Nitric Oxide, Lipid Peroxidation and Total Thiol Levels in Acute Appendicitis

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Background: The mechanisms involved in the pathology of acute appendicitis (AA) and the factors affecting the progression have still been investigated. Oxidative stress is one of the factors of interest. Nitric oxide (NO) and its role in AA has not been studied previously. **Methods:** Thirty-four patients who underwent operation with a perioperative diagnosis of AA and 16 age and sex-matched controls were included in the study. Serum thiobarbituric acid reactive substances (TBARS), thiol groups (SH), NO metabolites (NO_x), and conventional inflammation markers were determined. **Results:** NO_x, TBARS, C-reactive protein (CRP) levels, white blood cell

(WBC) count, and erythrocyte sedimentation rate (ESR) were significantly higher, and total SH was significantly lower in AA than in control group. NO_x, TBARS, and SH levels were comparable in acute phlegmonous appendicitis and advanced appendicitis. There was a significant positive correlation between NO_x and TBARS, CRP, ESR, WBC and a significant negative correlation between NO_x and SH. **Conclusions:** Serum NO_x levels and oxidative stress elevate in AA independent from the extent of the lesion. Increased NO may play a role in the increased oxidative stress in AA. *J. Clin. Lab. Anal.* 24:63–66, 2010. © 2010 Wiley-Liss, Inc.

Key words: nitric oxide; thiobarbituric acid reactive substances; thiol groups; oxidative stress; acute appendicitis

INTRODUCTION

The factors involving the progression of acute appendicitis (AA) after the onset of inflammation have still been investigated. One of these factors of interest is oxidative stress. The possible role of oxidative stress parameters in the progression of AA has been addressed in a few studies (1–3).

Nitric oxide (NO), as a mediator of cytokine activation, is produced by cytokine-activated cells in inflammation. It can react with superoxide and form peroxynitrite, which has been accused to implicate in a variety of immunopathological situations like septic shock (4).

Lipid oxidation gives rise to a number of secondary products. Malondialdehyde (MDA), one of the well-known secondary products of lipid peroxidation after exposure to reactive oxygen species and free radicals, may be used to evaluate oxidative damage (5,6). Reaction between MDA and thiobarbituric acid is used for determination of MDA concentration as thiobarbituric acid reactive substances (TBARS). Plasma thiols

are physiological free radical scavengers and they may serve as an antioxidant function by several mechanisms. It was reported that the measurement of plasma total thiol (SH) is a good reflection of excess free radical generation both in physiological and pathological conditions in human (7,8).

A possible correlation between oxidative stress and the extent of AA has been reported previously (2,3). However, the data in this field are limited and give inconsistent results in different parameters. To our knowledge, there is not any study investigating nitrosative and oxidative stress interactions in AA. We know that oxidative stress and nitrosative stress can interact together to cause increased tissue damage. We aimed to investigate the possible role of NO and

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whether there is a relation between oxidative stress and NO in AA.

SUBJECTS AND METHODS

Thirty-four patients (18 male, 16 female; age 28.3 ± 8.96 years) who underwent operation with a perioperative diagnosis of AA and 16 age and sex-matched controls (8 male, 8 female; age 31.6 ± 10.4 years) were included in the study. Preoperative blood samples were obtained in Vacutainer® tubes (BD, New Jersey), centrifuged at 1300g for 10 min and stored at -80°C until the analysis. erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and white blood cell (WBC) were measured on the day of sampling. For NO metabolites (NO_x), TBARS, and SH measurements, serum samples were frozen at -80°C until the working day.

All of the appendectomy specimens were examined by a pathologist who was unaware of the biochemical findings and the patients were classified in three groups: (1) Acute phlegmonous appendicitis (APA; $n = 22$); (2) advanced appendicitis including acute gangrenous appendicitis and perforated appendicitis (AA; $n = 8$); and (3) negative explorations (NE; $n = 4$).

WBC and ESR were determined with a Coulter LH 750 (Beckman-Coulter Inc., Fullerton, CA) and Alifax (Alifax SPA, Padova, Italy) analyzers, respectively. CRP was measured on an Immage nephelometer (Beckman Coulter Inc.). NO_x was measured with a modified Griess method. Briefly, nitrate was reduced to nitrite by nitrate reductase and the occurred color was measured at the end of the reaction of nitrite with Griess reagent; the resultant color changes were read at 540 nm; calibration curves were made with sodium nitrite standards prepared in deionized water (6.25 – $100 \mu\text{mol/L}$) (9). Serum TBARS levels, as indicators of MDA, were determined by the method described by Wasowicz et al. (10). After the reaction of thiobarbituric acid with MDA, the reaction product was extracted in butanol and was measured spectrofluorometrically at wavelengths of 525 nm of excitation and 547 nm of emission. 0 – $25 \mu\text{mol/L}$ 1,1',3,3'-tetraethoxypropane solutions was used as the standard. SH groups were measured by a colorimetric method by which thiol groups gave a chromogen compound with dithiobisnitrobenzoic acid in alkaline pH (11). SH concentrations were calculated by the use of molar absorptivity of reaction product, thionitrobenzoic acid ($\epsilon_{412} = 13.600 \text{ L mol}^{-1} \text{ cm}^{-1}$).

Statistics

Values are presented as mean $\pm$ SD, with a P less than 0.05 indicating significance. Clinical variables were compared using nonparametric Mann–Whitney U -test

between the appendicitis and control groups. Four NE were excluded from appendicitis group during the group comparisons. Kruskal–Wallis test was performed to determine the differences of the variables between APA, AA, and NE. Spearman correlation analysis was performed for the correlation of the variables. All statistical calculations were made using SPSS® for Windows 14.0 (SPSS Inc. Headquarters, Chicago, IL) software program.

