUC2).[3,4]

The release of mucin from goblet cells is controlled by acetylcholine (Ach), histamine, prostaglandin E_2 (PGE_2), and antigen mediators, resulting in a suitable mucus barrier. Acetylcholine regulates the rate at which mucin is released, histamine and PGE_2 induce mucus secretion in the colon. In contrast, antigen mediators induce goblet cells by forming *goblet cell-associated antigen passages*. Furthermore, coordinating mucin secretion, mucus, ions, fluids, and intestinal motility effectively removes harmful substances and pathogenic bacteria.[5,6]

Goblet cell depletion reduces mucin production, mucus thickness, increases epithelial permeability, and loosens tight junctions resulting in colitis.[7] Colitis is characterized by an infiltration of inflammatory cells, including macrophages, neutrophils, T cells, and lymphocytes, into the colonic mucosa.[8] Consequently, the inflammation produces Reactive Oxygen and Nitrogen Species (RONS). RONS can cause genetic and epigenetic changes which result in carcinogenesis.[9-11]

Research on the number of goblet and inflammatory cells with a normal colon has been done more in experimental animals than humans.[5,9,10] Based on those mentioned above, this study aims to evaluate the increase of inflammatory cells related to the increased incidence of colitis and colorectal cancer.

METHODS

The histopathological samples comprised 91 patients divided into 3 groups, which are 31 with colorectal cancer, 30 with colitis, and 30 with hemorrhoids as normal colon (control). These were obtained through surgical procedures or endoscopic biopsies.

Each histopathological sample was stained with Hematoxylin-Eosin (HE) and the total number of inflammatory cells, Polymorphonuclear (PMN), Mononuclear (MN), and goblet cells in 10 or all fields of view on endoscopic biopsies were counted using a light microscope with a magnification of 400x. Furthermore, each

sample was examined by two independent observers.

Statistical analysis was carried out using the SPSS version 26.0 for Windows with a 95% confidence level. Then, descriptive analysis of the number of goblet and inflammatory cells in each group was expressed as average ±SD. Meanwhile, Pearson's test was conducted to analyze the correlation between the number of goblet and inflammatory cells in each group while analyzing the relationship between the cells and the incidence of colorectal cancer and colitis compared to the normal colon in Multivariate Multinomial Logistic Regression. Furthermore, P-value less than 0.05 is considered statistically significant.

RESULTS

The number of goblet and inflammatory cells in colorectal cancer, colitis, and normal colon was depicted in Table 1. The average number of goblet cells (cells/field of view) was 41.74±25.51 in colorectal cancer, 53.67±22.98 in colitis, and 53.65±38.33 in normal colon, respectively (Table 1). In addition, the number of inflammatory cells (cells/field of view) was 148.40±57.37 in colorectal cancer, 157.76±64.74 in colitis, and 79.37±46.99 in normal colon, respectively (Table 1) (Figure 1). There was a significant correlation between the number of goblets and inflammatory cells in colorectal cancer, colitis, and normal colon (p>0.05) (Table 2).

Based on the multivariate multinomial logistic regression, an increase in inflammatory cells number is significantly associated with the incidence of colorectal cancer (p=0.000) and colitis (p=0.000) (Table 3).

DISCUSSION

This study shows a negative correlation between the number of goblet and inflammatory cells in the 3 groups. The decrease in goblet cells causes changes in the thickness of the mucus layer and increases the risk of inflammation in the intestinal epithelium.[12,13] Furthermore, goblet cell depletion in patients with colitis which is accompanied by a significant decrease in mucin levels, causes an increase in bacterial penetration into the colonic epithelium.[14]

Inflammatory cells are more significantly associated with colitis and colorectal cancer than goblet cells. An increase of 10 inflammatory cells per visual field increased the risk of colorectal cancer and colitis by 32% and 37%. Furthermore, infiltration of inflammatory cells plays a role in increasing marker Myeloperoxidase (MPO), an enzyme produced by inflammatory cells, both in colitis and colorectal cancer.[15]

