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Degree of neutrophil, atrophy, and metaplasia intestinal were associated with malondialdehyde level in gastritis patients

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Abstract. The main pathogenesis of gastritis is inflammation that closely related to free radicals. Malondialdehyde (MDA) is a free radical biomarker and is found to increase in gastritis patients. However, these studies are generally performed on experimental animals as well as MDA examination in gastric mucosa. This study aim was to determine the association of degrees of gastritis (degree of lymphocyte infiltration, neutrophil activity, atrophy, and intestinal metaplasia) with plasma MDA level. A cross-sectional study of 80 consecutive gastritis patients who came to an endoscopic unit of Adam Malik General Hospital in Medan, Indonesia, from May–September 2017. Assessed for severity of chronic inflammatory, neutrophil activity, atrophy, and intestinal metaplasia refers to Updated Sydney System. Plasma MDA levels were examined using an HPLC MDA kit. Univariate analysis, bivariate (chi-square and Fisher exact test), and multivariate (binary logistic regression test) were programmed with SPSS version 22. There was no significant association between degree of lymphocyte infiltration with MDA level. There were significant associations between degree of neutrophil activity, atrophy, and intestinal metaplasia with MDA level (p=0.039, 0.003, 0.021; respectively). The moderate-severe degree of neutrophil activity, atrophy, and intestinal metaplasia were associated with high level of MDA.

1. Introduction

Main pathogenesis of gastritis is inflammation that closely related to free radicals. Significant numbers of neutrophils and/or macrophages that infiltrate gastric mucosa during inflammation generate large amounts of reactive oxygen species (ROS). Neutrophils produce superoxide radical anion (O₂⁻), which belongs to a group of ROS. Superoxide radical anion reacts with cellular lipids, leading to the formation of lipid peroxides, that are metabolized to malondialdehyde (MDA). This end product of lipid peroxidation, MDA, has been suggested to act as a tumor promoter and a co-carcinogenic agent. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage.[1]

MDA level in the gastric mucosa is generally investigated regarding its association with degree of gastritis in many previous studies. Studies that evaluated plasma MDA concentrations usually focused on gastric malignancy patients. The aim of this study was to determine the association of gender, age,
overweight, gastritis degrees (degree of lymphocyte infiltration, neutrophil activity, atrophy, and intestinal metaplasia) with plasma MDA level.

2. Methods

2.1. Patient Selection
This was a cross-sectional study of eighty consecutive gastritis patients admitted to endoscopy unit at Adam Malik General Hospital in Medan, Indonesia from May until September 2017. Inclusion criteria are male or female aged ≥18 years old, diagnosed with gastritis on endoscopy and histopathologic examination, willing to be recruited in this study and signed the patient consent forms. Exclusion criteria were patients with systemic disease and malignancy. All patients have given informed consent, and the study was approved by the local ethics committee.

2.2. Histological Assessment of Gastritis
All patients underwent endoscopy examination, and asample of gastric corpus and antrum were taken by biopsy. The tissues then stained using Hematoxylin-Eosin. The degree of chronic inflammation, neutrophil infiltration, atrophy, and intestinal metaplasia was evaluated refer to the visual analog scale of the updated Sydney System.[2] The higher degree was used if differences of degree were found between gastric body and antrum.

2.3. Detection of Plasma MDA Level
High performance liquid chromatographic (HPLC) analysis was performed with the isocratic method using an Agilent 1200 HPLC system (San Jose, CA, USA) with a commercial MDA kit (Immunodiagnostic AG, Bensheim, Germany).[3] The levels above the mean were classified as high levels, and the levels below the mean were categorized as low levels.

2.4. Statistical Methods
SPSS version 22 (SPSS Inc., Chicago) was used for the analysis. The data were analyzed using univariate, bivariate (Fisher’s exact test, chi-square test), and logistic regression with 95% confidence interval. P<0.05 was considered statistically significant.

3. Results
A total of 54 patients (67.5%) were men with an average age of 49.4 years old (table 1).

Table 1. Basic characteristics of the subjects.

| Characteristics | n = 80 |
|-----------------|-------|
| Sex, n(%)       |       |
| Male            | 54 (67.5%) |
| Female          | 26 (32.5%) |
| Age (years) a   | 49.4±12.15 |
| Ethnic          |       |
| Batak           | 46 (57.5%) |
| Javanese        | 24 (30%) |
| Acehnese        | 10 (12.5%) |
| Occupation, n (%) |       |
| Entrepreneur    | 34 (42.5%) |
| Housewife       | 24 (30%) |
| Employee        | 20 (25%) |
| Farmer          | 2 (2.5%) |
| BMI (kg/m²) b   | 22.18±3.27 |
| Plasma MDA (µmol/L) b | 1.43 ± 0.31 |
Previous studies have shown different results. Garg et al. reported that there were chronic gastritis with high MDA level (p<0.05). There were no significant associations between gender and age with MDA levels (p>0.05) (table 2).

