Blood Zinc Profile and Fecal Analysis of Colitis Patients in Cipto Mangunkusumo General Hospital

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ABSTRACT

Background: *Colitis is a gastrointestinal disease that poses a major problem in Indonesia. Zinc (Zn) is an important trace element which plays role as antimicrobial in intestinal mucosa, increases gastrointestinal barrier, and immune function. Colitis can cause histologic changes in gastrointestinal tract epithelial which will disrupt the absorption and excretion of Zn causing zinc deficiency. This study aims to evaluate the difference of Zn level in colitis and healthy subjects, as well as the fecal analysis profile of colitis patients in Cipto Mangunkusumo General Hospital.*

Method: *The design of this study was cross-sectional and was performed in colitis and healthy subjects. Colitis patients were recruited consecutively in patients in Gastroenterology Clinic and Gastrointestinal Endoscopy Centre in Cipto Mangunkusumo General Hospital. Zinc examination from blood sample was performed using spectrophotometry. Fecal analysis was performed manually. The level of zinc from colitis subjects was compared to healthy subjects. Fecal analysis examination consists of macroscopic, microscopic, and chemical examinations of the feces.*

Results: *There were 40 colitis subjects and 16 healthy subjects in August 2019 - May 2020. There were significant differences in Zn levels between colitis and healthy subjects, which were 10.9 ± 1.9 mol/L and 12.3 ± 1 mol/L, respectively (p = 0.011). There were 45% (18/45) colitis subjects who were also suffering from Zn deficiency. The stool analysis in colitis subjects showed soft stool consistency in 90% subjects, slimy stools in 17.5%, increased in erythrocyte count in 60%, increased in leukocyte count in 5%, positive stool starch in 20%, positive stool fat in 7.5%, acid pH in 97.5%, positive stool occult blood in 37.5%, and fungi in stool in 7.5%.*

Keywords: *Colitis, zinc deficiency, faecal analysis*

ABSTRAK

Latar belakang: *Kolitis merupakan penyakit gastrointestinal yang masih menjadi masalah utama di Indonesia. Zinc (Zn) merupakan trace element penting yang berperan sebagai antimikrobial mukosa intestinal, meningkatkan barrier gastrointestinal, dan fungsi imun. Kolitis menyebabkan perubahan histologi pada epitel saluran gastrointestinal yang akan mengganggu penyerapan dan ekskresi Zn sehingga menimbulkan defisiensi*

Zn. Penelitian ini bertujuan mengetahui perbedaan kadar Zn antara subjek kolitis dan sehat, serta profil analisa tinja pada pasien kolitis di RSUPN dr. Cipto Mangunkusumo.

Metode: *Disain penelitian adalah potong lintang dan dilakukan pada subjek kolitis dan sehat. Subjek kolitis diperoleh secara konsekutif dari pasien di Poliklinik Gastroenterologi dan Pusat Endoskopi Saluran Cerna RSUPN dr. Cipto Mangunkusumo. Pemeriksaan Zn dari sampel darah dilakukan secara spektrofotometri. Pemeriksaan analisis tinja dilakukan secara manual. Kadar Zn dari subjek kolitis dibandingkan terhadap subjek sehat. Pemeriksaan analisis tinja meliputi pemeriksaan makroskopik, mikroskopik, dan kimia tinja.*

Hasil: *Didapatkan 40 subjek kolitis dan 16 subyek sehat pada bulan Agustus 2019 - Mei 2020. Didapatkan perbedaan bermakna kadar Zn pada subjek kolitis dan subjek sehat berturut-turut sebesar 10,9 ± 1,9 µmol/L dan 12,3 ± 1 µmol/L (p = 0,011). Subjek kolitis yang menderita defisiensi Zn sebesar 45% (18/45). Profil analisa tinja pada subjek kolitis ditemukan konsistensi tinja lembek 90%, tinja berlendir 17,5%, jumlah eritrosit meningkat 60%, jumlah leukosit meningkat 5%, amilum tinja positif 20%, lemak tinja positif 7,5%, pH asam 97,5%, darah samar tinja positif 37,5%, dan jamur dalam tinja 7,5%.*

