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ASSOCIATION BETWEEN HYPOXIC INDUCIBLE FACTOR (HIF) -1 ALPHA WITH HISTOPATHOLOGICAL FEATURES OF GASTRITIS

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Abstract

Background: The incidence of gastritis in Southeast Asia is around 583,635 of the total population each year. There are several features of histopathology of gastritis, namely chronic inflammation, acute inflammation, gastric mucosal atrophy, and metaplasia. The association between inflammation and hypoxia has been found since many years ago. Hypoxic Inducible Factor (HIF) is activated in response to hypoxia and inflammation. . In hypoxic conditions, there will be an increase in the production of HIF-1α. This study was conducted to determine the relationship of HIF-1α expression with gastritis degree, so that it can be known whether HIF-1α can be used as a parameter of the degree of gastric mucosa damage.

Methods: The design of this study was a cross-sectional study with independent variables such as histopathological severity of gastritis (degree of lymphocyte infiltration, degree of neutrophil activity, degree of atrophy, and degree of intestinal metaplasia) and the dependent variable was serum HIF-1α levels. This study was conducted at the Endoscopic Unit of H. Adam Malik GeneralHospital in Medan. Statistical analysis was carried out with the SPSS (version 22.0). **Results:** HIF-1α levels are higher in moderate + severe lymphocyte infiltration than normal + mild degrees. HIF levels are higher in moderate + severe neutrophil activity than normal + mild degrees. HIF-1α levels are higher in moderate + severe atrophy than normal + mild degrees. HIF-1α levels are higher in mild metaplasia degrees than without metaplasia.

Conclusions: Significant association was found between histopathological features of gastrits and serum HIF-1α levels..

Introduction

Gastritis is a state of inflammation or inflammation of the gastric mucosa that is acute, chronic, diffuse and local. It is caused by Helicobacter pylori, bile reflux, non-steroidal anti-inflammatory or allergic responses. World Health Organization (WHO) conducted a review of several countries in the world and got the percentage of gastritis in the world, including the UK 22%, China 31%, Japan 14.5%, Canada 35%, and France 29, 5%. In the world, the incidence of gastritis is around 1.8-2.1 million of the population every year. The incidence of gastritis in Southeast Asia is around 583,635 of the total population each year.[1]

There are several features of histopathology of gastritis, namely chronic inflammation, acute inflammation, gastric mucosal atrophy, and metaplasia. Chronic inflammation is found in mononuclear cell infiltrates, especially lymphocytes. Inflammatory infiltrates such as lymphocytes, plasma cells, histiocytes, and granulocytes are in the lamina propia and sometimes inside the gland. Acute inflammation is characterized by neutrophil infiltrates and eosinophils active inflammation of the gastric mucosa is characterized by the presence of neutrophils in the lamina propria and / or gland lumen. Gastric mucous atrophy, characterized by the loss of appropriate glands, where normal with their functional compartments, namely the antrum or corpus (appropriate glands). While changes in cell metaplasia that differentiate into other types of cells indicate adaptation to environmental stimuli.[2]

The association between inflammation and hypoxia has been found since many years ago. In some pathological situations, Hypoxic Inducible Factor (HIF) is activated in response to hypoxia and inflammation.[3] Hypoxic Inducible Factor (HIF) is a transcription factor involved in adaptation to low oxygen conditions.[4] At present, there are 3 types of identification of HIF α subunits, namely HIF-1α, HIF-2α and HIF-3α, but HIF-3α is not

widely known.[3]Under normoxia conditions, HIF-1α will affect the hydroxylation of proline residues with proline hydroxylase and sub-degradation through the proteasome pathway. In hypoxic conditions, where there is a low oxygen level, there will be a decrease in proline hydroxylase activity, so there will be an increase in the production of HIF-1α.[4]

Gastric cancer is still a big challenge in the world because the diagnosis is often late and in the later stages treatment options pose a high risk of death. Knowledge of precursor lesions from the development of intestinal type gastric cancer contributes significantly to the diagnosis of early gastric cancer when surgery and chemotherapy still produce a better prognosis.[5] This study was conducted to determine the relationship of HIF expression with gastritis degree, so that it can be known whether HIF can be used as a parameter of the degree of gastric mucosa damage. Understanding this is important to develop a strategy for detecting precancerous lesions earlier in order to reduce the incidence of gastric cancer and death from gastric cancer.

Method

2.1 Study Design

The design of this study was a cross-sectional study with independent variables such as histopathological severity of gastritis (degree of lymphocyte infiltration, degree of neutrophil activity, degree of atrophy, and degree of intestinal metaplasia) and the dependent variable was serum HIF-1α levels.This study was conducted at the Endoscopic Unit of H. Adam Malik General Hospital in Medan after obtaining approval from the Medical Research Committee for Health and related institutions. This research was conducted from March 2019 to August 2019.

