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Analysis of Images of Neutrophil Lymphocyte Ratio and Bacteria Density Level of Helicobacter Pylori in Tissue in Gastritis Patients

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ABSTRACT A chronic inflammatory condition, gastritis can extend for years. The most prevalent cause of gastritis and the primary cause of peptic ulcers, respectively, are infections with *Helicobacter pylori*. In comparison to non-*Helicobacter Pylori* gastritis, *Helicobacter Pylori* infection results in mononuclear (MN) and polymorphonuclear (PMN) cell infiltration and increases the production of proinflammatory cytokines in the stomach mucosa. Histopathological testing for *Helicobacter Pylori* to make the diagnosis. The aim of this study was to analyze the neutrophil lymphocyte ratio (NLR) in patients with gastritis caused by histological analysis, *Helicobacter Pylori* was diagnosed. This sort of study was conducted at the Anatomical Pathology Laboratory Installation, RSPAL dr. Ramelan Surabaya, using descriptive observational research and purposive sampling. The information used includes the outcomes of a biopsy of 60 samples that were identified as having gastritis with histopathological analysis of the identification of *Helicobacter pylori* bacteria in gastric tissue classified by the level of bacterial density using *The Updated Sydney System*, as well as haemogram data for complete blood examination to further calculate the neutrophil lymphocyte ratio (NLR). The data were processed using descriptive statistical tests to determine the average neutrophil lymphocyte ratio (NLR). The density of *Helicobacter Pylori* of the r-Spearman test statistics indicates that (Pv = 0.000) below < 0.005 indicates that there is a strong relationship between the neutrophil lymphocyte ratio (NLR) and the density of *Helicobacter Pylori* bacteria. The conclusion of this research is the neutrophil lymphocyte ratio (NLR) should be a parameter to check before the invasive treatment to Gastritis patient which is examining the histology of the tissue.

INDEX TERMS Neutrophil lymphocyte ratio (NLR), *Helicobacter Pylori* bacteria, Gastritis.

I. INTRODUCTION

Gastritis is an inflammatory disease that can heal itself and cannot heal itself and lasts for a fairly long time[1]. Inflammatory gastritis, pre-cancerous gastric atrophy and intestinal metaplasia are also associated with *Helicobacter Pylori* infection [2]. Gastritis can be caused by *Helicobacter Pylori* inflammation, bile reflux, non-steroidal anti-inflammatory drugs, autoimmunity, or an allergic response. *Helicobacter pylori* is the most common cause of gastritis with an incidence of more than 80%[3]. In most areas, the prevalence of *Helicobacter Pylori* increases with age, although recent time trend analyses of numerous large populations have shown a decline in the prevalence of *Helicobacter Pylori* infection. [4]. Gastric cancer is the third leading cause worldwide and *Helicobacter pylori* infection is responsible for 74.7% of all cases of no cardia gastric cancer.[5].

According to the World Health Organization (World Health Organization), the incidence of gastritis in the world reaches 1.8-2.1 million of the total population each year, in the UK (22%), China (31%), Japan (14.5%), Canada(35%), and France(29.5%). In Southeast Asia, close to 583, 635 of the total population annually. Based on the World Health Organization, gastritis incidence in Indonesia is 40.8%. With a frequency of 274,396 cases, the incidence rate of gastritis is relatively high in various parts of Indonesia. [6].

Following studies and observations made by the Ministry of Health of the Republic of Indonesia, the prevalence of gastritis is highest in Medan (91.6%), followed by Surabaya (31.2%), Denpasar (46%), Jakarta (50%) Bandung (32.5%), Palembang (35.3%), Aceh (31.7%), and Pontianak (31.2%). The incidence of gastritis is caused by an unhealthy diet[7]. *Helicobacter Pylori* is classified as a gram-negative bacterium, bacillus, rod curve. *Helicobacter Pylori* causes chronic gastric infection and gastritis in humans. Peptic ulcers and gastritis are recognized to be primarily brought on by *Helicobacter pylori* inflammation. [8].