RESULTS

The results of the biochemical tests are given in Table 1. ESR, WBC, CRP, NO_x , TBARS levels were significantly higher, total SH was significantly lower in AA than in control group. NO_x , TBARS, and SH levels were not significantly different in APA and AA subgroups. The results of the subgroup analysis are shown in Table 2.

There was a significant positive correlation between NO_x and TBARS ($r = 0.358$; $P = 0.020$), CRP ($r = 0.545$; $P < 0.001$), ESR ($r = 0.473$; $P = 0.001$), WBC ($r = 0.558$; $P < 0.001$) and a significant negative correlation between NO_x and SH ($r = -0.520$; $P < 0.001$). TBARS showed a significant positive correlation with CRP ($r = 0.488$; $P = 0.001$) and WBC ($r = 0.547$; $P < 0.001$) (Figure 1).

DISCUSSION

This study demonstrates for the first time that NO_x are increased in sera of the AA patients, together with the evidence of increased oxidative stress.

The possible role of oxidative stress parameters in AA has been reported in various studies. In an experimental study, the activities of antioxidant enzymes such as catalase and glutathione peroxidase were found to be increased in peripheral and mesenteric blood samples of rabbits that underwent a ligation of appendices 24 hr before sampling. However, catalase activity was also increased in the control group. The authors have suggested the results as oxygen free radical effect to activate the enzymatic defense system in AA (12). A few

TABLE 1. Comparison of the Biochemical Parameters

	Acute appendicitis	Control	P
ESR (mm/hr)	12.1 ± 8.42	7.53 ± 6.25	< 0.001
WBC ($\times 1,000$)	15.0 ± 4.09	7.04 ± 1.98	< 0.001
CRP (mg/L)	61.2 ± 85.5	1.83 ± 0.78	< 0.001
NO_x ($\mu\text{mol/L}$)	57.4 ± 15.2	24.8 ± 10.6	< 0.001
TBARS ($\mu\text{mol/L}$)	1.54 ± 0.35	0.87 ± 0.33	< 0.001
Total SH ($\mu\text{mol/L}$)	277 ± 65.6	482 ± 149	< 0.001

Results are given as Mean $\pm$ SD.

TABLE 2. Subgroup Analysis

	Advanced appendicitis	Acute phlegmonous appendicitis	P
NO _x (μmol/L)	53.0 ± 13.8	57.9 ± 15.9	>0.05
TBARS (μmol/L)	1.38 ± 0.14	1.60 ± 0.39	>0.05
Total SH (μmol/L)	277 ± 70.4	278 ± 65.6	>0.05

Results are given as Mean ± SD.

Fig. 1. Serum levels of TBARS (A), SH (B), and NO_x (C) in acute appendicitis and control groups.

clinical studies investigating the correlation between oxidative stress parameters and the extent of appendicitis have been done. Tissue superoxide dismutase (SOD) activity was found to be higher in phlegmonous and gangrenous appendix than catarrhal appendix, TBARS levels were found highest in gangrenous appendix (3), and these findings were supported by a study made in pediatric patients, which reported higher TBARS and SOD levels in peripheral blood in advanced

appendicitis than less advanced groups and controls (1). In another study TBARS levels were found to remain similar in appendicitis subgroups, whereas the total antioxidant capacity (TAC) was found significantly decreased in advanced appendicitis group compared with APA and concluded that TAC might be an indicator for the progression of inflammation in appendicitis (2). However, this finding has not been supported by the consecutive study, which reported that children with AA did not have decreased blood plasma antioxidant capacity (13). Our findings in this study indicate an increase in oxidative stress with an increase in TBARS and decrease in SH levels in appendicitis patients compared with controls; however, there was not a significant correlation of these parameters with the progression of inflammation.

NO system is regarded as an important component in mediating cytokine activation of macrophages in inflammation (14). NO has important roles ranging from immunomodulation to vascular tonus and capillary flow and capillary vasodilatation can potentially effect peritoneal transport and bacterial invasion. Peroxynitrite, a powerful oxidant, can be formed with the reaction of NO with superoxide and is implicated in a variety of pathological situations ranging from tissue damage to septic shock (4). The interaction of NO and oxidative stress is important and has not been previously investigated in AA. We found significantly higher NO_x levels in AA, which correlates strongly to oxidative stress and conventional inflammation markers. The source of the increased NO_x levels is thought to be the activated macrophages. Peroxynitrite anion is formed from the diffusion-controlled reaction between the free radicals NO and O₂⁻. Peroxynitrite can react with CO₂ to form CO₃⁻ and NO₂ radicals, which will initiate free radical biochemistry and a minor amount of peroxynitrite will undergo the proton-catalyzed homolysis to yield OH and NO₂, which may initiate lipid oxidation (15). Starting from these interactions, it may be concluded that increased synthesis of NO with the inflammation of appendix may be an important factor for the initiation or progression of oxidative stress in AA. Our findings in this study do not support the idea that oxidative stress parameters correlate with the extent of AA. The relatively small numbers of subjects in the

subgroups may be an explanation for this lack of correlation.

In conclusion, serum NO_x levels and oxidative stress are found to be increased independent from the extent of the lesion and increased NO, which has not been reported previously, may be the source or may have an important role in the increased oxidative stress in AA.

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