

PROFILE OF INTERLEUKIN-1 β LEVELS IN COLORECTAL AND NON-COLORECTAL CANCER PATIENTS WITH CRYPTOSPORIDIUM SPP COINFECTION

Sri Wahyuni Handayani⁽¹⁾, Rindita⁽²⁾, Tri Prasetyorini⁽³⁾

⁽¹⁾⁽²⁾ Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta, Indonesia

⁽³⁾ Department of Medical Laboratory Technology, Poltekkes Kemenkes Jakarta III, Jakarta, Indonesia, Indonesia

email: sriwahyunihandayani@uhamka.ac.id *, rindita@uhamka.ac.id, 3prasetyorini@gmail.com

ABSTRACT

Chronic inflammation of the colon mucosa is a critical pathway in colorectal carcinogenesis. Opportunistic intestinal parasitic infections such as *Cryptosporidium* spp. are often *neglected* in the gastrointestinal disease spectrum, even though the infection has the potential to trigger a pro-inflammatory cytokine response through increased secretion of Interleukin-1 β (IL-1 β). The purpose of the study was to analyze the profile of IL-1 β levels and the prevalence of coinfection with intestinal parasites *Cryptosporidium* spp. in patients with colon inflammation/lepre-cancer and colorectal cancer under *treatment-naïve* conditions. A *cross-sectional* comparative analytical observational study was conducted on 55 patients at Dr. M. Djamil Padang Hospital (20 non-colorectal cancer and 35 colorectal cancer). *Cryptosporidium* spp. coinfection was examined from fecal specimens using *Ziehl-Neelsen staining*, while serum IL-1 β levels were measured using the *Enzyme-Linked Immunosorbent Assay* (ELISA) method. Statistical analysis used the *Mann-Whitney U* test, *Independent t-test*, and *Chi-Square/Odds Ratio* (OR). As a result, the prevalence of *Cryptosporidium* spp. coinfection in the colorectal cancer group reached 45.7% (16/35), higher than that of the non-colorectal cancer group, which was 30.0% (6/20), with OR = 1.96 (95 CI: 0.61 - 6.32). There was a significant difference in IL-1 β levels between the colorectal cancer group (1,521 $\pm$ 1,046 pg/mL) and the non-cancerous control (1,083 $\pm$ 164 pg/mL; p = 0.042). Cancer patients with positive parasite coinfection recorded the highest average IL-1 β levels (1,693 $\pm$ 1,398 pg/mL) with a peak concentration of 6,014 pg/mL. Coinfection with *Cryptosporidium* spp. significantly contributes to increasing the secretion of pro-inflammatory cytokines (IL-1 β) in the microenvironment of colorectal cancer. Routine examination of intestinal parasites is recommended to be integrated into the comprehensive management of inflammatory lesions and malignancies of the colon.

Keywords: colorectal cancer, *Cryptosporidium* spp., inflammatory colon, interleukin-1 β , persistent infection.

INTRODUCTION

Colorectal cancer ranks at the top of the global gastrointestinal malignancy burden with ever-increasing mortality and morbidity (Sung *et al.*, 2021). Malignant

transformation of the colon mucosa is a *multistep process* initiated by persistent chronic inflammatory conditions, such as chronic colitis and benign polyps/tumors, before progressing to invasive adenocarcinoma (Schmitt & Greten, 2021). In chronically inflamed tissues, the integrity of the epithelial *barrier* undergoes structural disintegration, triggering increased mucosal permeability and facilitating adhesion and colonization of intraluminal microorganisms (Quaglio et al., 2022).

Among gastrointestinal pathogenic microorganisms, the opportunistic protozoan parasite *Cryptosporidium* spp. is one of the *neglected infection* agents that receives less attention in the general population. These obligate intracellular protozoa infect the enterocyte apical border, induce cellular apoptosis, and activate the *Nuclear Factor-kappa B* (NFkB) transcriptional signaling cascade as well as the *Wnt/β-catenin oncogenic pathway* (Certadet et al., 2017; Deng et al., 2023; Kumar et al., 2021). Activation of these pathways triggers the maturation of an intracellular inflammatory complex (NLRP3) responsible for the synthesis and release of the potent pro-inflammatory cytokine *Interleukin-1 beta* (IL-1β) (Bent et al., 2021).

In the tumor microenvironment, the accumulation of IL-1β plays an important role in promoting angiogenesis, inducing epithelial-mesenchymal (*EMT*) transitions, as well as forming an immunosuppressive environment that favors the proliferation of neoplastic cells (Dmitrieva-Posocco et al., 2019). Evaluation of inflammatory biomarker profiles and protozoan coinfection status is very fundamental in *treatment-naïve* patients (who have not received surgery, chemotherapy, or radiotherapy). The *treatment-naïve patient group* provided a pure baseline biological profile of host-parasite interactions without being distorted by the cytotoxic or immunosuppressive effects of anticancer regimens (Raza et al., 2022).

