

Detection of *Candida Spp.* Infection in Chronic Gastritis Tissue by Using PAS Stain

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Abstract

Background: Chronic gastritis is a disease that is often found in the community and can happen to anyone regardless of age and gender. The inflammatory process that occurs in the stomach can be infection caused by a certain microorganism. The purpose of this study was to detect possible causes of infection other than bacteria, namely fungi in chronic gastritis tissue by using PAS stain.

Methods: This is a descriptive observational study to detect the possibility of *Candida spp.* infection in case of chronic gastritis at Dr. Ramelan Naval Central Hospital Surabaya.

Results: As many as 32 paraffin blocks of chronic gastritis tissue during the 2019 period at Dr. Ramelan Naval Central Hospital which previously had Giemsa staining and four out of 32 paraffin blocks positive of *Helicobacter pylori* were stained with PAS. Yeast was found in 11 (34.3%) blocks where in one (3.1%) block there was co-existence between yeast and *H. pylori*.

Conclusion: *Candida spp.* can be detected from chronic gastritis tissue by using PAS stain.

Keywords: *Candida spp.*; yeast; *H. pylori*; Giemsa; PAS

1. Introduction

Chronic gastritis is one of the most common diseases, which can have serious consequences and can be fatal in humans. More than half of the world's population suffers from this disease, indicating that hundreds of millions of people in the worldwide may have chronic gastritis. The importance of chronic gastritis as a serious disease is because most likely cases are found in clinical practices (Sipponen and Maaros, 2015).

Fungal infections are more common in high-risk populations. Endemic fungal infections can be associated with climate changes, expansion of human habitat, ease of transportation, and movement of population. Population at risk for opportunistic fungal infections or disseminated endemic fungal infections include the categories of patients who have received transplants, immunosuppressive agents and chemotherapy, HIV patients, premature infants, the elderly, and patients undergoing major surgery (Guarner and Brandt, 2011).

The acidic atmosphere in the stomach was considered unfavorable for the colonization of bacteria and fungi in the gastric mucosa. Study showed an increased incidence of fungal colonization in the gastric mucosa, from 7-33% of specimens taken from patients without sign and symptoms of fungal infection (Zwolinska et al.,

1997). Fungal colonization of the gastric mucosa may prolong the period of clinical symptoms and healing process (Gong et al., 2012).

Culture alone cannot differentiate between colonization and tissue invasion. Observations with a microscope reveal a lot of information, for example, if *Candida albicans* was isolated from a sputum culture, it did not necessarily mean that it was the organism causing pneumonia unless *pseudohyphae*, yeast or both invading the lung parenchyma were also visible on direct microscopy. Pathogenicity had actually occurred, if invasion was seen (Gupta et al., 2009). There are several types of fungi that cannot be diagnosed by culture, so it can only be done by staining the tissue (Kawilarang, 2015). Biopsy can show that fungi can infect the tissue and not just contaminants or saprophytes (Mandell et al., 2020).

Periodic Acid Schiff (PAS) used to identify glycogen in cells, detecting aldehydes produced by oxidation with periodic acids, which in the presence of Schiff's reagent (pararosaniline and sodium metabisulfite), form intracellular complexes colored in magenta, which react with cytoplasmic stores of carbohydrates, mucopolysaccharides and glycoproteins. The structural morphology of *Candida spp.* was easily identified by the method that stains the carbohydrates which present in large quantities in the fungal cell walls. PAS is considered the best method for identification of *Candida spp.* in cytopathology, PAS had been shown to have high sensitivity in identified the structure of *Candida spp.* (Padilha et al., 2014).

The possibility of *Candida spp.* infection in the stomach will be investigated in this study, by using chronic gastritis tissue from endoscopic biopsy with PAS stain.

2. Matherial and methods

This study has been exempted under review of Dr. Ramelan Naval Central Hospital Health Research Ethical Committe (No. 03/EC/KERS/2021).

The samples of this study are chronic gastritis tissue paraffin blocks, stored in the Department of Pathology, Dr. Ramelan Naval Central Hospital Surabaya from January until December 2019.

Total sample included were 32 samples; 13 were males (40.7%) and 19 were females (59.3%). The sample individuals in this study, the youngest was 34 years old and the oldest was 77. The distribution of samples by age and gender can be seen in table 1.