Kata kunci: *Kolitis, defisiensi zinc, analisa tinja*

INTRODUCTION

Colitis is a disease of the digestive tract which is commonly found and is a major problem in developing countries, including Indonesia.[1,2] In 2010, the Ministry of Health of the Republic of Indonesia conducted a national survey and found that diarrhea, gastroenteritis, and colitis ranked first as the most common diseases in inpatient units and ranked fifth in the outpatient units of various hospitals in Indonesia.[3] Colitis is classified into infective and non-infective colitis.[2] Infective colitis is inflammation of the large intestine caused by infectious agents, such as bacteria, parasites, fungi, or viruses.[2,5,6]

Non-infective colitis is inflammation of the colon which is not caused by an infectious agent. Non-infective colitis includes inflammatory bowel disease (IBD) consisting of Crohn's disease (CD) and ulcerative colitis (UC), radiation colitis, ischemic colitis, and microscopic colitis.[7-9] In infective and non-infective colitis inflammation occurs in the intestinal mucosa resulting in changes of the anatomy and histology of the intestines.[2,5-9]Almost in all type colitis there is a damage to enterocytes due to the effects of bacteria or toxins causing inflammation, leading to clinical manifestations in forms of diarrhea, abdominal pain, mucus and bloody diarrhea, and fever.[2,5-9]

Zinc is a trace element in the human body which plays role in the immunity , including the immune response in the gut. Zinc is an essential element required for intestinal mucosal integrity, sodium transport, water, and immune function.[10-12] Stool analysis is a simple examination required to establish the diagnosis of colitis.[13] Data on Zn levels and stool analysis profiles in colitis patients are still limited. Therefore, this study aims to determine the levels of Zn in the blood and the profile of stool analysis in colitis subjects at RSUPN dr. Cipto Mangunkusumo.

METHOD

The design of this research was cross-sectional. A sample size of 39 people was required to determine Zn levels in subjects suspected of having colitis. The sample size to determine the difference in Zn levels between healthy and colitis subjects was 16 people for each group. Study results are presented using descriptive approach for stool analysis and Zn levels. Hypothesis testing to determine differences in Zn levels in healthy and colitis subjects were the unpaired Student T Test if the data distribution was normal or the Mann Whitney test if the data distribution was not normal. The limit of statistical significance used is alpha = 0.05.

The study was carried out in August 2019-May 2020. Study participants were obtained using consecutive sampling of patients who visited at the Gastroenterology Polyclinic or underwent colonoscopy at the Gastrointestinal Endoscopy Center (PESC) RSUPN dr. Cipto Mangunkusumo. Zn blood samples were examined using spectrophotometry with lot numbers of 9328/ZF01181JS, 0811801, 0821801, and 267/ZF01191H for standard, normal control, pathological control, and reagents from Dialab, respectively. [14] Stool analysis was done manually. Inclusion criteria were patients aged 18 years and diagnosed with colitis. Exclusion criteria were patients with hypoalbuminemia, alcohol consumption, pregnant women, and irritable bowel syndrome (IBS).

RESULTS

In this study, we carried out a performance test particularly accuracy and precision testing using within run test of Zn level with normal and pathological control materials. The results of the within-run accuracy test showed that the coefficient of variation (CV) of normal and pathological control materials was 1.3% and 1.2%, respectively. The results of the accuracy test showed that there were deviations (d) of normal and pathological control materials, which were 1.5% and 1.4%, respectively. Total errors of normal and pathological control examinations were 4.1% and 3.8%, respectively. The performance of the the equipment used for measuring Zn levels was very good, which was shown by the results of the within-run accuracy test that is in the CV range recommended by the company.[14] The results of the Zn level accuracy test showed that the total error value was still in the range of Allowable Total Error from Westgard, namely 4.1% and 3.8% against 13.5%.[15]

In this study, there were 40 colitis subjects and 16 healthy subjects. Colitis subjects consisted of 14 male subjects (14/40;35%) and 26 female subjects (26/40;65%). The median age of the subjects in the colitis group was 51 (21-80) years old. The characteristics of the colitis and healthy group of research subjects are presented in Tables 1 and 2.

