Oxidative Stress and the Severity of Gastroesophageal Reflux Disease: Is There Any Correlation?

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ABSTRACT

Background: *Oxidative stress plays a crucial role in the inflammatory response of the oesophageal epithelium; hence, therapies that affect oxidative stress may be a new approach in GERD management. The objective is to analyze and determine the correlation between Total Oxidant Status (TOS), Total Antioxidant Status (TAS), and Oxidative Stress Index (OSI) with severity of GERD.*

Methods: *This is an observational analytic study using a cross-sectional design,that conducted at the Endoscopic Unit of three hospitals in Surabaya, which held from June 2020 to March 2021. The 18 to 60 years old subjects who meet the GERD-Q score 8-18 and not meet the exclusion criteria undergo an endoscopic examination to determine the GERD severity and blood test. Data analysis was using the Spearman rank correlation test.*

Results: *This study indicates that most subjects are women, with mean age 39.31±10.99 years, most are GERD grade A, the mean value of TOS, TAS, ISO respectively is 5.18±1.60, 1.58±0.18, and 3.36±1.35. The laboratory test results show insignificant different based on GERD degree. There is a non-significant positive correlation between TOS and GERD severity, a significant negative correlation between TAS and GERD severity, and a significant positive correlation between OSI and GERD severity (respectively, r = 0.121, p = 0.266 vs r = -0.323, p = 0.044 vs r = 0.345, p = 0.033).*

Conclusion: *The TAS and OSI may be correlated to the GERD severity but still need further investigations.*

Keywords: *Gastroesophageal reflux disease, Oxidative Stress Index, Total Oxidant Status, Total Antioxidant Status*

ABSTRAK

Latar Belakang: *Stres oksidatif berperan penting dalam respons inflamasi epitel esofagus. Oleh karena itu, terapi yang memengaruhi stres oksidatif mungkin menjadi pendekatan baru dalam manajemen GERD. Tujuannya adalah untuk menganalisis dan menentukan korelasi antara Status Oxidant Total (SOT), Status Antioxidant Total (SAT), dan Indeks Stres Oksidatif (ISO) dengan tingkat keparahan GERD.*

Metode: *Ini adalah studi analitik observasional menggunakan desain potong lintang. Studi ini telah dilakukan di Unit Endoskopi di tiga rumah sakit di Surabaya yang dilaksanakan mulai Juni 2020 hingga Maret 2021. Subjek berusia 18 hingga 60 tahun yang memenuhi skor GERD-Q 8-18 dan tidak memenuhi kriteria eksklusi menjalani endoskopi untuk menentukan derajat GERD dan tes darah. Analisis data menggunakan uji korelasi peringkat Spearman.*

Hasil: *Studi ini menunjukkan bahwa sebagian besar subjek adalah wanita, dengan rata-rata usia 39,31±10,99 tahun, sebagian besar derajat A, nilai rata-rata TOS, TAS, ISO masing-masing adalah 5,18±1,60, 1,58±0,18, dan 3,36±1,35. Hasil tes laboratorium menunjukkan perbedaan tidak signifikan berdasarkan derajat GERD. Ada korelasi positif yang tidak signifikan antara TOS dan keparahan GERD, korelasi negatif yang signifikan antara TAS dan keparahan GERD, dan korelasi positif yang signifikan antara OSI dan keparahan GERD (masing-masing, r = 0,121, p = 0,266 vs r = -0,323, p = 0,044 vs r = 0,345, p = 0,033).*

Simpulan: *TAS dan OSI mungkin terkait dengan tingkat keparahan GERD tetapi masih memerlukan penyelidikan lebih lanjut.*

Kata Kunci: *Penyakit refluks gastroesofageal, Indeks Stres Oksidatif, Status Oksidan Total, Status Antioksidan Total*

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a condition of gastric contents refluxing into the oesophagus and causes inconvenient symptoms, leading to complications[1]. This disease is a common cause of upper gastrointestinal disorders that have three phenotypic presentations, namely non-erosive reflux disease (NERD), erosive esophagitis (EE), and Barrett oesophagus[2,3,4]. This disease has been a global burden[2,3] as worldwide prevalence estimates range from 8-33%[2], with a global prevalence of 13.98%[2] In Asian countries, including Indonesia, GERD and following complications are generally lower than in Western countries; however, recent data suggest its prevalence increases. There was a rise in GERD prevalence in Southeast Asia based on symptoms from 6.3% - 18.3% during 2005 – 20102[1]. In Indonesia, the number of survivors of esophagitis increased from 5.7% in 1997 to 25.18% in 2002, with an average of 13.13% per year.

