Intact Glycocalyx of Intestinal Mucosa in Intraabdominal Infection: an Investigation Using Blood Group Antigen

Toar J. M. Lalisang[1], Mohamad Sadikin[2], Ridho A. Syaiful[1], Novi S. Hardiany[2], Yefta Moenadjat[1]

[1] Department of Surgery, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2] Department of Biochemistry and Molecular Biology. Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Toar JM Lalisang, MD, PhD. Department of Surgery, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. email: toar.m@ui.ac.id.

ABSTRAK

Latar belakang: *glikokaliks usus berperan pada patogenesis translokasi bakteri penyebab sepsis yang berasal dari infeksi intraabdomen. Kondisi ini rentan terjadi pada golongan darah tertentu. Namun, sejauh ini belum diketahui hubungan antara glikokaliks usus pada golongan darah tertentu dengan infeksi intraabdomen. Penelitian ini bertujuan mengetahui kondisi glikokaliks usus pada sepsis intraabdomen pada golongan darah tertentu.* ***Metode:*** *penelitian deskriptif melibatkan subjek infeksi intraabdomen yang menjalani laparotomi. Sampel berupa spesimen usus. Dilakukan pengukuran ekspresi glikokaliks usus dengan metode ELISA menggunakan antigen golongan darah (A dan B). Data ekspresi pada sekretor dianalisis menggunakan uji Kolmogorov–Smirnov dilanjutkan dengan komparasi parametrik menggunakan ANOVA dan uji–t.* ***Hasil:*** *terdapat 32 subjek dengan infeksi intraabdomen yang diteliti pada studi ini. Seluruhnya berstatus sekretor serta mengeskpresikan antigen A dan B dengan kuat. Tidak ada perbedaan antara infeksi intraabdomen dengan komplikasi maupun tanpa komplikasi. Golongan darah O merupakan golongan darah yang dominan pada subjek (43,8%). Eschericia coli merupakan mikroba yang paling sering didapatkan (61,3%).* ***Kesimpulan:*** *penelitian ini menunjukkan tidak terjadi kerusakan glikokaliks pada usus pasien sepsis akibat infeksi intraabdomen.*

Kata kunci: *glikokaliks, infeksi intraabdomen, golongan darah.*

ABSTRACT

Background: *intestinal glycocalyx plays a role in bacterial translocation as the pathogenesis sepsis derived from intra-abdominal infections that vulnerable in certain blood types. However, the link between intestinal glycocalyx in specific types of blood groups and abdominal infections remains unknown. This study aims to find out the condition of intestinal glycocalyx in certain blood types with intraabdominal sepsis.* ***Methods:*** *descriptive study involved subjects with intraabdominal infections who underwent laparotomy. Samples are in the form of intestinal specimens. The measurement of intestinal glycocalyx proceeded by the ELISA method using blood group antigens (A and B). Expression data on the secretors were analyzed using the Kolmogorov – Smirnov test followed by parametric comparisons using ANOVA and t-tests.* ***Results:*** *there were 32 subjects with intra-abdominal infections studied in this study. All of them are secretors and express A and B antigens strongly. We found no difference between intraabdominal infections in those with complications or without complications. Blood type O is a predominant blood type found (43.8%). Escherichia coli is the most commonly found microbe in the culture (61.3%).* ***Conclusion:*** *this study shows there is no disrupted intestinal glycocalyx of sepsis patients caused by intraabdominal infection.*

Keywords: *glycocalyx, intraabdominal infection, blood type.*

INTRODUCTION

In the pathogenesis of gut-derived (abdominal) sepsis (recently attributed to intraabdominal infection), there are two main problems encountered and addressed in the clinical practice. The first issue is the phenomenon of bacterial translocation. Intraabdominal infection following gut perforation (or, complicated intraabdominal infections, cIAI) is a logical consequence. However, in the non–perforated viscus (non-complicated intraabdominal infections, or simply intraabdominal infections, IAI) – known as bacterial translocation – remains a mystery that needs more evidence.[1–3] Recent studies showed that disrupted tight junctions that play an essential role in maintaining the intestinal mucosal barrier is responsible for the translocation.

The second issue is sepsis that identified in patients with blood type O of the ABO blood typing system. Studies showed that blood group O is prone to a particular disease while the other blood group not. Our preliminary study showed that sepsis develops in subjects with blood group O comprise of 46.69% out of 213 subjects diagnosed as complicated intraabdominal infections. In contrast, blood type A was 24.66%, and blood group B 26.87%.[4] The phenomenon may clearly describe through the better knowledge of identified blood type antigen in the tissues other than blood cells (namely, secretor), using the blood typing system of the non–ABO system. The essential secretor of the blood type is glycocalyx of the gastrointestinal tract, as well as intestinal mucosa. Glycocalyx has a core peptide that binds the amino acid residues to the host's glycans.[5] The core peptide comprises either glycosylation–O that binds oxygen or glycosylation–N that binds other amino acids. The core peptide is found differently in each blood group.[6] This distinctive characteristic leads to different susceptibility to certain conditions, including sepsis.[7]

While disintegrated endothelial glycocalyx paralleled to endothelial dysfunction found in sepsis, the course of intestinal glycocalyx remains unclear. To find out the answer, we investigated the glycocalyx in the intestinal mucosa in specific blood types in those diagnosed as intraabdominal infection (both in IAI and cIAI).