Helicobacter pylori only infects humans and transmission occurs via the fecal-oral route. Colonization of pathogens infecting the gastric mucosa [9]. A study shows the reduced viability of *Helicobacter Pylori* bacteria when in an acidic pH especially at pH 3 as the lowest value, the factor that causes *Helicobacter Pylori* to survive up to pH 3 is the absence of urea, in the stomach *Helicobacter Pylori* neutralizes pH by secreting urease, this fact is related with the ecology of *Helicobacter Pylori* in the human stomach with a pH of 2.0 – 3.0. *Helicobacter Pylori* in several other genes can live and adapt in water (pH 6.5-7.4), milk (pH 6.6-6.8), and in the mouth (pH 5.5-7.4)[10].

Pathogenicity factors include motility to search for target cells, adhesion to gastric surface epithelial cells, urease will release ammonia from urea to facilitate the survival of cells in a highly acidic environment and Cytotoxin Vacuolizing (VACA) which destroys epithelial cells. Once the pathogen has infected the stomach tissue, acute gastritis occurs, the course of which may or may not involve obvious symptoms. The gastric mucosa is very well protected from bacterial infections. However, *Helicobacter Pylori* has excellent adaptability to the ecological environment of the stomach with a unique series of steps into the mucus, moves and is spatially oriented within the mucus, attaches to gastric epithelial cells, evades the immune response, and as a result colonization and persistent transmission occur [11]. The initial way to identify *Helicobacter Pylori* was histological examination, which is regarded as the gold standard in the direct identification of *Helicobacter Pylori* infection. [12]. The gold standard detection of *Helicobacter Pylori* bacteria in Indonesia generally uses tissue histopathology [13]. Several clinical studies have shown that inflammatory markers related to leukocyte count are the most widely used and simple. Neutrophils and lymphocytes are the main components of leukocytes [14]. Neutrophils play a role in active inflammation in acute gastritis; Lymphocytes and plasma cells accompany the inflammatory process in chronic gastritis [15].

As compared to non-*Helicobacter Pylori* gastritis, *Helicobacter Pylori* infection will result in mononuclear (MN) and polymorphonuclear (PMN) cell infiltration and stimulate the production of proinflammatory cytokines in the stomach mucosa. [16].The neutrophil-lymphocyte ratio can reflect an imbalance between overactive inflammation and protective regulation and has advantages over using neutrophils or lymphocytes alone [17]. The neutrophil lymphocyte ratio is measured the haemogram's absolute lymphocyte and neutrophil counts by each other. [15]. Staining is an important part of the histologic examination and several stains such as routine Hematoxcylin-Eosin stain, Giemsa, Warthine Starry, *Helicobacter Pylori* silver stain, toluidine blue, acridine orange, McMullen, Genta, Dieterle, and immunohistochemical stains have been used to detect *Helicobacter Pylori*. Additional staining is usually recommended for biopsy specimens showing moderate or severe chronic gastritis, but no Helicobacter Pylori was identified on Hematoxcylin-Eosin staining. Giemsa staining is the most popular technique in clinical application because it is proper, extremely sensitive, and affordable. [12].

The purpose of this study was to analyze the neutrophil lymphocyte ratio (NLR) in patients with gastritis caused by *Helicobacter Pylori* by histopathological examination diagnosis. Fadia Rakhmalia, Anik Handayati, and Evy Diah Woelansari are conceived of the presented idea. Fadia Rakhmalia developed the theory and performed the computations. Anik Handayati and Evy Diah Woelansari verified the analytical methods. Evy Diah Woelansari encouraged Fadia Rakhmalia to investigate the each variabels used and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

II. METHODHOLOGY

This research project was carried out from February to May 2022 at the Dr. Ramelan Surabaya Anatomical Pathology Laboratory Installation.. This type of research uses a retrospective cohort with a descriptive observational design. This study uses data from SIMRS RSPAL Dr. Ramelan Surabaya for the period January 2019 - February 2022.