The study of Imroati et al. (2019) at Dr Soetomo General Academic Hospital Surabaya showed that from 4058 upper endoscopies for three years (2016-2018), 424 patients with GERD were obtained, with an average of 10.45% per year. The study showed more women than men (52.36% vs 47.64%), with the highest age between 45-60 years. The youngest was 16 years, the oldest was 93 years, and the average age was 49.64±14, 44 years. This study showed that the most findings were being a grade A esophagitis (Los Angeles classification). Risk factors that may be associated include the use of NSAID medication, diabetes, obesity, hiatal hernia, and other factors related to herbal drugs, alcohol, cirrhosis hepatitis, hypertension, peptic ulcer disease, and others (data not yet published). The pathogenesis of oesophagal reflux is very complex, include hyperacidity, abdominal pressure, food, drugs, and anatomical factors. High gastric acid will activate macrophages, Proteinase-Activated Receptor 2 (PAR 2), Platelet-Activating Factor (PAF), and stimulate oesophagal epithelial cells to produce radical oxygen species (ROS) and cause oxidative stress. The oxidative stress will facilitate the inflammatory process, which eventually causes mucosal damage.

The role of oxidative stress (OS) in oesophageal mucosal injury has been interesting among researchers. Oxidative stress plays a crucial role in the inflammatory response of the oesophageal epithelium; hence, therapies that affect oxidative stress may be a new approach in GERD management, especially in patients with persistent symptoms despite receiving adequate treatment. Several studies on oxidative stress in GERD have been carried out using oesophageal tissue; however, study about the expression of oxidants and tissue antioxidants has not been examined in Indonesia. The OS condition can be seen from blood serum examination by the presence of increased total oxidant status (TOS), decreased total antioxidant status (TAS), and increased oxidative stress index (OSI). The low total antioxidant capacity may indicate oxidative stress or increase susceptibility to oxidative damage. The collaboration between antioxidants results in more excellent protection against reactive oxygen species (ROS) and reactive nitrogen species (RNS) attacks than each component. Then, it is essential to examine the

total antioxidant capacity (15). The SO parameters such as TOS, TAS, and OSI have been studied in breast and thyroid cancer (16), while research on GERD is still minimal. This study aimed to analyze and determine the correlation between total oxidant status, total antioxidant status and oxidative stress index with the degree of gastroesophageal reflux disease severity.

METHODS

An observational analytic study with a cross-sectional design was conducted in the Endoscopic Unit of the Gastroentero-Hepatology Division of the Department of Internal Medicine at RSUD Dr Soetomo, the Integrated Endoscopy Service Center at Airlangga University Hospital and Darmo Hospital Surabaya from June 2020 to March 2021. This study has obtained ethical clearance from the Ethical Committee of Health Research Dr Soetomo Hospital Surabaya, Indonesia (2025/KEPK/VII/2020). The GERD severity includes non-erosive reflux disease (NERD) if there is no erosion/inflammation from endoscopic examination and esophagitis if there is erosion or mucosal break. The grade of erosion was differentiated according to Los Angeles criteria, which was divided into four grades. Grade A when there is one (or more) mucosal break of not more than 5 mm that does not extend between the crests of the two mucosal folds. Grade B is one (or more) mucosal break of more than 5 mm that does not grow between the crests of the two mucosal folds. Grade C if there is one (or more) mucosal break that continues between the crests of 2 or more mucosal folds but is less than 75% of the esophageal circumference. Grade D is one (or more) mucosal breaks involving more than 75% of the esophageal circumference.

