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Increased oxidative stress and deficient antioxidant levels may be involved in the pathogenesis of idiopathic recurrent acute pancreatitis

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ABSTRACT

Background: Increased Oxidative Stress (OS) is implicated in the pathogenesis of Chronic Pancreatitis (CP). Whether or not OS contributes to disease progression through the stages of Recurrent Acute Pancreatitis(RAP), to CP is not known. Increased OS, if present in RAP could be an important therapeutic target in preventing progression of RAP to CP.

Objective: To assess the oxidative stress and antioxidant status in patients with idiopathic RAP.

Methods: 50 consecutive patients with Idiopathic Recurrent Acute Pancreatitis (IRAP) were included. Markers of OS [4-hydroxynonenol (4-HNE), malondialdehyde (MDA) and serum SOD (S-SOD)] and antioxidant status [ferric reducing the ability of plasma (FRAP), Glutathione peroxidase (GPX) and Vitamin C (Vit C)] were measured in quiescent phase and during an episode of pancreatitis. Their levels were compared with those in age and sex matched healthy controls and patients with CP.

Results: The mean age of patients with IRAP was 22.2 ± 7.7 years and 39 (78%) were males. Levels of 4-HNE were significantly increased in patients with IRAP compared with healthy controls (3.03 ± 2.35 vs. 2.12 ± 1.29 ng/ml; p = 0.03) and were even higher during an episode of acute pancreatitis (5.21 ± 3.51 ng/ml; p = 0.03). Antioxidant levels were reduced in IRAP compared with healthy controls as measured by FRAP (707.0 ± 144.9 vs. 528.8 ± 120.0 μmol/Fe2+liberated; p = 0.0001) and GPX (1472 ± 375.7 vs. 910.0 ± 558.5 pg/ml; p = 0.001). OS and antioxidant profiles were similar in IRAP and CP with no significant difference.

Conclusion: OS is increased in patients with IRAP, more so during an acute episode. Antioxidant levels are also reduced suggesting that OS may play a role in the pathogenesis of IRAP and its progression to CP.

Introduction

The pathogenesis of idiopathic CP is not well understood. According to the necrosis-fibrosis hypothesis, it is the recurrent attacks of acute pancreatitis that result in progressive parenchymal injury and fibrosis leading to CP. However, what is not known is if increased Oxidative Stress (OS) contributes to disease progression through the stages of Recurrent Acute Pancreatitis (RAP) to CP. Raised OS has been implicated in the pathophysiology of CP and has been well documented. Antioxidant supplementation in CP has been shown to be beneficial but is debatable. We and others have shown that micronutrient antioxidant therapy is associated with a reduction in pain in patients with CP although there are also studies which show that antioxidant therapy has no effect on pain in this condition [1,2].

Oxidative stress may be induced by increased exposure to oxidants. Xenobiotics are detoxified in the body through phase I and phase II pathways, chiefly in the liver [3]. Increased exposure to xenobiotics such as alcohol, nicotine, and petrochemical fumes may overwhelm the capacity of phase I and phase II detoxification pathways and result in oxidative stress [4]. The pancreatic acinar cells are the main site for generation of free radicals and therefore are exposed to the detrimental effects of the same [5]. A large number of mitochondria within the acinar cell is the site of the electron transport chain and is a major contributor to redox balance within the cell. The oxidative stress in the acinar cell may result from generation of free radicals through CYP induction, concurrent exposure to a chemical that undergoes bioactivation, and insufficiency of micronutrients that are required to sustain antioxidant

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(AO) capacity. Oxidative stress can cause cell damage either directly by cell membrane destruction, depleting the cells of antioxidants; by toxicity from free radical peroxidation products; or through altering signaling pathways, including redox regulation of genes [6,7]. It is possible that the imbalance between the antioxidant capacity and oxidative stress, when present triggers acinar cell damage and recurrent attacks of pancreatitis [8].

Recurrent acute pancreatitis may eventually lead to CP. In a prospective cohort study of patients with idiopathic recurrent acute pancreatitis (IRAP), we found that 47% of patients developed CP over a 5-year follow-up period suggesting that IRAP is an earlier stage of CP [9]. If oxidative stress plays a role in the pathogenesis of RAP during its progression to CP, there might be an opportunity for therapeutic intervention at an early stage before the development of established CP.

Thus, the objective of the present study was to evaluate oxidative stress and antioxidant capacity in patients with IRAP.

Methods

Study design

Clinical cohort study.