The samples of this study were all data on the results of neutrophil cell counts, lymphocyte cell count results, and gastric tissue histopathology results that had been classified using The Updated System Sydney classification for identification of *Helicobacter Pylori* bacteria with gastritis at the Anatomical Pathology Laboratory Installation, RSPAL Dr. Ramelan Surabaya for the period January 2019 - February 2022 which meets the inclusion criteria. The sampling technique is purposive sampling where the

researcher has determined certain conditions under which the sample can be used as research material. SIMRS data for gastritis patients, who have been filtered according to inclusion criteria, are then processed and collected for data analysis. The inclusion criteria in this study were gastritis patients who had histopathological examination of gastric tissue and complete blood examination. Complete blood count data on the results of the neutrophil blood cell count and the lymphocyte blood cell count, the Neu/Lim ratio was calculated so that the results of the neutrophil lymphocyte ratio could be obtained. Furthermore, a normality test was performed on the results of the neutrophil lymphocyte ratio (RNL) to determine the distribution of the data. Using the Kolmogrov-smirnov test if the sample is > 50 samples and using the Shapirowilk test if the sample used is < 50 samples. Determining the minimum and maximum limit values for the neutrophil lymphocyte ratio value using the ROC curve, so that numerical data can be converted into categorical values according to the maximum and minimum limit values. Through the normality test, the distribution of the data used will be known.

III. RESULTS

A result of research conducted at RSPAL dr. Ramelan Surabaya using SIMRS data on history of gastritis patients with histopathological identification and complete blood count as a source of data on the ratio of neutrophils to lymphocytes.

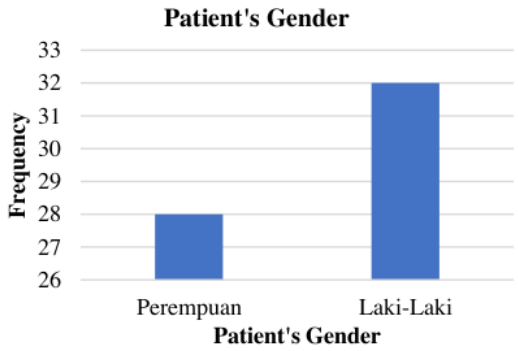

FIGURE 1. Frequency of Sex of Gastritis Patients From Histopathological Examination and Neutrophil Lymphocyte Ratio (NLR).

A. CHARACTERISTICS OF PEOPLE WITH GASTRITIS BY GENDER

TABLE 1
Gender Demography of Gastritis Patients From Histopathological Examination and Neutrophil Lymphocyte Ratio (NLR)

Male (n)	Female (n)
28	32

TABLE 1 Based on the demographic data of 60 gastritis sufferers in this study, they are presented in table 1 and table 2. Gastritis patients aged 15-83 years and have been examined at the Anatomical Pathology Laboratory and Clinical Pathology Laboratory RSPAL dr. Ramelan Surabaya (FIGURE 1).

B. CHARACTERISTICS OF PEOPLE WITH GASTRITIS BY AGE

TABLE 2 age data is presented which is categorized into 7 different categories. Thus, the data can be distributed according to the categories provided to determine the frequency of each category. In the age range 51-60 has the highest frequency in patients diagnosed with gastritis with a frequency of 18 patients. The incidence frequency of male gastritis patients is smaller than the frequency of female patients, which is 28 patients (FIGURE 1).

TABLE 2
Demographic Age of Gastritis Patients From Histopathological Examination and Neutrophil Lymphocyte Ratio (NLR).