METHODS

The investigation proceeded in intestinal and blood specimens taken from subjects who diagnosed with an intraabdominal infection that underwent a surgical procedure in 2017. The diagnosis based on the findings: sepsis syndrome with GI perforation (cIAI) and with no perforation (IAI). Primary peritonitis, particularly abdominal tuberculosis, and those who are not the candidate for gut resection, and pediatrics excluded. The subjects enrolled consecutively as he/she agreed to be enrolled and signed the consent. The subjects' characteristics, namely, age, gender, and diagnosis recorded. Blood specimens taken for some tests, particularly blood typing and peripheral blood count. Intraoperatively, gut specimens taken for investigation purposes — the samples subjected to mucin analysis for the expression of antigen A and B of blood group and bacterial culture to find out the microbial pattern.

Intestinal mucosa scraped and subjected to the investigation for the concentration of protein in glycocalyx. For ELISA purposes, we used anti A serum (Blood Group A Antigen Monoclonal Antibody, Human, Thermo Fisher, catalog HE–193) and anti B serum (Blood Group B Antigen Monoclonal Antibody, Human, Thermo Fisher, catalog 89–F) and secondary antibody Goat anti–Mouse IgG (H+L) Secondary Antibody, HRP (Thermo Fisher, catalog 31430). Antigen expression on ELISA expressed in % intensity with reference of ABO blood group (transferase A, alpha 1–3–N–acetylgalactosaminyltransferase; transferase B, alpha 1–3–galactosyltransferase) of a homosapiens is 0.472813 (data Glycocalyx gene set).[8]

Data descriptively presented in two categories i.e., subjects with perforation and those not. The analysis preceded with a distribution test for numerical one using Kolmogorov Smirnov, a normal distribution defined as p-value >0.05. Binary data expressed in mean (SD) and categorical data presented in the table describing the frequency in percentage. Statistical analysis proceeded using a parametric compare of two means, namely ANOVA and non–parametric t-test.

The committee of ethics Faculty of Medicine, Universitas Indonesia approved the study No.104/UN.F1/ETIK/2017 and the permission to conduct a study from Research Bureau of dr. Cipto Mangunkusumo General Hospital No.LB.02.01/X.2/66/2017.

RESULTS

Thirty–two subjects met the criteria enrolled in the study. All subjects treated empirically using intravenous aminoglycoside (namely gentamycin sulfate 80 mg b.i.d.) and metronidazole 1,500 mg b.i.d. Subjects' characteristics presented in **Table 1**.

Table 1. Subjects' characteristics in the study (n = 32)

Demographic characteristics	Total
Gender*, n (%)	
- Males	18 (56.2)
- Females	14 (43.8)
Age (mean (SD)), years	52.3 (12.7)
Blood type*, n (%)	
- A	10 (31.2)
- B	8 (25.0)
- AB	0 (0.0)
- O	14 (43.8)
Blood tests, mean (SD), cells/mm^3	
Leukocytes	15,223 (5,702)
Platelet	343,748 (163,634)

In the pathology point of view, GI infection, and the bowel obstruction found of most. The abdominal pathology both of GI– and non–GI origin presented in **Table 2**.

Bacterial culture (n = 31) were monomicrobial (7 subjects, 22.6%) and polymicrobial (24 subjects, 77.4%) with the commonest bacteria found was Escherichia coli (19 subjects, 61.3%), Klebsiella pneumonia (5 subjects, 15.2%), Pseudomonas aeruginosa (2 subjects, 6.1%) Bacteroides fragilis (2 subjects, 6.1%), and Acinetobacter (1 subject, 3%). Besides, Candida albicans also found (2 subjects, 6.1%). Bacterial growth on the disc found in both cIAI (11 subjects, 35.48%) and IAI (21 subjects, 67.74%). The difference between the two groups showing p = 0.119 CI 95% (-0.373-3.145).