Zn levels in colitis and healthy subjects were 10.9 ± 1.9 and 12.3 ± 1 (µmol/L), respectively and there was a significant difference of Zn levels between colitis and healthy subjects (p = 0.011). There was no significant difference between infectious and non-infectious colitis groups (p = 0.9), between infective and mixed type colitis (p = 0.5) and between non-infective and mixed type colitis (p = 0.3). The description of Zinc levels (µmol/L) in healthy subjects and colitis can be seen in Table 3 and Figure 1.

In this study, we obtained that the most frequent bacteria causing colitis were *Klebsiella pneumoniae* (10/23; 25%) and *Klebsiella oxytoca* (9/23; 22.5%), as described in Table 5.

In this study, a stool analysis profile of colitis patients was obtained, as shown in Table 6. The stool consistency was soft (36/40, 90%) in most of the subjects. Mucus in the feces of colitis subjects was found in 7 of 40 subjects (17.5%). The number of erythrocytes in the feces was increased in most colitis subjects (24/40, 60%). The number of leukocytes in the stool was normal in most of the participants (38/40, 95%). The results of the chemical examination of feces found pathological pH (39/40, 97.5%), sugar in faeces (1/40, 2.5%), and positive occult blood (15/40, 37.5%).

Table 1. Characteristics of research subjects in colitis group

Characteristics	n (%)
Age (years old)	
18-30	8 (20)
31-40	6 (15)
41-50	6 (15)
>50	20 (50)
Sex	
Male	14 (35)
Female	26 (65)
Clinical manifestations	
Diarrhea	9 (22.5)
Constipation	3 (7.5)
Bloody feces	22 (55)
Fever	3 (7.5)
Abdominal pain	30 (75)
Vomitting	4 (10)
Colitis type	
Total colitis	40 (100)
Infective colitis	
Bacterial colitis	6 (15)
Fungal diarrhea	1 (2.5)
Non-infective colitis	
IBD	
Ulcerative colitis	2 (5)
Crohn's disease	4 (10)
Unclassified IBD*	2 (5)
Radiation colitis	3 (7.5)
Unclassified colitis **	7 (17.5)
Mixed type colitis ***	
Ulcerative colitis + bacterial colitis	5 (12.5)
Crohn's disease + bacterial colitis	1 (2.5)
Unclassified IBD + bacterial colitis	5 (12.5)
Radiation colitis + bacterial colitis	2 (5)
Unclassified IBD + bacterial colitis + fungal colitis	2 (5)

*Unclassified IBD: IBD which has not been categorized into ulcerative colitis or Crohn's disease based on history taking, physical examination, colonoscopy, and/or histopathology results.

**Unclassified colitis: Colitis which has not been categorised into a type of colitis based on

Unclassified type of collitis based on history taking, physical examination, faecal culture result, colonoscopy, and/or histopathology results.

*** Mixed type colitis: Presence of ≥ 2 types of colitis in 1 subject.

Table 2. Characteritics of healthy subjects (n = 16)

Characteristics	n (%)
Age (years old)	
18-30	5 (31.25)
31-40	11 (68.75)
Sex	
Male	8(50)
Female	8(50)

Table 3. Zn level (µmol/L) in healthy and colitis subjects

Group	n (%)	Zinc level (µmol/L)
Total healthy subjects	16(100)	12.3 ± 1
Total colitis patients	40(100)	10.9±1.9
Infective colitis		
Bacterial colitis	6(15)	12.6(7.2-13.3)
Fungal colitis	1(2,5)	9.5
Non-infective colitis		
IBD		
Ulcerative colitis	2(5)	11.4±0.5
Crohn's disease	4(10)	12.7±2.3
Unclassified IBD*	2(5)	10.2±1.1
Radiation colitis	3(7,5)	10.5±0.8
Unclassified colitis**	7(17,5)	10.4±2.2
Mixed type colitis***		
Ulcerative colitis + bacterial colitis	5(12,5)	11.1±1.5
Crohn's disease + bacterial colitis	1(2,5)	8.0
Unclassified IBD + bacterial colitis	5(12,5)	10.1±1.7
Radiation colitis + bacterial colitis	2(5)	10.9±3.9
Unclassified IBD + bacterial colitis + fungal colitis	2(5)	11.5

* Unclassified IBD: IBD that cannot be categorized as ulcerative colitis or Crohn's disease based on history, physical examination, colonoscopy and/or histopathology.