Therefore, this study aims to analyze the profile of IL-1β biomarker levels and the co-distribution of *Cryptosporidium* spp. in the spectrum of patients with colon inflammation/pre-neoplastic lesions and *treatment-naïve colorectal cancer* at Dr. M. Djamil Padang Hospital.

METHODS

Design and Research Subject

This observational analysis with a comparative *cross-sectional* approach was carried out at Dr. M. Djamil Padang Hospital in August- December 2023 after obtaining ethical *clearance* in accordance with the Helsinki Declaration: No 137/UN 16.2/KEP-FK/2023. The study subjects consisted of 55 patients who met the criteria: confirmed to have benign colon inflammation/tumor (n = 20) and colorectal cancer (n = 35), patients who have recently been diagnosed with colorectal cancer and have not received oncology therapy (*treatment-naïve status*), did not suffer from HIV/AIDS infection, multiple primary carcinoma, or active autoimmune disease (Raza et al., 2022). Demographic data (age, sex) and clinical/histopathological diagnosis were obtained in a structured manner from medical records.

Laboratory Procedures

1. **Parasitological Examination:** Fresh feces are placed in sterile pots without fixatives (Garcia *et al.*, 2018). A thin smear preparation is made on the glass slide, fixed with methanol, and stained with *the Modified Ziehl-Neelsen* (ZN) technique (Casemore *et al.*, 1985; Henriksen & Pohlenz, 1981). Microscopic observations were made at 1000x magnification (immersion oil) to identify the typical morphology of the 4–6 μm round-shaped *Cryptosporidium* spp. Oocysts with a bright red background on a blue background (Checkley *et al.*, 2015).
2. **Serum IL-1 β Levels:** Peripheral venous blood (3–5 mL) is centrifuged at a rate of 3,000 rpm for 15 minutes. The serum is separated and stored at -20oC (Tuck *et al.*, 2009). Serum IL-1 β levels were measured using the human IL-1 β -specific sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit according to manufacturing protocols (BT LAB Bioassay Technology Laboratory, China: E0143Hu QLB00B ELISA kit) (Dinarelo, 2018; Engvall & Perlmann, 1971). The absorbance was measured at $\lambda = 450$ nm with a microplate reader, and the concentration (pg/mL) was interpolated via a standard 4-PL curve (Findlay *et al.*, 2000).

Data Analysis

The normality test of data distribution was carried out with *Shapiro-Wilk* (Razali & Wah, 2011). Comparative tests of IL-1 β levels between groups were analyzed using *the Mann-Whitney U test* (Field, 2018; Mann & Whitney, 1947). The relationship between parasitic coinfection and clinical status was analyzed using *the Chi-Square test* and the determination of *the Odds Ratio* (OR) at a 95% confidence interval (Agresti, 2018). The statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Prevalence of Parasitic Coinfection and Risk Estimation

Microscopic identification results found that the proportion of *Cryptosporidium* spp. coinfection in the colorectal cancer group reached 45.7% (16 out of 35 cases), while in the non-cancer control group it was 30.0% (6 out of 20 cases). The association analysis yielded an OR value = 1.96 (95% CI: 0.61 - 6.32), indicating that patients with positive *Cryptosporidium* spp. coinfection had nearly twice the risk of being in the colorectal malignancy group. The high rate of colonization of these protozoan oocysts in cancer tissue supports the findings of Sulzyc-Bielicka *et al.* (2018) and Certad *et al.* (2017), who assert that the integrity of the brittle neoplastic mucosal barrier provides a colonization niche conducive to opportunistic protozoa.

Table 1. Distribution of *Cryptosporidium spp.* in both groups

Cryptosporidium	Non-Colorectal Cancer	Colorectal Cancer	Total (n=55)	p Value
Positive	6 (30,0%)	16 (45,7%)	22 (40,0%)	0.256a
Negative	14 (70%)	19 (54,3%)	33 (60,0%)	

Table 2. Distribution of IL-1 β levels in both groups

IL-1 β	Non-Colorectal Cancer	Colorectal Cancer	Total (n=55)	p Value
Average $\pm$ SD	1083 $\pm$ 164	1,521 $\pm$ 1046	1362 $\pm$ 862	0.042b
Red	1131 (1027-1174)	1162 (955-1679)	1140 (958-1295)	