**Table 2.** Association between gender, age, overweight, the degree of gastritis with MDA level.

| Variable                  | MDA High | MDA Low | Total | P     | OR (95% CI) |
|---------------------------|----------|---------|-------|-------|-------------|
| Gender                    |          |         |       |       |             |
| Male                      | 27 (50%) | 27 (50%)| 54 (100%)| 0.575| 3.33 (0.47-11.81) |
| Female                    | 6 (23.1%)| 20 (76.9%)| 26 (100%)|     |             |
| Age                       |          |         |       |       |             |
| ≥50 years                 | 16 (42.1%)| 22 (57.9%)| 38 (100%)| 1.000| 1.07 (0.23-4.37) |
| <50 years                 | 17 (40.5%)| 25 (59.5%)| 42 (100%)|     |             |
| Overweight                |          |         |       |       |             |
| Yes                       | 13 (54.2%)| 11 (45.8%)| 24 (100%)| 0.039 | 2.12 (1.98-8.14) |
| No                        | 20(35.7%) | 36 (64.3%)| 56 (100%)|     |             |
| Lymphocyte                |          |         |       |       |             |
| Normal+ Mild              | 18 (34.6%)| 34 (65.4%)| 52 (100%)| 0.032 | 2.18 (1.52-9.52) |
| Moderate+ Severe          | 15 (53.6%)| 13 (46.4%)| 28 (100%)|     |             |
| Neutrophil                |          |         |       |       |             |
| Normal+ Mild              | 23 (35.9%)| 41 (64.1%)| 64 (100%)| 0.002 | 2.97 (2.14-18.74) |
| Moderate+ Severe          | 10 (62.5%)| 6 (37.5%) | 16 (100%)|     |             |
| Atrophy                   |          |         |       |       |             |
| Normal + Mild             | 23 (33.8%)| 45 (66.2%)| 68 (100%)| <0.001| 9.78 (3.4 – 37.65) |
| Moderate+ Severe          | 10 (83.3%)| 2 (16.7%) | 12 (100%)|     |             |
| Intestinal Metaplasia     |          |         |       |       |             |
| Normal+ Mild              | 28 (37.8%)| 46 (62.2%)| 74 (100%)| 0.001 | 8.21 (3.76-24) |
| Moderate+ Severe          | 5 (83.3%) | 1 (16.7%) | 6 (100%) |     |             |

*p<0.05

There were significant associations between degree of atrophy, intestinal metaplasia, neutrophil activity with high MDA level (p<0.05). There were no significant associations between degree of lymphocytes infiltration and overweight with MDA level (p>0.05) (table 3).

**Table 3.** Multivariate analysis of factors associated with high level of MDA.

| Variable                  | P        | OR (95% CI) |
|---------------------------|----------|-------------|
| Atrophy                   | 0.003*   | 7.026 (1.348-26.625) |
| Intestinal metaplasia     | 0.021*   | 4.294 (1.215-10.326) |
| Neutrophil Activity       | 0.039*   | 1.628 (1.085-4.726)  |
| Lymphocyte Infiltration   | 0.215    | 1.089 (1.030-1.151)  |
| Overweight                | 0.799    | 1.006 (0.962-1.051)  |

*p<0.05

4. Discussion

All patients in this study had chronic gastric inflammation with mild, moderate, and severe lymphocyte infiltration. Sixty-five percent had mild lymphocyte infiltration, followed by moderate lymphocyte infiltration (28.75%), and severe lymphocyte infiltration (6.25%). Neutrophil infiltration, atrophy, and intestinal metaplasia were found in 47.5%, 25%, and 25% specimens respectively. Previous studies have shown different results. Garg et al. reported that there were chronic...
inflammations in 100% of subjects, neutrophil infiltration in 33.33%, atrophy in 12.33%, and intestinal metaplasia in 7%. [4] Zhang et al. reported chronic inflammation in 90.3% specimens, neutrophil infiltration in 56.2% specimens, atrophy in 36.8% specimens, and intestinal metaplasia in 37% specimens. [5] Choudhury et al. found chronic inflammation and neutrophil infiltration in 100% of specimens, atrophy and intestinal metaplasia in 19.23% of specimens. [6]