** Unclassified colitis: Colitis that cannot be categorized as colitis based on history, physical examination, stool culture results, colonoscopy and/or histopathology.

*** Mixed colitis: There are 2 types of colitis in 1 study subject.

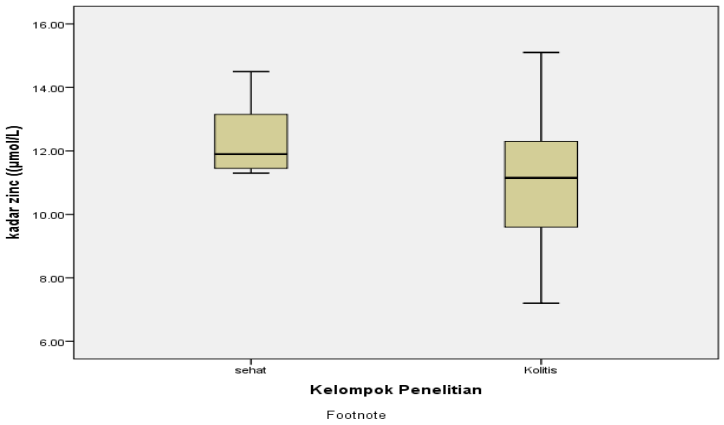

Figure 1. Box plot of Zn levels in healthy and colitis group

Table 4. Comparison of Zn level in Different Groups

Group	Frequency (n)	Zinc level (µmol/L)	p
Colitis	40	10.9 ± 1.9	**0.011**
Healthy	16	12.3 ± 1.0	
Infective colitis	7	11.2 ± 2.2	0.9
Non-infective colitis	11	11.4 ± 1.7	
Infective colitis	7	11.2 ± 2.2	0.5
Mixed type colitis	15	10.6 ± 1.8	
Non-infective colitis	11	11.4 ± 1.7	0.3
Mixed type colitis	15	10.6 ± 1,8	

Table 5. Pathogen bacteria in faecal culture of colitis patients

Bacteria	n (%)
Klebsiella pneumoniae	10 (25)
Klebsiella oxytoca	9 (22.5)
Escherichia coli patogen	1 (2.5)
Pseudomonas aeruginosa	1 (2.5)
Proteus mirabilis	1 (2.5)
Proteus vulgaris	1 (2.5)

Table 6. Fecal analysis profile in colitis group

	n (%)
Macroscopic	
Consistency	
Soft	36 (90)
Watery	4 (10)
Slimy	7 (17.5)
Bloody	0 (0)
Oily	0 (0)
Frothy	0 (0)
Microscopic	
Erythrocyte	
Normal	16 (40)
Increased	24 (60)
Leukocyte	
Normal	38 (95)
Increased	2 (5)
Parasite	0 (0)
Amylum	8 (20)
Fat	3 (7.5)
Muscle fiber	14 (35)
Plant fiber	17 (42.5)
Chemistry	
pH	
Normal	1 (2.5)
Pathologic	39 (97.5)
Sugar	1 (2.5)
Occult blood	15 (37.5)
Microbiology	
Fungi	3 (7.5)

DISCUSSION

Colitis is a disease of the gastrointestinal tract characterized by acute or chronic inflammation of the large intestine. Based on the etiology, colitis is divided into two groups, namely non-infective colitis and infective colitis. Non-infective colitis includes inflammatory bowel disease (Crohn's disease and ulcerative colitis), radiation colitis, ischemic colitis and microscopic colitis. Infective colitis includes tuberculous colitis, amoebic colitis, shigellosis, pseudomembranous colitis, and colitis caused by viruses, parasites, or other bacteria.[2,4-6] Colitis causes damage to enterocytes caused by bacteria or toxins that cause inflammation and clinical manifestations of diarrhea, abdominal pain, mucus and bloody diarrhea, and fever.[2,4-9]