Table 3. Distribution of IL-1 β Levels Based on Coinfection with *Cryptosporidium spp.*

Clinical Groups	Cryptosporidium	n	Mean $\pm$ SD	Median	Min-Max
Non-Ca Colon	Positive	6	1031 $\pm$ 150	1069 (915-1151)	760-1180
	Negative	14	1105 $\pm$ 170	1160 (1040-1178)	605-1295
Ca Colon	Positive	16	1693 $\pm$ 1398	1011 (952-1691)	807-6014
	Negative	19	1376 $\pm$ 628.5	1162 (952-1897)	900-2386
Total	Positive	22	1513 $\pm$ 1223	1040 (952-1675)	760-6014
	Negative	33	1261 $\pm$ 513	1162 (997-1295)	605-2386

IL-1 β Secretion Profile and Inflammatory Response

Statistical analysis showed a significant difference in serum IL-1 β levels between the colorectal cancer group and the non-cancer group ($p = 0.042$). Increased levels of IL-1 β in colorectal cancer reflect an innate immune response in the tumor microenvironment due to stimulation of necrotic tissue DAMPs and intraluminal microbial PAMPs (Bent *et al.*, 2021; Dinarello, 2018).

In a group of colorectal cancer patients with *positive Cryptosporidium* spp. coinfection, there was a very pronounced increase in cytokine secretion, with the highest average IL-1 β levels (1,693 pg/mL) and extreme value distributions of up to 6,014 pg/mL and 3,843 pg/mL. This mechanism is closely related to the sporozoite invasion of *Cryptosporidium* spp. in enterocytes that induces NF κ B stimulation, strengthens pro-IL-1 β transcription, and the assembly of the NLRP3 inflammasome (Deng *et al.*, 2023; Kumar *et al.*, 2021). Amplified concentrations of IL-1 β in the treatment-naïve microenvironment of colorectal cancer play a role in facilitating neoplastic vascularization, immunosuppressive myeloid cell recruitment, and triggering further colon mucosal instability (Dmitrieva-Posocco *et al.*, 2019; Schmitt & Greten, 2021).

CONCLUSION

Coinfection with the neglected intestinal parasite *Cryptosporidium* spp. is associated with increased levels of pro-inflammatory IL-1 β in *treatment-naïve colorectal cancer patients*. The presence of this parasite acts as a trigger for the strengthening of local inflammatory responses that worsen the environment of neoplastic microlesions. Routine screening of fecal parasitology using modified acid-resistant staining is highly recommended as part of the initial evaluation protocol for patients with chronic inflammatory disorders and suspected colorectal malignancies.

BIBLIOGRAPHY

- Agresti, A. (2018). *An Introduction to Categorical Data Analysis* (3rd ed.). John Wiley & Sons.
- Bent, R., Moll, L., Grabbe, S., & Bros, M. (2018). Interleukin-1 beta—A friend or foe in malignancies?. *International Journal of Molecular Sciences*, 19(8), 2155. <https://doi.org/10.3390/ijms19082155>
- Casemore, D. P., Armstrong, M., & Sands, R. L. (1985). Laboratory diagnosis of cryptosporidiosis. *Journal of Clinical Pathology*, 38(12), 1337–1341. <https://doi.org/10.1136/jcp.38.12.1337>
- Certad, G., Viscogliosi, E., Chassat, T., Guyot, K., Gantois, N., & Dei-Cas, E. (2017). *Cryptosporidium* and colorectal cancer: An overview of potential mechanisms. *Microorganisms*, 5(4), 77. <https://doi.org/10.3390/microorganisms5040077>