Characteristic	Gastritis
Ages :	Histopathology & Neutrophil Lymphocyt Ratio (%) n = 60
10-20	2
21-30	4
31-40	8
41-50	6
51-60	18
61-70	11
71-80	7
81-90	4
Total	60

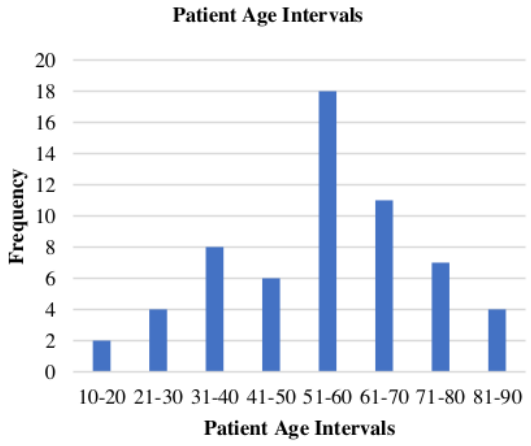

FIGURE 2. Frequency Interval Age of Gastritis Patients From Histopathological Examination and Neutrophil Lymphocyte Ratio Examination.

C. HISTOPATHOLOGICAL CLASSIFICATION OF HELICOBACTER PYLORI BACTERIA DENSITY IN GASTER TISSUE OF PEOPLE WITH GASTRITIS USING THE UPDATED SYDNEY SYSTEM

Based on TABLE 3 Histopathological classification of gastric tissue in this study was adjusted using *The Updated Sydney System* by Dixon et al using normal (0) i.e. no *Helicobacter Pylori* anywhere on biopsy, mild (1) i.e. only a few *Helicobacter Pylori* in single or multiple foci, moderate (2) i.e. Many *Helicobacter Pylori* seen in separate foci areas, severe (3) that is > 50% of the surface area covered by *Helicobacter Pylori* (FIGURE 3).

TABLE 3
Histopathological Tissue Classification of Gastritis Patients Using *The Updated Sydney System* with Giemsa staining Special Stain Identification of *Helicobacter Pylori* Bacteria.

	Gastritic Tissue Histopathology			
	Helicobacter Pylori (+) n = 27		*Helicobacter Pylori* (-) n = 33	
	Grade	Frequency (n)	Grade	Frequency (n)
***Helicobacter Pylori* Bacteria Density**	0	0	0	33
	1	18	1	0
	2	5	2	0
	3	4	3	0

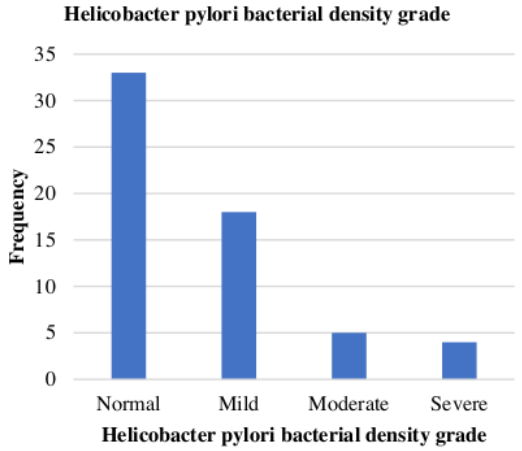

FIGURE 3. Bacterial Density Grade *Helicobacter pylori* in gastric tissue according to the classification of *The Updated Sydney System.*

D. THE RELATIONSHIP OF NEUTROPHIL LYMPHOCYTE RATIO WITH THE DENSITY LEVEL OF THE BACTERIA HELICOBACTER PYLORI USING THE CLASSIFICATION SYSTEM THE UPDATED SYDNEY SYSTEM

The normality test in this study is presented in TABLE 4, showing the normality test for the Neutrophil Lymphocyte Ratio which has been examined in the anatomical pathology laboratory and clinical pathology laboratory, RSPAL dr. Ramelan Surabaya is not normally distributed. SPSS output results obtained a significant value (p-value) for the Neutrophil Lymphocyte Ratio is 0.000. When compared with the value (0.05) then the p-value < α so it can be concluded that the data is not normally distributed.