Figure 1 shows that the inflammatory cells fill the lamina propria due to the lack of goblet cells in colorectal cancer. Lymphocytes are visible in both colorectal cancer and colitis. RONS produced by inflammatory cells then directly induce the oxidation and deamination of DNA bases which causes alkalization through lipid peroxidation and DNA damage. Meanwhile, mutations cause carcinogenesis and exacerbate inflammatory processes.[16-18]

The effect of goblet cells in triggering colitis and colorectal cancer does not show statistically significant results as it is observed that an increase in the number of goblet cells is associated with a reduced 18% (OR=0.82) risk of colorectal cancer compared to colitis with a P-value close to 0.05 (p=0.065). Therefore, the increase in goblet cells is more likely to occur in colitis than in colorectal cancer.

Individuals with susceptibility to goblet cell depletion such as downregulation on Hath1 or healthy individuals triggered by *environmental triggers* (gastrointestinal infections, toxins, and long-term NSAID users) give rise to an inflammatory process which then sets off an immune response. Furthermore, the goblet cell inflammatory process increases with the release of mucin, Relm-β, and Tff3, which is significant for achieving homeostasis. This inflammatory process leads to stress on the Endoplasmic Reticulum (ER) which then brings about apoptosis and impaired maturation of goblet cells. However, there is impaired mucin glycosylation and decreased mucin synthesis. Consequently, this causes a decrease in the quality and amount of mucus as a mucosal barrier and facilitates the occurrence of colorectal inflammation.[3,7,9,19] Individuals with genetic disorders or ER goblet cell damage may have a much lower goblet cell number than a normal one or when the body is still achieving homeostasis.

Weak goblet cell relationships are also shown by Periyakoil P et al. and Makkink MK et al., where the number of goblet cells can remain large in colorectal cancer

Table 1. Number of Goblet and Inflammatory Cells in Colorectal Cancer, Colitis, and Normal Colon.

Variables	Minimum	Maximum	Median	Mean±SD
Number of Goblet Cells (cells/field of view)				
Colorectal Cancer	3.40	104.30	35.30	41.74±25.51
Colitis	18.00	100.50	47.10	53.67±22.98
Normal Colon (Hemorrhoids)	3.00	146.40	45.00	53.65±38.33
Number of Inflammatory Cells (cells/field of view)				
Colorectal Cancer	49.20	300.60	140.30	148.40±57.37
Colitis	66.70	340.00	151.20	157.76±64.74
Normal Colon (Hemorrhoids)	13.20	182.30	67.00	79.37±46.99

Table 2. The correlation coefficient between the number of goblet and inflammatory cells in colorectal cancer, colitis, and normal colon using the Pearson correlation test.

Parameter	Colorectal Cancer	Colitis	Normal Colon (Hemorrhoids)
Coefficient correlation (r)	-0.236	- 0.040	- 0.333
p-value	0.200	0.833	0.073

and colitis but differ in morphology and ability to produce MUC2.[20,21] Moreover, several conditions can affect the total goblet cells such as the presence of fecal diversion in patients with psychological stress conditions.[22-24] In contrast to the study by Leow CC et al., using *Alcian blue* and immunofluorescence, it was observed that the number of goblet cells and mucin in the colon adenocarcinoma decreased dramatically when compared to the healthy colon.[19] Hence, these numbers are important in the pathogenesis of colitis and colorectal cancer. However, changes in morphology and the ability to produce MUC2 also have an important role.

We do have several limitations to this study. There was no goblet cell morphology assessment, no histochemical staining other than HE and immunofluorescence. Also, not examining the condition before a biopsy or surgery can affect the study results. In conclusion, the number of inflammatory cells is associated with the incidence of colorectal cancer and colitis, while the number of goblet cells is not.