Population and Sample

The population was patients with gastroesophageal reflux disease who will undergo endoscopy at the location of study. At the same time, consecutive sampling was an approach to recruited samples. The inclusion criteria included patients aged 18-60 years old; the GERD questionnaire scored 8 to 18; endoscopy results showed NERD or esophagitis; willing to sign Informed Consent. Meanwhile, the exclusion criteria were patients with chronic disease, people with infection, people indicating obesity and pregnancy, have contraindications to endoscopy, consuming long-term NSAIDs, antioxidant supplements, alcohol, and smoking.

Patient and Public Involvement

This study included 29 patients with GERD as the subject of research, which is the total sample obtained during the duration of the study. The researchers had conducted the study following relevant guidelines and regulations**.** All participants had signed the informed consent for this study. Patients involved in this study were as objects to be observed and collected the needed samples of blood.

Procedures

A gastroenterologist carried out the endoscopic examination from the Gastroenterology-Hepatology Division of the Internal Medicine Faculty of Airlangga University-Dr. Soetomo Surabaya. The endoscope used is Olympus (Olympus Optical Co. Ltd. Japan) and was sterilized with a 2% Activated Glutaraldehyde (Cidex) solution.

The patients were given a local anaesthetic combined with 10% Xylocaine which was sprayed into the oesophagus. Prior to blood samples, patients had to fast for at least six hours before the endoscopy procedure. If the patients were restless, an intravenous injection of Midazolam 1 mg (1 ml) was given, or sedation with propofol was administered under anaesthesia.

Total Oxidant Status (TOS) Procedure

Venous blood was taken in a tube and stored at four °C; then, the serum was separated by centrifugation at 3000 rpm for 10 minutes. The serum was stored at -80°C until it was ready to be used (it can last up to 1 year). When all samples have been collected, Reagents 1 and 2 are prepared. The first reagent was a buffer solution containing H_2SO_4 with a concentration of 25 mM and a pH of 1.75. Meanwhile, the second reagent was a substrate solution consisting of 25 mM H_2SO_4, pH 1.75, 5 mM ferrous ion, and 10 nM o-dianisidine. The calibration standard was H_2O_2 10 mol/L. Total oxidant status was measured by the Rail Assay Diagnostics method (Prodia Laboratory) with a typical value range of 4-6 mol/L (400-600 mmol/hL).

Total Antioxidant Status (TAS) Procedure

The procedure was quite similar to the TOS test, and the difference was about the reagent used. In this TAS test, the serum was poured into the three reagents:

(1) a buffer solution containing phosphate-buffered saline with a concentration of 80 mmol/L and a pH of 7.4,

(2) a chromogen containing metmyoglobin 6.1 mol/L and ABTS® (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) 610 mol/L,

(3) a substrate containing hydrogen peroxide (in a stable form) with a concentration of 250 mol/L. The calibration standard used 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid with a concentration according to lots. Total antioxidant status/total antioxidant capacity was measured using the Randox method (Prodia Laboratory) with an average value range of 1.30-1.77 mmol/L.

Oxidative Stress Index (ISO) Calculation Procedure

The ratio of total oxidant status levels to total antioxidant capacity has been accepted as an oxidative stress index. Oxidative stress index value is calculated based on the formula below:

$$\text{OSI (}\textit{arbitrary unit}\text{)} = \frac{\text{TOS (μmol } H_2O_2 \text{ Equiv./L)}}{\text{AS (mmol/L)}}$$

Data Analysis

To determine the relationship between total antioxidant status, total oxidant status, oxidative stress index and the degree of gastroesophageal reflux disease severity, the Spearman rank correlation test using SPSS software version 20.0 (SPSS.Inc., Chicago, IL) was applied. The Kappa Cohen consistency test was conducted to assess the consistency of the determination of GERD severity. The *p*-value <0.05 indicates a significant difference.

RESULTS

Characteristics of Subjects

The disparity of gender between subjects was relatively small, in which female was 16 (55.2%) subjects, and male consisted of 13 (44.8%) subjects, with a mean age of 39.31±10.99 years. According to the data, the grade A of GERD was more likely experienced by subjects (79.3%) than the non-erosive disease (17.2%) and grade B (3.4%). The female subjects were more likely to diagnose non-erosive reflux disease and grade B of GERD than men who experienced more to the grade A.