Table 1. The distribution of samples by age and gender

Age (years)	Male n (%)	Female n (%)	Total n (%)
30-39	2 (6.3)	0	2 (6.3)
40-49	2 (6.3)	6 (18.7)	8 (25)
50-59	6 (18.7)	9 (28.1)	15 (46.8)
60-69	1 (3.1)	4 (12.5)	5 (15.6)
70-79	2 (6.3)	0	2 (6.3)
Total	13 (40.7)	19 (59.3)	32 (100.0)

3. Results

As many as 32 samples were examined for *H. pylori* with Giemsa stain, and four samples were positive. *H. pylori* was identified as reddish-purple microorganisms against the background of other cells that are blue and pale blue (Sandhika, 2019). Sample distribution based on Giemsa stain for *H. pylori* are shown in table 2.

Table 2. Sample distribution based on Giemsa staining for *H. pylori*

<i>Helicobacter pylori</i>	Number of Sample	Percentage (%)
Positive	4	12.5
Negative	28	87.5
Total	32	100.0

As many as 32 samples were examined with PAS staining, and 11 samples were positive of yeast. Sample distribution based on PAS staining for *Candida spp.* are shown in table 3.

Table 3. Sample distribution based on PAS staining for *Candida spp*

Yeast	Number of Sample	Percentage (%)
Positive	11	34.4
Negative	21	65.6
Total	32	100.0

As many as 32 samples examined for *H. pylori* with Giemsa staining and *Candida spp.* with PAS staining, found one sample (3.1%) which indicate the co-existence of *H. pylori* and *Candida spp.*

4. Discussion

This study found the presence of yeast which was quite high in chronic gastritis tissue, 34.3% compared to several other studies which only found less than 10% (Karczewska, 2009; Saikumar and Manonmoney, 2014). The presence of *Candida spp.* in the stomach, even in small amounts, can cause injury to the epithelium and

mucosal changes due to the proteolytic action of enzymes produced by fungi *blastoconidia* or *spores*. Attachment of *Candida* to host tissues is one of the first steps in the disease process. Invasion may be accompanied by phase of transitions (*yeast* and *hyphae* interconversion) and secretion of enzymes that promote tissue penetration by organisms. The relationship between *Candida spp.* and the epithelial surface involved attachment at an early stage, followed by insertion of *yeast* cells into the subepithelial tissue, cavitation, and finally invasion of organisms with *hyphae* formation (Bernhardt et al., 2001).

This study found that infection in chronic gastritis tissue caused by microorganisms other than *H. pylori*, namely *Candida spp.* with microscopic morphology, *yeast*, has a clear result and can be detected by PAS staining (figure 1.). This study also provides important information by the presentation of *Candida spp.* in the gastric mucosa which was detected in the context of co-existence with *H. pylori* that can cause intensification of inflammatory changes in gastric mucosal tissue. Co-existence of *Candida spp.* with *H. pylori* was demonstrated in one sample (3.1%). *Candida spp.* can be a facilitator that carries *H. Pylori* in the *yeast* vacuole (Siavoshi et al, 2019; Sánchez-Alonzo et al, 2020).

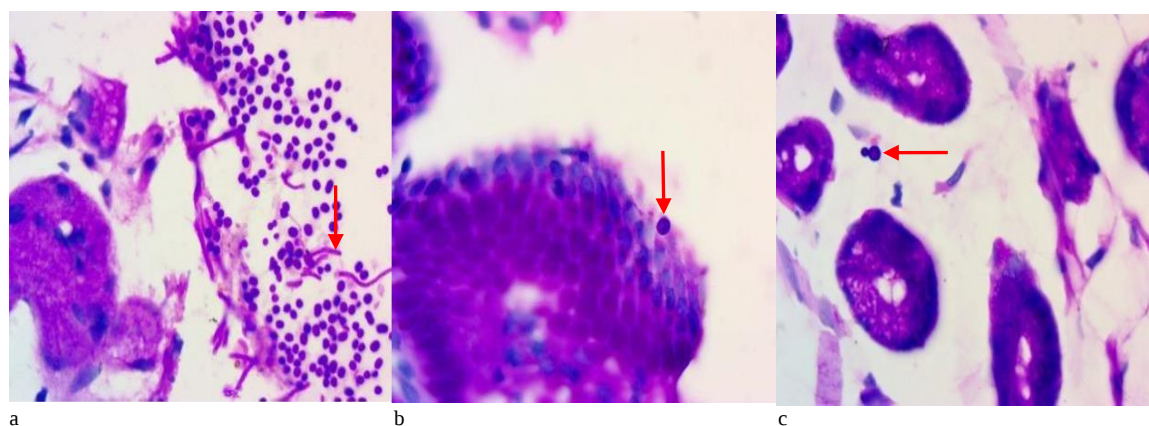


Figure 1. Histopathology result (red arrow) (a) *hyphae*; (b) *yeast* and (c) budding cell; using PAS stain with 400x magnification

5. Conclusion

Detected *Candida spp.* the cause of infection with the presence of *yeast* in the tissue from samples stained with PAS as many as 11 (34.4%) of 32 samples of chronic gastritis tissue paraffin blocks.