In this study, we found that there were more female than male patients, which was 65% (Table 2). Studies by Cosnes et al and Betterige et al reported similar results with this study.[16,17] However Prideaux et al found equal proportion of males and females in ulcerative colitis patients and male predominance in CD.[18] The oldest study participants was found in colitis subjects (age > 50 years as much as 50%), with a median age of 51 (21-80) years (Table 2). The age of the subject of this study is almost the same as that obtained by Kelsen et al, who reported 2 peaks in the age of IBD occurrence, namely the first peak at age 20-30 years for CD and age 30-40 years for KU, and the second peak at age 60-70 years.[19]

The most common clinical symptoms in colitis subjects was abdominal pain (30/40, 75%) (Table 1). Hendrickson et al found that abdominal pain was the second most common symptom after bloody bowel movements in UC patients, and the most clinical symptom in CD patients.[20] Abdominal pains described by UC patients are usually in the lower abdominal region and cramps that are felt especially when there is a bowel movement. The location of abdominal pain on CD depends on the extent of the colon involved. Abdominal pain is felt in the left lower quadrant if the colon is involved distally, and if pancolitis occurs, abdominal pain is felt in all quadrants of the abdomen.[20] Papaconstantinou et al found that the main symptom of bacterial colitis is abdominal pain. The presence of exudative ulceration in bacterial colitis causes abdominal pain.[21]

The second most common clinical symptom in this study was bloody stools (22/40, 55%) (Table 1), instigated by ulcerative colitis, Crohn's disease, unclassified IBD, radiation colitis, bacterial colitis, and unclassified colitis (Table 1). Hendrickson et al and Ozin et al, reported the same findings in clinical symptom in UC patients, particularly bloody diarrhea.[20,22] Lesions in UC are generally diffuse and superficial, but deep lesions may also be present. Ulcers in the UC bleed easily, causing bloody stools.[18] Hendrickson et al also found symptoms of bloody stools in CD patients.[20] Bloody stools are often found in IBD, further leading to anemia due to the bleeding.[20]

In this study, all patients with radiation colitis had bloody stools. In radiation colitis, there is repeated injury to the intestinal mucosa by ionizing radiation causing fibrosis, obliterative endarteritis, edema, and perforation. Changes in the anatomy of the intestinal mucosa in radiation colitis can cause symptoms of bloody stools.[1,23-25]

In this study, we found that there were non-infectious colitis (27.5%), infectious colitis 17.5%, and mixed colitis 37.5% (Table 1). Prideaux et al in a systematic review obtained information on the increasing prevalence of IBD in various countries in Asia.[18] In Japan, the prevalence of UC increased from 7.85/1000 population (1984) to 63.6/1000 population (2005). In China, the prevalence of CD increased from 1.3/1000 population (1990) to 2.29/1000 population

(2005), while in Korea, the prevalence of UC increased significantly from 7.6/1000 population (1997) to 30,9/1000 population (2005).[18] Simadibrata et al reported that 100 of 207 patients with chronic diarrhea (48.3%) in Indonesia were chronic infective diarrhea.[1]

The group of healthy subjects in this study consisted of 16 people with 8 men and 8 women. The mean age of the subjects in the healthy group was 32.9 ± 3.3 years. This healthy group was taken as a control group and a comparison with the colitis group to evaluate the differences in Zn levels. All healthy subjects had normal Zn levels, i.e., > 10.7 mol/L. The mean Zn level in the healthy subject group was 12.3 ± 1 mol/L (Table 3).

In this study, the proportion of colitis subjects with Zn deficiency was 18/40, 45%, with an average Zn level of 10.9 ± 1.9 mol/L (Table 3). The proportion of IBD subjects with Zn deficiency was 8/40, 20%, with an average Zn level of 11.7 ± 1.9 mol/L. The results of this study were similar to those obtained by Vagianos et al, particularly the proportion of Zn deficiency in IBD patients which was 15.2% with Zn levels of 11.6 ± 2.1 mol/L.[26]