The sample of this study is an affordable population that meets the inclusion and exclusion criteria taken by consecutive sampling, where all samples that meet the criteria for acceptance and rejection are included in the sample until the number of samples is met. The inclusion criteria are : Age ≥ 18 years old, gastritis patients and receive voluntary and written information and participation approval for a physical examination, endoscopy and biopsy of gastric tissue that is known and approved by the Health Ethics Research Committee. The exclusion criteria are : the patient is not cooperative, pregnant, has a systemic disease and malignancy, have H.pylori eradication therapy in the last 6 months, take antibiotics in the last 2 weeks, and take PPI class drugs in the last 2 weeks.

2.2 Statistical Analysis

All data were analyzed with statistical software SPSS 22.0 using univariate, bivariate and multivariate analysis with 95% confidence interval. Univariate analysis to determine the percentage of chronic inflammatory degrees, neutrophil activity, atrophy, and intestinal metaplasia that is normal, mild, moderate, and severe. Bivariate analysis to analyze the association of serum HIF-1α levels with gastritis severity (normal, mild, moderate, severe). The normality of the data was determined using the Kolmogorov-Smirnov test. Statistical analysis in this study using independent T test, but if the data is not normally distributed, the data is analyzed using the Mann Whitney test.

Results

Demographics of Respondents

There were 80 subjects of this study, consisted of 49 males (61,3%) and 31 females (38,8%). Batak is the most ethnic group of research subjects with 46 people (57,5%), the other are Javanese 28 people (35%) and Aceh 6 people (7.5 %). Majority of the subject's education were senior high school with a total of 41 people (51.3%), then junior high school 20 people (25%), university 16 people (20%) and elementary school 3 people (3.8%). Most of the subject are employee (43,8%), then entrepreneur (27,5%, housewives (22,5%), and civil government employees (6,3%). The mean of Body Mass Index of th subjects is 23,36 kg/m2. The median of HIF serum level is 62,5 with minimum level is 34 and maximum level is 119 pg / ml.

Tabel 1. Characteristics of Subjects

Characteristic	Frequency (n=80)	Percent (%)
Sex		
Male	49	61,3
Female	31	38,8
Ethnic		
Batak	46	57,5
Javanese	28	35
Aceh	6	7,5
Occupation		
Employee	35	43,8
Housewife	18	22,5
Enterpriser	22	27,5
Government employees	5	6,3
Education		
Primary school	3	3,8
Junior high school	20	25,0
Senior high school	41	51,3
University	16	20,0
BMI, mean (SD), kg/m2		23,36
HIF (pg / ml), median (min-max),		62,5 (34-119)

Gastritis Degrees According to Updated Sydney System

From the histopathological results of gastric mucosal biopsy specimens, lymphocyte infiltration was present in all subjects (100%), most of which had mild lymphocyte infiltration (55%), followed by moderate lymphocyte infiltration (32,5%), and severe lymphocyte infiltration (12,5%). In a total of 58,7% of specimens with neutrophil infiltration, 37,5% were included in mild infiltration degrees and 17.5% moderate degrees, and 3.8% severe degrees. Of the 16.2% specimens with atrophy, the degree of mild atrophy was 11,2%, and the degree of moderate atrophy was 5%. While from 5% of specimens with intestinal metaplasia, all were with mild metaplasia.

Table 2. Lymphocyte Infiltration Degree, Neutrophil Activity, Atrophy, and Intestinal Metaplasia

Variable	Gastritis Severity Degrees				Total
	0 (Normal)	**1 (Mild)**	**2 (Moderate)**	**3 (Severe)**	
Lymphocyte	0 (0%)	44 (55,0%)	26 (32,5%)	10 (12,5%)	80 (100%)
Neutrophil	33 (41,3%)	30 (37,5%)	14 (17,5%)	3 (3,8%)	80 (100%)
Atrophy	67 (83,8%)	9 (11,2%)	4 (5,0%)	0 (0%)	80 (100%)
Metaplasia	76 (95%)	4 (5%)	0 (0%)	0 (0%)	80 (100%)

From table 3 we can see that HIF levels are higher in moderate + severe lymphocyte infiltration than normal + mild degrees. HIF levels are higher in moderate + severe neutrophil activity than normal + mild degrees. HIF

levels are higher in moderate + severe atrophy than normal + mild degrees. HIF levels are higher in mild metaplasia degrees than without metaplasia.

Table 3. Association between HIF Levels and Normal + Mild, moderate + severe Gastritis Degrees

Gastritis Severity Degrees	Median	Min-Max	p
Lymphocyte			
Normal+Mild	56,00	34 – 112	0,045*
Moderate+Severe	66,00	36 – 119	
Neutrophil			
Normal+Mild	56,00	34 – 97	<0,001*
Moderate+Severe	96,50	54 – 119	
Atrophy			
Normal+Mild	58,00	34 – 104	0,001*
Moderate+Severe	112,00	75 – 119	
Metaplasia			
Normal	58,00	34 – 106	0,001*
Mild	113,00	104 – 119	

Significant association was found between the degree of lymphocyte infiltration and HIF levels ($p = 0.045$). There was a significant association between the degree of neutrophil activity with HIF levels ($p < 0.001$). There was a significant association between the degree of atrophy with HIF levels ($p = 0.001$). There was a significant association between the degree of intestinal metaplasia with HIF levels ($p = 0.001$).