The TOS value obtained an average of 5.18 mol/L, while the TAS value gained approximately 1.58 mmol/L, both of which were included in the normal limit. Meanwhile, the OSI value reached an average of 3.36 with a standard deviation of 1.35. Generally, individual factors, such as age, body mass index (BMI), systolic blood pressure, diastolic blood pressure, leukocytes, gra nulocytes, random blood sugar and albumin, did not show significant differences (p>0.05) neither in males nor in female patients. Meanwhile, for the profile of Hb levels, erythrocyte sedimentation rate (ESR), high-density lipoprotein (HDL) and uric acid were concluded to have significant differences (p<0.05) both in male and female patients. Other results for the profile of TAS, TOS and OSI values have no significant difference (p>0.05) between male and female patients.

Table 1. Profile of Subjects Based on Gender

Profile	Male (n=13)			Female (n=16)			p
	%	Median (Range)	Average±SD	%	Median (Range)	Average±SD	
GERD severity (n=29)							
NERD*	3.45			13.79			
Grade A	41.38			37.93			
Grade B	0			3.45			
Age		-	42.9±11		-	36.4±10.5	0.120
BMI*		-	23.1±3.5		-	23.7±3.6	0.779
SBP		-	113.9±12.9		-	114.3±14.4	0.938
DBP		-	71.9±9.8		-	75.8±9.8	0.305
Haemoglobin		-	14.5±0.7		-	12.8±1.3	0.000
Leucocytes		-	7.1±1.7		-	7.7±2.7	0.483
Granulocytes		-	55.0±12.4			63.0±11.0	0.095
ESR*		6 (3-28)	-		21 (5-32)	-	0.013
Random Blood Sugar		94 (78-172)	-		103 (76-185)	-	0.351
Albumin		4.5 (4.1-4.8)	-		4.4 (4.0-4.6)	-	0.232
HDL*		-	40.4±8.2		-	50.0±8.5	0.005
Uric acid		-	5.8±1.1		-	4.7±1.3	0.031
TAS*		-	1.6±0.2		-	1.5±0.2	0.066
TOS*		4.7 (3.4-9.9)	-		4.8 (3.2-10.3)	-	0.559
OSI*		3.1 (2.4-6.0)	-		3.6 (2.2-8.9)	-	0.215

x* NERD, non-erosive reflux disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index.

Profile of Subjects Based on the GERD Severity

Interpretation of endoscopy results to determine the degree of GERD was carried out by two gastroenterohepatologists. Kappa Cohen analysis of the first and second expert interpretation results for consistency obtained a value of 0.824 (p = 0.000). Referring to these results, the consistency of the measurement can be stated as good. In general, the research profile did not show a significant difference at each level of GERD, except for the profile of systolic and diastolic blood pressure values, which showed significant differences in which the most considerable value was in the degree of GERD severity. Diastolic blood pressure was the highest in grade B.

Distribution of TOS, TAS and OSI by GERD Severity

The comparison analysis results were carried out using the non-parametric Kruskal Wallis test method because the number of samples in each group was very small. The results of the comparison analysis of SAT, SOT and ISO values based on the degree of GERD concluded that there were no significant differences in values.

Analysis of the Correlation between TOS, TAS and OSI with the GERD Severity

The correlation between the TAS value and the GERD severity obtained a coefficient of -0.323, which indicates a negative relationship. This correlation means that if the TAS value is higher, the GERD severity will be less severe. The p-value for the correlation between TAS and GERD was 0.044, which was smaller than 0.05, then it means the negative correlation between TAS and GERD was significant.

The correlation between the TOS value and the GERD severity obtained a coefficient of 0.121, indicating a positive relationship. This correlation means that the GERD severity will also be more severe if the TOS value is higher. As the p-value for the correlation between TOS and GERD is 0.266, it can be concluded that the positive correlation between TOS and GERD is not significant.

The correlation between the OSI value and the GERD severity obtained a coefficient of 0.345, indicating a positive relationship. This correlation means that the GERD severity will also be more severe if the OSI value is higher. The p-value for the correlation between OSI and GERD reaches 0.03; it can be concluded that the positive correlation between OSI and GERD is significant.