TABLE 4
Neutrophil Lymphocyte Ratio (NLR) normality test for gastritis patients at the Anatomical Pathology Laboratory and Clinical Pathology Laboratory, RSPAL dr. Ramelan Surabaya.

	Kolmogorov-Smirnov	
Neutrophil Lymphocyte Ratio (NLR)	Sig	N
	.000	60

TABLE 5
Cut-off Value of Neutrophil Lymphocyte Ratio Data for Gastritis Patients Analyzed Using ROC Curve

Cut-off	Status
< 4,44	Minimum
< 4,55	Maximum

TABLE 5 presented analysis of neutrophil lymphocyte ratio data to determine the minimum and maximum values of neutrophil lymphocyte ratio using cut-off values with ROC curves. So that the data can be converted into categorical form for further data analysis.

TABLE6
The relationship between neutrophil lymphocyte ratio and histopathological examination results for identification of bacterial infections *Helicobacter Pylori* Density Levels of *Helicobacter Pylori* Bacteria in Gastritis Patients at RSPAL Dr. Ramelan Surabaya.

		Helicobacter Pylori Bacteria Density
Neutrophil Lymphocyte Ratio (NLR) Gastritis Patient	Correlation Coefficient	-,960
	Sig.(2-tailed)	,000
	N	60

Based on TABLE 6 showed that there was an association between the RNL of patients who were infected and not infected with *Helicobacter Pylori* and Density of *Helicobacter Pylori* (p – value) = 0.000. This relationship is in accordance with the decision making requirements for the r-spearman test, the significance value (p - value) < 0.05. In the guidelines for the strength of the relationship, there was a very strong correlation of 0.960 at (0.76 – 0.99) with a negative direction for the amount of neutrophil lymphocytes in patients compared to the density of stomach tissue with *Helicobacter Pylori* bacteria. The meaning of the correlation coefficient sign in the results of data analysis shows a negative sign (-) indicating an inverse relationship,

the higher the neutrophil lymphocyte ratio value, the lower the density level of *Helicobacter Pylori* bacteria in the gastric tissue of gastritis patients at RSPAL dr. Ramelan Surabaya.

IV. DISCUSSION

Research on the analysis of the neutrophil lymphocyte ratio (RNL) and the density level of *Helicobacter Pylori* bacteria in the tissues of gastritis patients at RSPAL dr. Ramelan Surabaya. The 60 samples used in this study were divided into two, namely; gastritis patients with *Helicobacter Pylori* infection were 27 patients and 33 patients were not infected with *Helicobacter Pylori* bacteria. The highest frequency of gastritis incidence is in the age range of 51-60 as many as 18 patients, this is in accordance with research conducted by (Ariefiany et al., 2014)[19] with a percentage of age 51-60 years of 26.7% of 30 respondents. According to Pramita et al., (2016) [20] In the elderly, the stomach wall becomes thinner, the production of mucus as a barrier decreases, so that the gastric mucosal layer is easily damaged and easily infected with *Helicobacter Pylori*. Autoimmune diseases are also common in the elderly, and at this age tend to have a bad diet.

Infection caused by *Helicobacter Pylori* bacteria in gastric tissue in the lamina propria layer causes gastritis inflammation which is characterized by inflammatory cell infiltration that predominates among inflamed tissue cells. Gastritis caused by infection with *Helicobacter Pylori* bacteria is chronic gastritis. *Helicobacter Pylori* bacteria produce VAC (Vacuolating Cytotoxin Cell). Thus, it will cause "vacuoles" or bubbles in the infected gastric tissue. The more cytotoxic the strain, the more active, clear, and more aggressive in chronic gastritis [21].