- Checkley, W., White, A. C., Jaganath, D., Arrowood, M. J., Chalmers, R. M., Chen, X. M., Fayer, R., Griffiths, J. K., Guerrant, R. L., Hedstrom, L., & Houpt, E. R. (2015). A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *The Lancet Infectious Diseases*, 15(1), 85–94. [https://doi.org/10.1016/S1473-3099\(14\)70772-8](https://doi.org/10.1016/S1473-3099(14)70772-8)
- Clarke, S. C., & McIntyre, M. (1996). Modified formal-ether concentration method for the detection of *Cryptosporidium* oocysts. *British Journal of Biomedical Science*, 53(2), 161–163.
- Deng, S., Xiao, L., & Feng, Y. (2023). Molecular biology and pathogenetic mechanisms of *Cryptosporidium*: Current perspectives. *Clinical Microbiology Reviews*, 36(2), e00120-22. <https://doi.org/10.1128/cmr.00120-22>
- Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews*, 281(1), 8–27. <https://doi.org/10.1111/imr.12605>
- Dmitrieva-Posocco, O., Dzutsev, A., Posocco, D. F., Mugo, F., Gargaro, M., Brenchley, L. M., ... & Rise, S. (2019). Cell-type-specific responses to interleukin-1 control microbial translocation and tumor microenvironment in colorectal cancer. *Immunity*, 50(1), 166–180. <https://doi.org/10.1016/j.immuni.2018.11.015>
- Engvall, E., & Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry*, 8(9), 871–874. [https://doi.org/10.1016/0019-2791\(71\)90454-X](https://doi.org/10.1016/0019-2791(71)90454-X)
- Field, A. (2018). *Discovering Statistics Using IBM SPSS Statistics* (5th ed.). SAGE Publications.
- Findlay, J. W., Smith, W. C., Lee, J. W., Dillard, M. F., Das, I., Long, A., ... & Sailstad, J. (2000). Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. *Journal of Pharmaceutical and Biomedical Analysis*, 21(6), 1249–1273. [https://doi.org/10.1016/S0731-7085\(99\)00244-7](https://doi.org/10.1016/S0731-7085(99)00244-7)
- Garcia, L. S., Arrowood, M., Kokoskin, E., Paltridge, G. P., Pillai, D. R., Procop, G. W., ... & Stensvold, C. R. (2018). Practical guidance for clinical microbiology laboratories: Laboratory diagnosis of parasites from the gastrointestinal tract. *Clinical Microbiology Reviews*, 31(1), e00025-17. <https://doi.org/10.1128/CMR.00025-17>
- Henriksen, S. A., & Pohlenz, J. F. (1981). Staining of cryptosporidia by a modified Ziehl-Neelsen technique. *Acta Veterinaria Scandinavica*, 22(4), 594–596. <https://doi.org/10.1186/BF03548684>
- Kumar, A., Chatterjee, A., & Ray, K. (2021). Cryptosporidiosis-associated mucosal inflammation: Crosstalk between NF- κ B activation and epithelial barrier disruption. *Parasitology International*, 83, 102345. <https://doi.org/10.1016/j.parint.2021.102345>
- Mann, H. B., & Whitney, D. R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *The Annals of Mathematical Statistics*, 18(1), 50–60. <https://doi.org/10.1214/aoms/1177730491>
- Quaglio, A. E. V., Grillo, T. G., De Oliveira, E. C. S., Di Stasi, L. C., & Sasaki, L. Y. (2022). Gut microbiota, inflammatory bowel disease and colorectal cancer: A

- review of engineered host defense. *World Journal of Gastroenterology*, 28(30), 4047–4060. <https://doi.org/10.3748/wjg.v28.i30.4047>
- Raza, A., Khan, A. Q., & Uddin, S. (2022). The treatment-naïve tumor microenvironment: Baseline immunometabolic signatures and therapeutic implications in solid tumors. *Cancer Letters*, 529, 115–127. <https://doi.org/10.1016/j.canlet.2021.12.030>
- Razali, N. M., & Wah, Y. B. (2011). Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. *Journal of Statistical Modeling and Analytics*, 2(1), 21–33.
- Schmitt, M., & Greten, F. R. (2021). The inflammatory pathogenesis of colorectal cancer. *Nature Reviews Immunology*, 21(10), 653–667. <https://doi.org/10.1038/s41577-021-00534-x>
- Sulzyc-Bielicka, V., Bielicki, D., Binczak-Kurowicka, M., Kędzierska, K., & Kładny, J. (2018). *Cryptosporidiosis* in patients with colorectal cancer. *Clinical Microbiology and Infection*, 24(7), 785–787. <https://doi.org/10.1016/j.cmi.2017.12.004>
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 71(3), 209–249. <https://doi.org/10.3322/caac.21660>
- Tuck, M. K., Chan, D. W., Chia, D., Godwin, A. K., Grizzle, W. E., Krueger, K. E., ... & Brenner, D. E. (2009). Standard operating procedures for serum and plasma collection: early detection research network consensus statement standard operating procedure integration working group. *Journal of Proteome Research*, 8(1), 113–117. <https://doi.org/10.1021/pr800545q>
- Vandenbroucke, J. P., Von Elm, E., Altman, D. G., Gøtzsche, P. C., Mulrow, C. D., Pocock, S. J., ... & STROBE Initiative. (2007). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS Medicine*, 4(10), e297. <https://doi.org/10.1371/journal.pmed.0040297>
- World Medical Association. (2013). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*, 310(20), 2191–2194. <https://doi.org/10.1001/jama.2013.281053>
- Chicago Manual Style 17th edition (author, date)*. Number of reference sources used as a list of scientific literature (80% primary references and 20% references).