The results of aerobic faecal culture in the colitis subject group showed that in 21 of 40 fecal cultures, the growth of pathogenic bacteria (21/40, 52.5%) was seen (Table 5). From 21 samples of faeces, the growth of 23 bacteria was found because there were 2 cultures which had 2 types of bacteria and there was 1 type of bacteria per culture in the remaining cultures. The most common bacteria that grew were *Klebsiella sp*, namely *Klebsiella pneumoniae* (10/23, 43%) and *Klebsiella oxytoca* (9/23, 39%). Other pathogenic bacteria that grew were pathogenic *Escherichia coli*, *Pseudomonas aeruginosa, Proteus mirabilis*, and *Proteus vulgaris* each with 1/23; 4.3%. Simadibrata et al reported that 48.3% of 207 adult chronic diarrheal patients had infectious diarrhea caused by pathogenic *Escherichia coli* (34.8%), *Aerobacter aerogenes* (3.6%), *Klebsiella oxytoca* (3.6%), *Mycobacterium tuberculosis* (3.6%). 3.6%), *Salmonella paratyphy* (2.9%), and *Klebsiella pneumoniae* (2.9%).[1,2] Lai et al reported an increase in the prevalence of *Klebsiella pneumonia* as a cause of gastrointestinal disorders, and there was a relationship between *Klebsiella pneumonia* and types of IBD, UC and CD.[27] *Klebsiella oxytoca* is the second most common type of bacterial pathogen in the colitis subject group. *Klebsiella oxytoca* is a normal flora of the gastrointestinal tract. *Klebsiella oxytoca* causes colitis in people who were given quinolone and cephalosporin antibiotics.[28,29] In this study, data on the use of antibiotics were not sought; hence, the reason for *Klebsiella oxytoca* was not known to be the second most common.

The consistency of stool in colitis subjects was soft (90%) and watery (10%) (Table 6). Petryszyn et al found that stool consistency in CD patients was watery (60.6%) and soft (9.1%), while stool consistency in UC patients was watery (63.2%) and soft (13.2%).[30] Rao et al found that the stool consistency in UC subjects was watery (65%), soft (15%), and solid (19%).[31] In colitis subjects, 17.5% slimy stools were found (Table 6). Rao et al found slimy stools in active and inactive UC, 96% and 12%, respectively.[31] Slimy stools can be found in bacterial colitis caused by Enteroaggregative *E. coli* and *Shigella*.[18,22]

In colitis patients, there was an increase in the number of erythrocytes in the stool in 60% of the subjects (Table 6). Positive stool occult blood was obtained in 37.5% of the subjects. Harris et al reported an increase in erythrocyte count in 2 of 55 UC patients (3.6%).[40] Rao et al reported positive stool occult blood in active and inactive UC patients, particularly in 96% and 8%, respectively.[32] Gastrointestinal bleeding is characterized by an increase in the number of erythrocytes in the stool and positive occult blood. Gastrointestinal bleeding occurs due to changes in the intestinal mucosa which could be in the form of edema, ulceration, and perforation. The proportion of positive occult blood (37.5%) were lower than the proportion of increase in the number of erythrocytes (60%) in UC patients. This could be due to several factors, which included low sensitivity of occult blood tests using the Guaiac method, presence of intermittent bleeding, degeneration of intratestinal hemoglobin and sampling of stools which do not meet the requirements (mixed with urine or water).[33]

The number of leukocytes in colitis subjects was mostly normal (95%) (Table 6). Bacterial colitis accompanied by an increase in the number of leukocytes in the stool, is mostly caused by *Salmonella, Shigella*, and enteroinvasive *E. coli*.[1,2,4] Pereira et al found an increase in the number of faecal leukocytes in patients with chronic bowel disease, particularly in 34/42 subjects (80.9%), UC patients (2.4%), CD patients (9.5%), amoebic colitis (7.1%), and colonic adenocarcinoma (61.9%). 7.2%.[34] Harris et al found an increase in the number of leukocytes in 3.6% UC patients and 7.2% in bacterial colitis patients.[32]

Lugol's examination was performed to detect carbohydrate maldigestion and Sudan III examination to detect fat maldigestion. In colitis subjects, we

found positive Lugol's examination (positive starch) in 20%. Maldigestion of carbohydrates may occur due to intestinal hypermotility in diarrhea; so that, the contact time of food substances with digestive enzymes that break them down is not enough.[31] A positive examination of fat in feces in colitis subjects was found in 7.5% patients. Binder et al reported decreased absorption of various amino acids, folic acid, and fat in UC patients. Although UC patients have been in remission status, the colon continues to experience changes in secretory capacity. The proximal colonic mucosa tends to be more sensitive to cAMP-dependent secretion and less sensitive to Ca^{2+}-dependent secretion, resulting in persistent diarrhea.[35]