There was no association between gender and MDA level (p=0.575). This result was supported by previous studies. Chole et al. reported that higher MDA levels in healthy women (4.79 nmol/ml) than in healthy men (3.77 nmol/ml) but not significantly different (p>0.05). [7] Beg et al. showed there was no significant difference in MDA levels between men and women (1.9±0.08 vs. 1.9±0.1). [8]

There was no association between age and MDA level (p=1.000). This result was in accordance with previous research. There was no significant correlation between MDA level and age either in male or female. [9] In an earlier study about the association of MDA level and aging, found that MDA levels were higher in older people but not significantly different. [10]

Hamma et al. reported that MDA level was positively correlated with BMI (r=0.149, p<0.05) in 187 healthy people in Constantine, Algeria. MDA increase significantly in obese patients. There was no significant difference in MDA levels between overweight and normal BMI. Increased lipid peroxidation in obese individuals reflects significant oxidative stress as one of the complications related to obesity. [11] From multivariate analysis, there was no significant association between overweight and MDA level, so overweight became a weak or non-dominant variable in influencing high level of MDA.

Under normal circumstances, free radicals will be formed in a small quantity. Free radicals then will be reduced by endogenous antioxidants (reduced glutathione, superoxide dismutase, and catalase) as a compensatory mechanism to prevent further tissue damage. Recruitment of phagocyte cells in gastritis will induce an increase in free radicals. Anion superoxide radicals (O₂⁻) are generated by neutrophil infiltration to cellular lipid membranes that lead to lipid peroxidation formation that is metabolized into MDA. [12] These lipid peroxidation reactions by damaging cell membranes that cause the release of intracellular components such as lysosomal enzymes cause further tissue damage and will cause degradation of the epithelial basement membrane, disrupt cell metabolism, and MDA reactions with DNA from the mutagenic malondialdehyde deoxyguanosine (Mi-dG). [13-16]

Turkkan et al. (2009) conducted a study of 35 dyspeptic patients undergoing endoscopy proving that MDA levels of gastric mucosa were significantly higher in chronic inflammatory degrees (p=0.04). [17] Result from Turkkan et al. was in agreement with the results of bivariate analysis in this study that there was a significant association between MDA level with lymphocyte degree, but the relationship was insignificant in multivariate test. This suggests that lymphocytes are weak inflammatory cells to induce expression of MDA. Everett et al. found statistically weak associations between MDA level and inflammatory scores of lymphocytes may be suspected because lymphocytes are less significant source of ROS formation. [18]

There was a significant association between degree of neutrophil and MDA level on bivariate analysis, and remained significant through multivariate analysis. Gastritis patients with moderate or severe neutrophil activity at risk of 1.63 times had high level of MDA compared to normal+low grade neutrophil activity. These results supported previous theories. The presence of neutrophil infiltration to cellular lipid membranes, which leads to the formation of lipid peroxidation, metabolized into MDA. Everett et al. analyzed MDA levels in gastric mucosa, they obtained MDA concentrations in gastric mucosa positively correlated with neutrophil infiltration in gastric antrum, where higher mucosal gastric MDA levels, the greater the infiltration of neutrophils in gastric antrum (r=0.32; p=0.05). [18] Demir et al. reported that high levels of gastric mucosal MDA may indicate increased neutrophil activity in patients with peptic ulcer and gastritis. [1]

There was a significant association between degree of atrophy and MDA level, where moderate and severe atrophy at risk of 7.03 times had high MDA level compared to normal+low-grade atrophy. There was a significant association between degree of intestinal metaplasia and MDA level, where moderate-severe intestinal metaplasia at risk of 4.29 times had high MDA level compared to...
normal+low-grade intestinal metaplasia. Chronic atrophic gastritis, intestinal metaplasia, and dysplasia are included in premalignant gastric lesions, but only atrophy and intestinal metaplasia are included in the assessment of gastric degrees according to the Updated Sydney System, which is the degree of gastritis accepted universally by the Anatomical Pathologist in the world.[2] The risk of having higher MDA levels was greater in patients with both moderate+severe atrophy and intestinal metaplasia compared with moderate+severe neutrophil activity. This indicated that elevated MDA level was important parameter for detecting severe tissue damage, and it has been reported from previous studies that MDA levels were associated with mutagenesis and carcinogenesis.[19]

5. Conclusion
There were significant associations between moderate+severe degree of neutrophil activity, atrophy, and intestinal metaplasia with a high level of MDA.

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