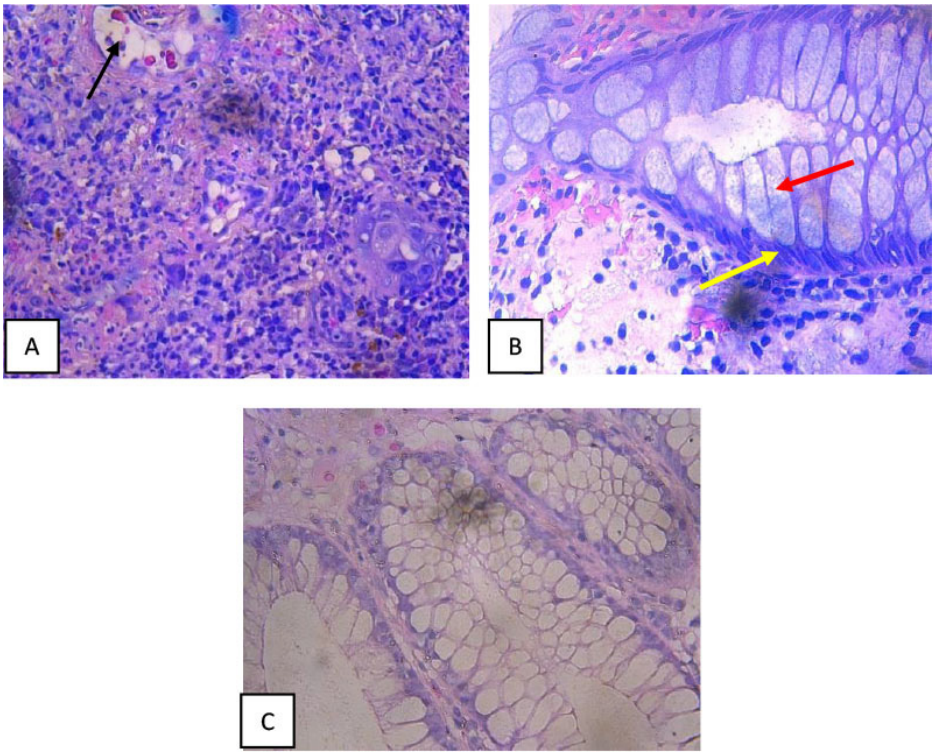

Figure 1. Colorectal cancer. (A) minimal goblet cell (black arrow); (B) Colitis: goblet cell count (red arrow) is still quite abundant among inflammatory cells (yellow arrow); and (C) Healthy colon: high goblet cell number.

Table 3. The relationship between goblet and inflammatory cells with the incidence of colorectal cancer, colitis, and healthy colon.

Variables	OR	95%CI	p
Colitis vs. normal colon			
Goblet cell number	1.160	0.940-1.433	0.167
Inflammatory cell number	1.374	1.192-1.583	0.000*
Colorectal cancer vs. normal colon			
Goblet cell number	0.956	0.770-1.188	0.685
Inflammatory cell number	1.326	1.155-1.521	0.000*
Colorectal cancer vs. colitis			
Goblet cell number	0.824	0.671-1.012	0.065
Inflammatory cell number	0.965	0.885-1.052	0.417

*Multivariate multinomial logistic regression: Statistically significant if p-value less than 0.05; OR: Odds Ratio; CI: Confidence Interval.

CONCLUSION

An increase in inflammatory cells is significantly associated with the incidence of colorectal cancer and colitis. However, future studies with a bigger sample size and prospective study design are suggested to clarify the causal effect.

ETHICAL CLEARANCE

This study was declared ethically feasible by the Health Research Ethics Commission, Medicine Faculty, University of Lambung Mangkurat, Banjarmasin, Indonesia. (No.633/KEPK-FK ULM/EC/VI/2021).

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHOR CONTRIBUTIONS

All authors equally contribute to the study from the conceptual framework, methodology, validation, formal analysis, review and editing until reporting the study results through publication.

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