Most colitis subjects in this study had acidic stool pH (39/40, 97.5%) (Table 6). This is caused by disorders of the digestive tract and absorption of monosaccharides in the small intestine; thus, the unabsorbed disaccharides will be fermented by bacteria in the large intestine and produce an acidic and gaseous atmosphere.[36] Black et al found the incidence of carbohydrate malabsorption in non-infectious colitis was 61.1%.[36]

In colitis subjects, 7.5% fungus was found (Table 6), but no identification of the type of fungus was carried out. Fungal infectious colitis occurs due to the use of immunosuppressive drugs and broad-spectrum antibiotics in the treatment of IBD and infectious diarrhea. Pranenararat et al, found that the proportion of fungi in colitis subjects including Paracoccidioidomycosis was 29%, Histoplasma 28%, Candida 20%, and Aspergillus 9.2%.[37]

Zinc is a trace element that plays an important role in gene expression, transcription factors, DNA synthesis and replication, RNA transcription through zinc-finger transcription factors, regulates enzyme activity, regulates apoptosis, cell and hormonal homeostasis, and immune function. Zinc also acts as an antimicrobial barrier to the intestinal mucosa which may inhibit the growth of bacteria.[10,38,39]

Zinc plays a role in maintaining the integrity of tight junctions and the innate (non-specific) and adaptive (specific) immune systems. Zn deficiency may cause intestinal hyperpermeability, particularly in the tight junction (TJ) of the intestinal epithelium, namely in the zonular occludens, resulting in an increase in nitric oxide and oxidative stress which increases the incidence of diarrhea.[16] Deficiency of vitamins A, B, C, D, and E, and the micronutrient Selenium , Magnesium, and Zinc may damage the mucosal epithelial cells in the gastrointestinal tract, making it easier for viruses or bacteria to enter the bloodstream (Figure 6). [40]

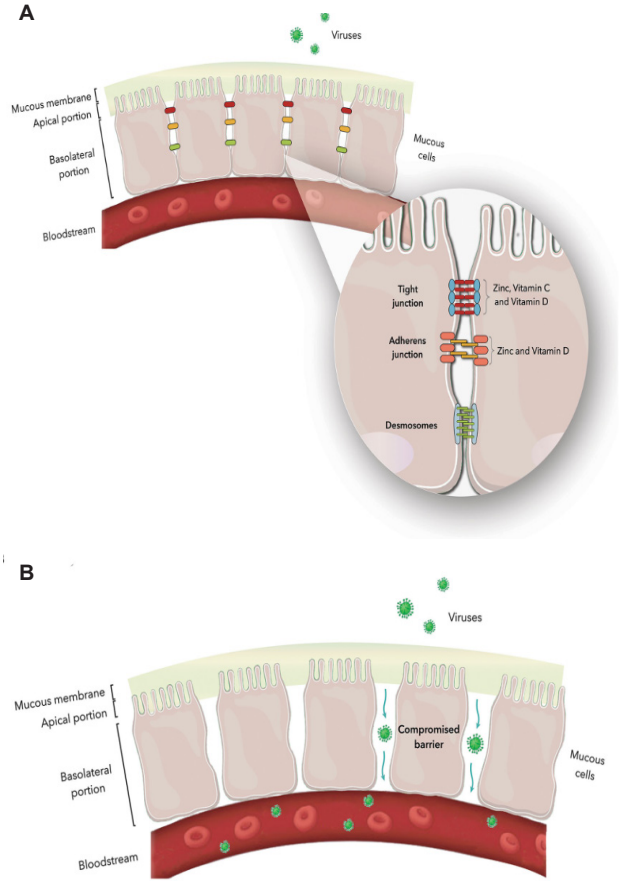

Figure 6. A. Junctional Complex of epithelial cells showing the role of Vitamin C, D dan Zinc B. Dysfunction of Junctional Complex causes virus to penetrate epithelial cells.[40]