Table 2. Intraabdominal pathology in the study (n = 32)

Abdominal pathology	n (%)	
- GI origin	29 (90.6)	
- Gut perforation (cIAI)	11 (34.4)	Perforated appendicitis, Perforated ileum, and Perforated colon
- Non–perforation (IAI)	21 (65.6)	GI obstruction
- Non–GI origin	3 (9.4)	Perforated uterus Pelvic abscess

Table 3. Expression of antigen A and antigen B in the study (n = 32)

Antigen expression		P value
Normal	0.472813 (Glycocalyx gene set)	
Antigen A, mean (SD)	2.33 (1.374)	0.467
Antigen B, mean (SD)	1.77 (1.334)	

Table 4. Antigen expression between cIAI and IAI

Group	n	mean (SD)	P value
Antigen A			
- cIAI	11	1.5435 (0.59473)	0.130
- IAI	21	1.2928 (0.32272)	
Antigen B			
- cIAI	11	1.4246 (0.45230)	0.250
- IAI	21	1.2775 (0.26075)	

On the study of antigen expression (n = 32), namely antigen A (using anti–A serum) and antigen B (using anti–B serum), all subjects (100%) expressed both antigens; denoting all subjects were the secretors.

In the study, we found both antigen A and B were strongly expressed in most subjects (n = 32); indicating the oligosaccharide complex constructing the glycans was intact. Using ANOVA test proceeded to find out the difference between cIAI (n = 11) and IAI group (n = 21), we found p = 0.130 for antigen A and p = 0.250 for antigen B; although box–whisker plot graphic showing the antigen expression in cIAI group a little bit stronger than those of IAI subjects (**Figure 1**).

Using independent samples test, we found there was no significant correlation of antigen A and B with cIAI and IAI; there's no significant difference found.

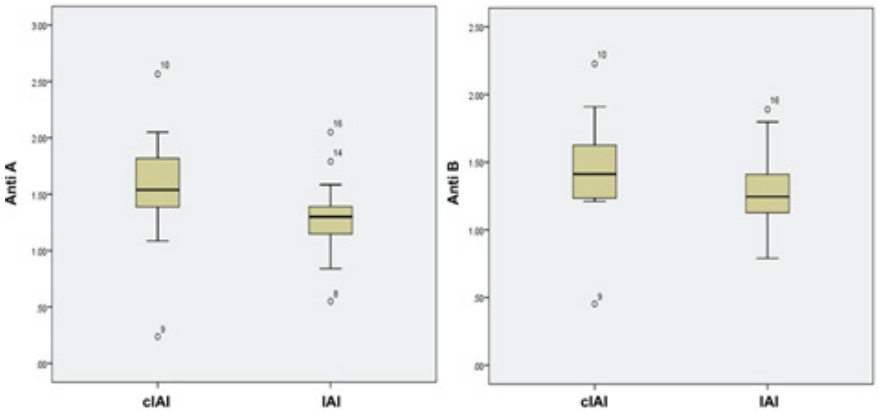

Figure 1. Box–Whisker plot graphic showing antigen expression in IAI and cIAI group. It is shown that the expression in the cIAI group is slightly stronger than IAI even though outliers were found in both of groups; statistically, this difference was not significant.

DISCUSSION

The important findings in the study never been reported. Firstly, strong expression of antigen means an intact glycocalyx found both in IAI group (representing the theory of bacterial translocation) and perforated group as well. Secondly, using immune enzyme method we were unable to identify tissue blood type, but the secretor. In the study we found the dominant blood type in intraabdominal infection was blood type O, and the dominant bacteria found to translocated Escherichia coli, which is the commensal bacteria in the GI.

A study showed disrupted endothelial glycocalyx in sepsis, which identified through an investigation under hematoxylin-eosin stained specimens.[9,10] There is plenty of research that showed disrupted endothelial glycocalyx in sepsis, including those correlated to the fluid management in sepsis.[10] However, the intestinal glycocalyx has distinctive characteristics to endothelial. The mucin domain of the glycocalyx integrates with the deeper mucus layer on the mucosal surface and interacts with the bacterial residence, i.e., commensal bacteria. This deeper mucus layer contains mucin (produce of Goblet cells), and antimicrobial peptide (produce of Paneth cells), together with commensal bacteria, protects against pathogenic bacteria.[11] It is known that the peptidoglycans of the glycocalyx consist of core peptides where the amino acids residues bound to glycans in two types, namely oxygen–linked glycosides (or, O–glycosylation) or N–glycosides (or, N–glycosylation) which are found differently in each blood type.[12] This specific characteristic leading to different susceptibility to a certain disease,[13] including sepsis. Glycans are the contact site of microbes to the host cell, and the microbes take the energy required for metabolic activity, to grow and develop.[7] The interaction between microbes and host proceeds in glycans that linked to O (namely, O–linked glycans)[12] that contact to lectins in bacteria (hemagglutinins, ligands, adhesins).[14] The secrets (enzymes) released by the bacteria were able to modify glycosylated glycans in the mucin bound the ligands, and uptake and utilizes glucose for the microbial metabolic purposes. The interaction between microbes and mucus layers allowing a prolonged colonization.[15]