Patients

All consecutive patients with recurrent acute pancreatitis who attended the pancreas clinic or were admitted to the gastroenterology ward at the All India Institute of Medical Sciences, New Delhi were screened for inclusion. Patients who fulfilled the inclusion criteria and consented to be a part of the study were included in the study.

Inclusion criteria

Patients presenting with 2 or more attacks of acute pancreatitis who were not found to have any definite cause of pancreatitis and diagnosed to have IRAP were included in the study.

Exclusion criteria

Patients with the following conditions were excluded: (a) taking antioxidant therapy or having received it earlier in the past 6 months, (b) evidence of CP in the form of pancreatic calcification or pancreatic duct dilatation (c) co-morbid diseases such as liver disease, chronic renal failure, malignancy, and hypertension that might affect the antioxidant status and oxidative stress levels, (d) complications of pancreatitis such as pseudocyst, biliary obstruction, (e) age <12 years or >60 years.

Healthy controls

Age and sex-matched healthy subjects were included in the study to compare the markers of oxidative stress and antioxidant levels between patients with IRAP and healthy controls. They were free from any chronic disease, and were non-alcoholic and non-smokers.

Diseased controls

Twenty-five patients with Idiopathic CP were included in this study as diseased controls.

Diagnosis of IRAP

The diagnosis of acute pancreatitis was made in the presence of suggestive clinical features, increased serum amylase and/or lipase (>3 times the upper limit of normal) levels, and evidence of pancreatitis on imaging i.e. ultrasonography (USG) and/or computerized tomography (CT) scan of the abdomen. Patients with 2 or more attacks of acute pancreatitis were diagnosed to have RAP. Acute Pancreatitis was considered to be idiopathic when no definite cause for it was detected, such as gallstones, alcoholism, hyperlipidemia, hypercalcemia, drugs known to cause acute pancreatitis, and viral infection after clinical evaluation and appropriate investigations. All patients underwent a standard set of investigations, including hematological, serum biochemical tests such as liver function tests, serum calcium, lipid profile, and abdominal ultrasonography and if required a CT scan of the abdomen. Further investigations in the form of endoscopic ultrasonography (EUS)/magnetic resonance cholangiopancreatography (MRCP) were done if indicated, as part of their further work-up [6]. All patients included in the study underwent imaging tests in the form of CT (n = 34) or MRI (n = 12) scan of the abdomen and/or Endosonography (n = 42) to rule out evidence of chronic pancreatitis. Only patients with no evidence of chronic pancreatitis in the form of parenchymal or ductal changes such as dilatation/calcification were included as patients with IRAP in the study.

Estimation of markers of oxidative stress and antioxidant capacity

The markers of oxidative stress estimated in the present study were malondialdehyde (MDA) and 4-hydroxynonenal (4HNE) and serum Superoxide Dismutase (S-SOD). The markers of antioxidant status assessed were glutathione peroxidase (Gpx) and total antioxidant capacity (measured as ferric reducing ability of plasma-FRAP) and Vitamin C (Vit C).

The methods to measure these markers were standardized in our laboratory including quality control assays. Samples of patients with IRAP were collected during the quiescent phase and also during the time of pain within the first 24–48 h of onset of pain. The blood samples were collected in the morning after an overnight fast. 10 mL of blood were drawn into vacutainers. The plasma and serum were separated within 2 h of blood collection and stored at −80 °C till analyzed.

4-HNE and MDA represent markers of lipid peroxidation. 4-HNE was measured by using kit based ELISA (Mybioscience Catalog No MBS2516118, San Diego, California) following manufacturer's guidelines. MDA was estimated by using the reaction with 1-methyl 2-phenylindol and estimated by colorimetric analysis as described by Gérard-Monnier et al. [10]. Serum SOD was assessed by the method given by Marklund and Marklund [11]. FRAP was assessed by the method described by Benzie and Strain [12]. Vitamin C levels were assessed by using the method described by Okamura [13]. Glutathione peroxidase was estimated by ELISA (Mybioscience Catalogue MBS2516156, San Deigo, California) following manufacturer's guidelines.

Outcome measure

The difference in the levels of markers of oxidative stress and antioxidant status between patients with IRAP and controls was the primary outcome measure.

Statistical analysis

Descriptive statistics i.e. mean, standard deviation and frequency distribution were calculated for each variable in the study. Data are presented as mean ± SD. To compare the two groups, Mann-Whitney *U* test or Kruskal Wallis test for quantitative variables and chi-square test for qualitative variables were applied as

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