In this study, the mean Zn levels in the colitis and healthy subjects were 10.9 ± 1.9 mol/L and 12.3 ± 1.0 mol/L, respectively (Table 4). The Zn level in the colitis group was significantly lower than the healthy group ($p = 0.011$). The results of Zn levels in this study were similar to those obtained by other researchers, such as Ainley et al, McCalin et al, and Ojuawo et al, who had lower Zn levels than the healthy control group.[41-43] Ainley et al found a significantly lower mean of Zn level between the CD group (13.5 ± 2.2 mol/L) and the control. [41] Ojuawo et al reported that the mean Zn levels of CD subjects (11.01 ± 2.49 mol/L) were significantly lower than the control subjects.[43] In contrast, the mean Zn level in UC subjects (12.39 ± 3.0 mol/L) was not significantly different from the control subjects. McClain et al reported the presence of Zn deficiency in CD subjects in 40% of 52 patients. [42] Comparison of Zn levels in this study and other studies is shown in Table 7.

In this study, we found 22.5% and 55% subjects complaining of diarrhea and bloody stools, respectively. From the stool examination, we found that the consistency of watery, slimy stools, and an increase

in erythrocytes were found in 10%, 17.5%, and 60%, respectively. Stool culture examination confirmed that 7/40 subjects (17.5%) had infective colitis. Absence of growth in cultures did not rule out the possibility of infective colitis, due to the limitations of identification of bacteria or fungi in faeces through stool culture examination. Thus, based on complaints and examination of feces, possibility of Zn deficiency in the subjects of this study increases, although it still had to be proven by further research.

In colitis there is a change in the histology of the intestinal mucosa. Changes in intestinal histology cause absorption disorders, resulting in deficiencies of various food substances required by the body, including Zn. Zn deficiency in colitis occurs due to inadequate intake of Zn from food due to nausea and excessive Zn excretion through the gastrointestinal tract due to chronic vomiting and diarrhea.[7-11,44] In this study, subjects complaining of diarrhea and bloody stools were 22.5% and 55%, respectively. Stool examination found the consistency of watery, slimy stools, and an increased in erythrocytes in the faeces in 10%, 17.5%, and 60%, respectively. Stool culture examination confirmed that 7/40 subjects (17.5%) had infective colitis. Absence of growth in cultures did not rule out the possibility of infective colitis, due to the limitations of identification of bacteria or fungi in faeces through stool culture examination. Thus, based on complaints and examination of feces, possibility of Zn deficiency in the subjects of this study increases, although it still had to be proven by further research.

Table 7. Comparison of Zn level in colitis in various studies

No	Study	Subjects in the study	Zinc level
1	This study	Colitis	10,9 ± 1,9 µmol/L
2	Ainley et al[41]	*Crohn's disease*	13,5 ± 2,2 µmol/L
3	Ojuawo et al[43]	*Crohn's disease*	11,01 ± 2,49 µmol/L
4	Ojuawo et al[43]	Ulcerative colitis	12,39 ± 3,0 µmol/L
5	McClain et al[42]	*Crohn's disease*	< 10,7 µmol/L

Limitations of this study include the use of reference values for Zn levels from overseas populations, faecal culture, which may not be appropriate for the Indonesian adult population. Therefore, it is recommended to study the reference value of Zn levels in the adult population in Indonesia. It is also necessary to do further research related to Zn deficiency from various types of colitis by increasing the sample size and the possibility of giving Zn therapy to colitis patients with proven Zn deficiency to accelerate the healing of colitis patients.

CONCLUSION

In this study, the Zn levels in colitis patients at the RSUPN. Dr. Cipto Mangunkusumo were 10.9 ± 1.9 mol/L. Zn levels in colitis subjects were significantly lower than healthy subjects. Several abnormalities in stool examination in colitis subjects were found, including soft stool consistency in 90% subjects, slimy stools in 17.5%, increased erythrocyte count in 60%, increased leukocyte count in 5%, positive stool starch in 20%, positive stool fat in 7.5%, acid pH in 97.5%, positive stool occult blood in 37.5%, and fungi in stool in 7.5%.

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