Normally, those of pathogenic remain in the superficial layer. However, changes in the luminal atmosphere such as antibiotics let the pathogenic invades the deeper layer.[16,17] The flagella, the representative of microbial virulence contact to the glycocalyx. This contact is leading to disruption of glycocalyx results in a defect of signals transducing to the epithelial cell-to-cell junctions. The molecules of the junction found disassembled, and the barrier is no longer maintained; formerly attributed to intestinal hyperpermeability. The micro molecules may migrate across the barrier.[18,19]

The above paragraphs explain the bacterial translocation focused on bacterial virulence, as many studies do. However, to date, the

pathophysiology of bacterial translocation remains unclear. Most believe it as a phenomenon rather than a true pathology.[20] In addition to limited study focused on the exact mechanism of bacterial translocation that remain scanty, studies on intraabdominal sepsis continue focused on the virulence of various bacteria, the microbial resistance, and the effort to find out the superior antibiotic.[21] One may have difficulty to explain how the bacteria may spread across the barrier with an intact glycocalyx.

There was a thought that glycocalyx of the intestinal mucosa in those of blood type O recognizes the antigens of the intestinal microbes let the microbes crossing the mucosal barrier with no host's immune response. Whereas, when the antigen is not recognized, then the inflammatory response may lead to an excessive one, as found in the sepsis, which is destructive. To this thinking, we carried out the study focused on blood types approach. Glycans associated O referred to the structure rolled as the secretor of blood types. Both of antigen A and antigen B expressed on glycocalyx of intestinal mucosa of the secretor and blood group AB. Otherwise, these antigens not expressed in non–secretor and blood group O.[22] The critical thinking is to distinguish blood group O to the non–secretor in non–expressed antigens; however, a more evidence is required. Our findings showed these antigens expressed in all subjects (100%) denoting all these subjects were the secretor, while the previous study showed only 70% of the population.[23,24] Theoretically, the carbohydrate-based antigens that have been identified as histo–blood group antigens (HBGA), blood group ABH and Lewis antigen also expressed in red blood cells are also expressed in other cells.[25] Those other cells attributed as the secretor. Known secretors are the epithelium and endothelium. All epithelium throughout the body is the secretor, except the epithelium covering the cerebrospinal fluid. The last-mentioned epithelium found in the form of soluble oligosaccharides. Various pathogens use histo–blood groups antigen to be attached to the host cells. Studies show that this pathogen–HBGA relationship promotes the different antigens currently known.[24,25] Each individual has a variety of active glycosyltransferases. This variation is leading to a different oligosaccharide expression in the tissue. Among the epithelial of rolled as the secretors, mucin–coated glycocalyx in the GI epithelium known to be the most HBGA contained HBGA. The study of Marionneau (2000) showed that the H antigen in the blood group ABH, which is a characteristic possessed by blood group O (of the ABO blood group system), might change antigen A or B antigen through the role of glycosyltransferases A or B. ABH and antigen precursors were able to let fucosylated of GlcNAc (glycans) and forming Lewis antigen. Fucosyltransferase secretor/FUT2 (small intestines) will be added resembling fucose secretor. This enzyme found in the secretors.[26]

In this study, all subjects were the secretor and exposed to intraabdominal infection. The infection was proven by the evidence of growing Escherichia coli (61.3%) in culture media taken from the peritoneum; beyond the lumen. This evidence, however, should be elaborated and critically appraised. Firstly, this finding did not eliminate the possibility that those of non–secretor is free from a potential to have an intraabdominal infection, because there were all the secretor has no control in this study. Secondly, those with blood O type were not the only ones who experienced intraabdominal infection, even though those with O type were found dominant (43.8%).

Investigation using this immune enzyme method is different from the current qualitative procedure using hemagglutination to identify blood type in tissues (i.e., secretors).[8,27] The technique is quite challenging. Despite encountering optimation procedures that took plenty of time, detection of the antigen expression referred to challenging one, because any value regarding the standard threshold should be obtained and set as the reference point.[8] A total of thirty–two subjects showing a strong expression (mean 1.374 and 1.77 for antigen A and B, respectively) or in another word 2.9 times (antigen A) and 3.7 times (antigen B) to normal.

The small-sized and inequality of samples was the limitation of the study; good candidates unwilling to be enrolled in the study; no resection indicated was the characteristic of problems

in surgical research. Other limitations in this study, including 1) the blood group test that not carried out simultaneously, even though the same serum used; thus, might lead to a bias. 2) the study on saliva using the same antigen, which proposed early, was found unable to be proceeded due to constraining. Finally, no study on histomorphology carried out. On the other hand, the authors believe that this study, which focused on glycocalyx on the intestinal mucosa, has not been ever studied, expected to encourage future studies.

CONCLUSION

In intraabdominal infection, the glycocalyx was intact showing bacterial translocation without disrupting the barrier. However, the link with blood type could not be explained in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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