Table 2. Profile of Subjects based on GERD Severity

Profile	NERD*	Grade A	Grade B	p
	Median (Range)	Median (Range)	Median (Range)	
Age	45.0 (20-49)	38 (18-57)	36 (36-36)	0.783
BMI*	24.9 (21.5-26.8)	23.0 (13.3-29.1)	20.8 (20.8-20.8)	0.224
SBP*	132 (121-133)	108 (85-138)	122 (122-122)	0.013
DBP*	82 (78-87)	71 (53-89)	90 (90-90)	0.016
Haemoglobin	13.2 (10.7-14.6)	13.8 (10.7-15.4)	12.3 (12.3-12.3)	0.322
Leucocytes	6.7 (6.0-8.5)	7.0 (3.6-14.2)	7.2 (7.2-7.2)	0.839
Granulocytes	56.8 (42.6-67.4)	62.4 (26.6-84.1)	72.1 (72.1-72.1)	0.259
ESR*	15 (5-22)	17 (3-32)	24 (24-24)	0.635
Random Blood Sugar	91 (76-149)	99 (78-182)	185 (185-185)	0.156
Albumin	4.3 (4.2-4.8)	4.4 (4.1-4.8)	4 (4-4)	0.192
HDL*	51 (42-65)	43 (30-61)	53 (53-53)	0.144
Uric acid	5.8 (2.9-7.3)	5.1 (3.1-7.3)	4.4 (4.4-4.4)	0.617

*NERD, non-erosive reflux disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein.

Table 3. Distribution of SAT, SOT, ISO by GERD Severity

Variables	GERD	Grade A	Grade B	p
	Median (Range)	Median (Range)	Median (Range)	
SAT	1.7 (1.5-1.8)	1.6 (1.2-1.9)	1.5 (1.5-1.5)	0.212
SOT	4.7 (3.2-4.9)	5.1 (3.4-10.3)	4.5 (4.5-4.5)	0.433
ISO	2.7 (2.2-2.9)	3.1 (2.4-8.9)	3.0 (3.0-3.0)	0.145

Table 4. Spearman Rank Correlation TAS, TOS, OSI and GERD

Independent Variables	Dependent variable	P
	GERD	
Total Anti-oxidant Status (TAS)	-0.323	0.044
Total Oxidant Status (TOS)	0.121	0.266
Oxidative Stress Index (OSI)	0.345	0.033

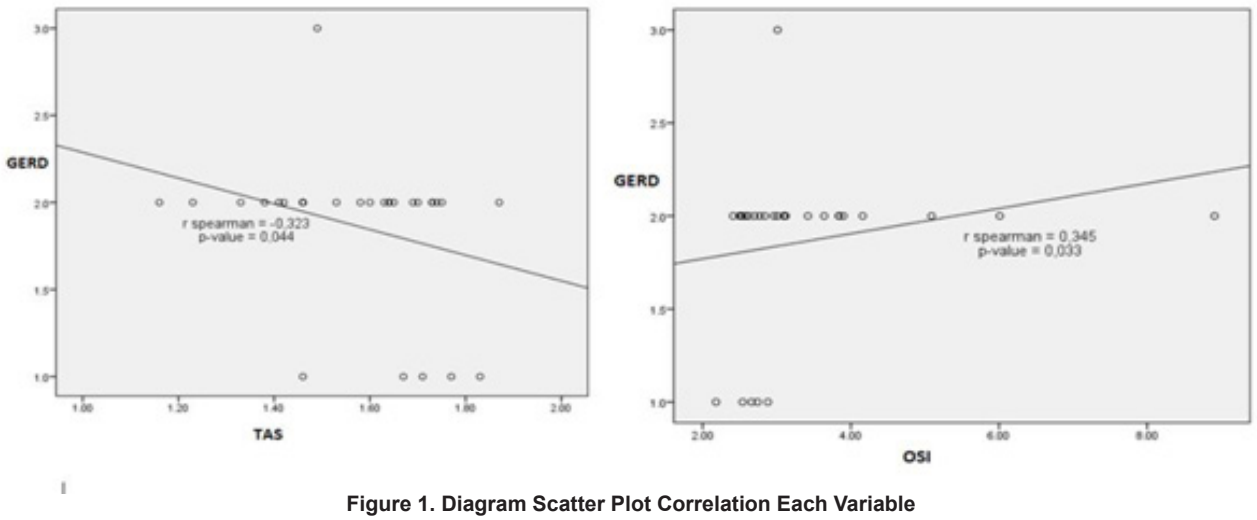

Figure 1. Diagram Scatter Plot Correlation Each Variable

This study showed a weak significant correlation between TAS and the GERD severity (r = -0.323, p = 0.044) and a weak significant correlation between OSI and the GERD severity (r = 0.345, p = 0.033). **(Figure 1)**