Different virulence factors laid by *Helicobacter pylori*, including urease, flagella, vacuolating cytotoxin A (VACA), and cytotoxin associated gene A (CagA), are crucial for invasion, colonization, and proliferation. proliferation of cells and gastric mucosal metaplasia. The increase in the degree of inflammatory cell infiltration caused by the production of cytotoxin associated gene A (CagA) by *Helicobacter Pylori* bacteria in gastric tissue is supported by research conducted by (Dja'far et al., 2019) [22].

Helicobacter pylori cause persistent inflammation of the stomach. This inflammatory response initially consists of neutrophil withdrawal, followed by T and B lymphocytes, plasma cells, and macrophages, along with epithelial cell damage [21]. The immune response that occurs due to infection by the bacterium Helicobacter Pylori is the withdrawal of neutrophils in the tissue and an increase in neutrophils in the blood. Withdrawal of neutrophils from the tissue aims to reduce the occurrence of reactive oxygen species (ROS). The high ROS in the tissue will cause cell inflammation from DNA/RNA oxidation and initiate degenerative diseases [23].

The expression of MN (Mononuclear) cells in the tissue has increased. Gastritis sufferers on the expression of MN cells in the tissue, showing the number of MN cells in the tissue [24]. The increase is initiated by macrophages (mature monocytes) that produce the hormones interleukin-8 and interleukin-12 and send a response with Th0 cells to expand Th1 forming an intracellular response and Th2 extracellular response by B lymphocytes. in the tissue increases, to function as Cellular Mediated Immune in reducing inflammation in gastric tissue [25].

In this study, the histopathological analysis slides reviewed under the 400x objective so that it would be easy to identify *Helicobacter Pylori* bacteria in gastric tissue. The results of the haemogram of gastritis patients used in this study, the value of neutrophils and lymphocytes also showed that the value of neutrophils and lymphocytes was higher. Thus, it can be processed entirely into the ratio of neutrophils to lymphocytes. The high value of the neutrophil lymphocyte ratio in patients with *Helicobacter Pylori* bacterial infection was proven in a study conducted by Jafarzadeh et al., (2013) [26]. with the results of the neutrophil lymphocyte ratio value of 34% above the negative control (28%).

According to the study's findings, there is a significant correlation between the density of *Helicobacter pylori* bacteria and the ratio of neutrophil lymphocytes as measured by the r-Spearman test.. These results are in accordance with research conducted by Atayan & Hacisalihoglu, (2017) [27]. This relationship is followed by the direction of the sign of the coefficient of negative (-) or the opposite, the lower the value of the neutrophil lymphocyte ratio, the higher the density of *Helicobacter Pylori* bacteria in the gastric tissue of gastritis sufferers at RSPAL dr. Ramelan Surabaya.

The total sample used in this research is 60 samples divided into two genders, female and male. The female is 28 samples and 32 samples from male. The sample needs to meet with the criteria which should be have histopatology result from gastric tissue and hemogram data for complete blood. The method used is descriptive observational and process with SPSS statistic 25. The result by processing the samples shown in the r-Spearman test, there is a significant correlation between the neutrophil lymphocyte ratio (NLR) and the density of *Helicobacter Pylori* bacteria (Pv = 0.000) when it is below 0.005.

V. CONCLUSION

The aim of this study was to analyze the neutrophil lymphocyte ratio (NLR) in patients with gastritis caused by *Helicobacter Pylori* by histopathological examination diagnosis. The result of this research is supporting the past research to the newest and got result a very strong relationship between the ratio of neutrophil lymphocytes (RNL) with the density level of *Helicobacter Pylori* bacteria. For the next research suggested to use a wide population represent the huge population. Therefore, it can be a strongest valid of a research in this topic.

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Analysis of Images of Neutrophil Lymphocyte Ratio and Bacteria Density Level of Helicobacter Pylori in Tissue in Gastritis Patients

GRADEMARK REPORT

FINAL GRADE

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GENERAL COMMENTS

Instructor

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