Discussion

From the results of the study, it was found that the number of men suffering from gastritis was greater than women namely 49 (61,3%) and 31 (38,8%). This result is in accordance with previous studies. Fareed et al and Mustapha et al also stated that in their study chronic gastritis was more dominated by male sex.[6,7] Sultana et al reported that among 105 cases of chronic gastritis, 72.3% were male and 27.6% were female.[8] A study in India also reported a comparison of men and women with gastritis was 2.1: 1.[9]Choudhury et al reported that the incidence of chronic gastritis was more common in men than women in a ratio of 1.85: 1. The increased incidence of chronic gastritis in men may be caused by higher stomach acid production and stress factors.[10]

All study subjects (100%) experienced chronic inflammation in which all specimens were found to have mild, moderate and severe lymphocyte infiltration. Most had mild lymphocyte infiltration (55%), followed by moderate lymphocyte infiltration (32,5%), and severe lymphocyte infiltration (12.5%). While neutrophil infiltration was 58,7%, atrophy 16.2%, and metaplasia 5%. These results are consistent with the study of Kalebi et al who reported that the most commonhistopathological picture of gastritis in Kenya is chronic inflammation characterized by lymphocyte infiltration, which is as much as 93%, followed by neutrophil activity as much as 86%,glandular atrophy as muchas 15%, and metaplasia as much as 2%.[11] Garg et al. Also reported that chronic inflammatory conditions were the most common histopathological presentation, in 100% of subjects, followed by neutrophil infiltration of 33.33%, atrophy at 12.33%, and intestinal metaplasia in 7%.[9]This result is also in accordance with the research of Siregar et al (2018) who found that most of the histopathological features of gastritis in Medan were lymphocyte infiltration, followed by neutrophil, atrophy, and metaplasia.[12]

There is a significant relationship between the degree of lymphocyte infiltration with HIF-1α levels ($p = 0.023$). There was a significant relationship between the degree of neutrophil activity with HIF-1α levels ($p < 0.001$). This is consistent with the results of previous studies. Ling et al (2009) found that the expression of HIF-1α increased significantly in the state of active inflammation and chronic inflammation. Inflammatory tissue is characterized by low oxygen and glucose levels, but is also followed by high levels of lactate and other residual metabolites. To maintain viability and function, cells must adjust their metabolic pathways. HIF-1α regulates

inflammatory reactions by regulating the pathways needed for energy homeostasis in cells and creating well-oxygenated surrounding environments.[13]

There was a significant relationship between the degree of atrophy with HIF-1α levels (p = 0.001). There was a significant relationship between the degree of intestinal metaplasia with HIF-1α levels (p = 0.001). These results are consistent with the results of research by Griffith et al (2007) which says that the expression of HIF-1α increases in areas experiencing metaplasia.[5] Atrophic gastritis, intestinal metaplasia, and dysplasia are histological premalignant lesions that are considered as cascading precursors of the development of gastric cancer.[14]

Gastric carcinogenesis develops from a gradual process, starting from chronic gastritis through atrophic gastritis, intestinal metaplasia, and dysplasia before becoming cancer.[15] Zhang et al and Jiang et al found that HIF-1α regulation was very likely involved in the pathogenesis of gastric cancer. Excessive expression of HIF-1α in gastric tumor tissue triggers the expression of abnormal genes that support the development of cancer. In that study it was found that the level of HIF-1α expression was significantly higher in tumor tissue than in adjacent normal tissue.[16,17] In addition, serum HIF-1α levels are also significantly higher in gastric cancer patients than normal healthy people.[16]

HIF-1α has a key role for tumor cells to produce energy to maintain metabolism. High levels of HIF-1α trigger the process of angiogenesis, cell proliferation, progression and resistance of tumor tissue through growth promoter regulation, oncogenes, glycolytic pathways and pH regulation.[18]HIF-1α has been shown to be overexpressed in various types of cancer, including ovarian, breast, uterine, cervical, and oropharyngeal cancers. Overexpression often positively leads to a poor prognosis. Jiang et al also revealed that the survival time of patients with gastric cancer with high HIF-1α levels was shorter than patients with lower HIF-1α expression. In addition, increased expression of HIF-1α also correlates with tumor size, TNM stage, and depth of tumor invasion.[17]

Conclusion

Based on the results and discussion in this study, it can be concluded that moderate + severe neutrophils, lymphocyte infiltration and atrophy is associated with higher HIF-1α levels than normal + mild degree. HIF-1α levels of mild metaplasia is higher than gastritis with no metaplasia. Significant association was found between histopathological features of gastrits and serum HIF-1α levels.

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