DISCUSSION

Researchers have not found other studies in adults that are similar to this study. A previous study by Deng *et al.* (2019) that has conducted on 60 pediatric patients (aged seven months - 16 years) regarding the relationship between the severity of reflux esophagitis and changes in oxidative stress, serum inflammation, VIP, and motilin, found that OSI levels were related to the degree of mucosal damage.

This study found a non-significant positive correlation between TOS and the GERD severity, a weakly significant negative correlation between TAS and GERD severity, and a weakly significant positive correlation between ISO and the GERD severity. These results support the theory that oxidative stress can affect the severity of GERD. However, the serum SAT, and SOT are examined, not the oesophagal tissue with GERD. Excessive production of free radicals and reactive oxygen species in the gastrointestinal tract will cause oxidative damage to gastrointestinal tissues by developing various pathological conditions and clinical symptoms.

The main oxidant product in oesophageal reflux is superoxide anion; there is a positive correlation between the degree of mucosal damage and superoxide anion levels (highest in patients with oesophageal reflux oesophagal barret). Decreased antioxidant activity of *superoxide dismutase* causes increased superoxide anion and mucosal peroxynitrite radicals that play a role in oesophagal and oesophageal Barrett damage in patients with oesophagal reflux so that *superoxide dismutase* administration may be a therapeutic target for esophagitis and oesophagal barret patients. In this study, the TAS for all subjects was still within normal limits. This shows a significant negative correlation with the degree of GERD severity. Meanwhile, TOS has a positive correlation with the degree of GERD, but it is not significant. The antioxidant protection activated in this population may be sufficient to maintain redox homeostasis. The pro-oxidant and antioxidant values did not increase significantly in GERD patients even though the population was exposed to oxidative stress. A previous study found a significant correlation between pro-oxidant and antioxidant, BMI and waist circumference; thus, the increase in pro-oxidant and antioxidant in patients needs to be re-confirmed in other studies.

This study shows that the proportion of females is higher than men. These results are consistent with previous studies that GERD is more common in women than in men in the Iranian population with a ratio of 1/1.06 and 1.94/1 in patients with reflux esophagitis, and GERD (2,17) examined the different presentations of GERD also support the finding of this study, in which from 88 subjects, about 53.40% were women, and 45.59% were men. This characteristic is similar to the study that examined TAS and chemokines' relationship in adults. Based on a systematic review, it was stated that the prevalence of GERD did not differ between women (19.4 ± 2.3%) and men (18.9 ± 2.4%, P = 0.87) or by region (countries) (19). Research-based on endoscopy results showed more reflux esophagitis in men than women with a ratio of 1.57 (95% CI, 1.40-1.76). At the same time, GERD was more common in women, Barrett's oesophagus was more common in

men. This happens because, in men, alcohol, hiatal hernia, history of H. pylori eradication, and BMI 25kg/m2 are risk factors for reflux esophagitis, while in women, smoking, age <40 years and 60 years, blood sugar 126 mg/dL, BMI <23 kg/m2, use of antibiotics, stooped posture, and income < $1000 are risk factors for GERD (19). The hormone oestrogen in women is thought to increase nitric oxide levels, which result in a decrease in LES muscle tone and a prolonged relaxation time of the LES, thereby increasing the risk of gastroesophageal reflux disease .

The most degree of GERD severity in this study was grade A (79.30%). This finding is in line with the study by Cătinean et al. (2017);35.22% of study subjects had erosive esophagitis (83.87% degrees A or B; 16.12% degrees C or D), and 64.77% were found to have non-erosive esophagitis (consisting of 15.78% GERD; 84.21% functional pyrosis), and there was no significant difference between the two sexes based on certain classes of esophagitis.