PAS staining is the best staining and has high sensitivity in identifying the structure of *Candida spp.* It is important for clinicians to consider whether or not to give antifungal therapy to patients whose tissues have been detected by *Candida spp.*

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Conflict of Interests

None

References

- Bernhardt, J., Dan, H., Michael, S., and Richard, C. 2001. Adherence and invasion studies of *Candida albicans* strains, using in vitro models of esophageal candidiasis. *The journal of infectious diseases*, 184(9), 1170–1175. DOI:10.1086/323807
- Gong, Y-B., Zheng, J-L., Jin, B., Zhuo, D-X., Huang, Z-Q. 2012. 'Particular *Candida albicans* strains in the digestive tract of dyspeptic patients, identified by multilocus sequence typing', *PLoS ONE* 7(4): e35311. DOI: 10.1371/journal.pone.0035311.
- Guarner J and Brandt ME. 2011. 'Histopathologic diagnosis of fungal infections in the 21st century', *Clinical microbiology reviews*. 247–280. American Society for Microbiology
- Gupta, E., Bhalla, P., Khurana, N., and Singh, T. 2009. Histopathology for the diagnosis of infectious diseases. *Indian Journal of Medical Microbiology*, 27(2):100-6 DOI:10.4103/0255-0857.49423
- Karczewska, E., Wojtas, I., Sito, E., Trojanowska, D., Budak, A., Zwolinska-wcislo, M., Wilk, A. 2009. 'Assessment of co-existence of *Helicobacter pylori* and *Candida* fungi in diseases of the upper gastrointestinal tract', *Journal of physiology and pharmacology*. 60, Suppl 6, 33-39 www.jpp.krakow.pl
- Kawilarang, A. P. 2020. Mycology lecture materials, accessed 13 November 2020, <https://mikologi.com/diktat-mikologi>.
- Mandell, Douglas, and Bennett's *Principles and Practice of Infectious Diseases*. 2020. 9th ed. Elsevier. Philadelphia.
- Padilha, C. M. L., Bruna Lavinias Sayed Picciani, B. L. S., Santos, B. M., Arley Silva, A., and Dias, e. p. 2014. Comparative analysis of Gram's method and PAS for the identification of *Candida* spp. samples from the oral mucosa. *J Bras Patol Med Lab*, v. 50, n. 5, p. 352-358, outubro. 10.5935/1676-2444.20140039
- Saikumar, C. and Manonmoney, J. 2014. 'Co-Existence of *Helicobacter Pylori* and *Candida* in upper gastrointestinal diseases', *Research journal of pharmaceutical, biological and chemical sciences*. March - April RJPBCS 5(2) Page No. 749.
- Sánchez-Alonzo, K., Parra-Sepúlveda, C., Vega, S., Bernasconi, H., Campos, V.L., Smith, C.T., Sáez, K., and García-Cancino, A. 2020. 'In Vitro Incorporation of *Helicobacter pylori* into *Candida albicans* caused by acidic ph stress', *Pathogens*. 9, 489; DOI: 10.3390/pathogens9060489.
- Sandhika, W. 2019. 'Detection of *Helicobacter pylori* infection in chronic gastritis biopsy specimen using warthin-starry and modified giemsa stain in dr. Soetomo hospital Surabaya', *Indonesian Journal of Tropical and Infectious Disease*. Vol. 7 No. 6 Sept-Dec. 150–154.
- Siavoshi, F., Heydaria, S., Shafieea, M., Ahmadia, S., Sanieeb, P., Sarrafnejad, A., and Kolahdoozand, S. 2019. 'Sequestration inside the yeast vacuole may enhance *Helicobacter pylori* survival against stressful condition. Infection', *Genetics and Evolution*. 69 127–133.
- Sipponen, P. and Maaroos, H. I. 2015. 'Chronic gastritis', *Scandinavian journal of gastroenterology*. 50: 657–667.
- Zwolinska, W. M., Budak, A., Trojanowska, D., Bogdal, J., and Stachura, J. 1998. 'Fungal colonization of the stomach and its clinical relevance', *Mycoses*. 41: 327-334.