In this study, the haemoglobin (Hb), ESR, HDL and uric acid levels had a significant difference (P<0.05), in which the mean Hb for males was higher, the median ESR was more elevated for females, the mean HDL was higher for females, and uric acid levels were higher in men. However, all were still within the normal range. Women's Hb levels are about 12% lower than men's, probably due to a direct effect of sex hormones (estrogens and androgens) on erythropoiesis, where estrogens dilate, and androgens constrict the kidney microvasculature. Increasing and decreasing blood hematocrit can affect oxygen delivery per unit of red cell mass unit and provide a mechanism for varying red cell masses without compensatory changes in erythropoiesis. The erythrocyte sedimentation rate in healthy people is higher in women than in men and increases according to age. This is due to increased levels of fibrinogen. The erythrocyte sedimentation rate is inversely correlated with haemoglobin but directly associated with fibrinogen levels. Leukocytosis was found in two patients with grade A. Still, neither clinical nor the source of infection was found, so they were included in the study, and there was no significant difference between the three degrees of GERD. Leukocytes are one of the pro-inflammatory factors (in addition to cytokines (IL-6 and IL-8) and oxidative stress) and are involved in the development of GERD. Women's HDL levels are higher than men's in Canada, the United States, Russia, Poland, China and Korea, partly because estrogen decreases macrophage metabolic activity by fat accumulation. In contrast, testosterone stimulates it, but other factors still need to be investigated.

Total antioxidant status in this study was not significantly different between men and women. Total antioxidant status was higher in men than women at any age. Albumin and uric acid levels in men, women and girls, and total protein in men are significant determinants of TAS levels. This is different from the results of this study, with a non-significant difference between men and women (P = 0.066), with albumin and uric acid levels still in the normal range.

Uric acid is a product of purine nucleotide metabolism, which has both antioxidant and pro-oxidant capacities. In plasma, half the total antioxidant capacity (as measured by the whole radical scavenging assay) has been attributed to uric acid acting (as a scavenger of singlet oxygen, peroxyl radicals and hydroxyl radicals). On the other hand, in the pro-oxidant environment, especially intracellularly, uric acid forms free radicals in various radical-generating systems. The pro-oxidant potential of uric acid supports that uric acid is a risk factor for diseases including cardiovascular disease, hypertension, and metabolic syndrome. Uric acid, albumin, bilirubin, total protein, ferritin, transferrin and haptoglobin are considered tertiary antioxidants because they sequester transition metals such as copper and iron and prevent them from participating in oxidative processes. Uric acid accounts for more than 60% of total plasma antioxidant power in healthy subjects, and several studies have shown a positive correlation to SAT. Thus, it is essential to ensure normal uric acid and albumin levels as long as they do not affect TOS, TAS, and ISO.

CONCLUSION

There is a non-significant positive correlation between TOS and GERD severity. On the contrary, a significant negative correlation is founded between TAS and GERD severity; meanwhile, a significant positive correlation has been proven between OSI and GERD severity. The TAS and OSI may be corrrelated to the severity of GERD. However, further research needs to be conducted to prove it.

LIMITATION

This study cannot be generalized because the sample was only taken from three hospitals in a city and did not get all the degrees of GERD severity. This study uses only the GERD questionnaire and

endoscopic examination to diagnose GERD; hence, it could not afford the best variation in GERD severity. The tissue oxidants and antioxidants levels have not been examined because Indonesia has no tool for this examination. In addition, this study used subjects who had received Proton Pump Inhibitors that have antioxidant effects; hence they might affect the results. Several confounding variables are known from the results of a very subjective history, such as a history of smoking, alcohol intake, chronic diseases (coronary heart disease, stroke, pregnancy, malignancy), and a history of drugs and antioxidants consumption.

FUNDING

This research received funding from Universitas Airlangga, Surabaya, Indonesia (No. 402/UN3.14/PT/2020)

AUTHORS CONTRIBUTION

T.A.I., T.S., B.W., E.V.N., and I.D.N.W., conceived, designed, supervised the study, analyzed the data, and wrote the first draft of the manuscript. All authors critically reviewed the manuscript, read and approved the final version to be published.

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