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ORIGINAL ARTICLE

Assessment of oxidative status in chronic pancreatitis and its relation with zinc status

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Abstract
Background Oxidative stress-induced free radicals have been implicated in the pathology of chronic pancreatitis (CP).
Aim We aimed to estimate oxidative stress and antioxidant status in tropical chronic pancreatitis (TCP) and alcoholic chronic pancreatitis (ACP) and correlate with zinc status.
Methods One hundred and seventy-five CP patients (91 TCP, 84 ACP) and 113 healthy subjects were prospectively studied. Disease characteristics and imaging features were recorded. Erythrocyte reduced glutathione, glutathione peroxidase (GPx), superoxide dismutase (SOD), plasma vitamin C, and erythrocyte thiobarbituric acid reactive substance (TBARS) were estimated by spectrophotometry. Erythrocyte zinc was estimated by flame atomic absorption spectrophotometry.
Results Enhanced lipid peroxidation with concomitant decrease in antioxidant status was observed in both TCP and ACP patients ($p < 0.05$). The findings were comparable in both diabetic and non-diabetic CP patients. Significantly, lower plasma vitamin C and elevated levels of erythrocyte TBARS was noted in TCP as compared to ACP patients. The erythrocyte zinc significantly correlated with SOD activity ($r=0.450$, $p<0.001$).
Conclusions Our study corroborates the role of oxidative stress in CP and suggests some differences in oxidative status in TCP and ACP patients. Zinc deficiency appears to affect oxidative status in CP patients.

Keywords Antioxidants · Chronic pancreatitis · Diabetes mellitus · Zinc

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Introduction

The pathophysiologic mechanisms of chronic pancreatitis (CP) are not fully understood. Most available data indicate that the primary site for the development of CP is the pancreatic acinar cell [1]. The role of reactive oxygen species (ROS) has been studied in both experimental and human CP [2, 3]. ROS play a role in perpetuating the pancreatic inflammation and the development of extra pancreatic complications [4]. However, there is limited literature in identifying oxidant status in tropical chronic pancreatitis (TCP) as compared to alcoholic chronic pancreatitis (ACP).

Zinc deficiency has been reported to impact pancreatic function [5]. Zinc is an important element in numerous proteins and plays a pivotal role in several essential cell functions, such as cell proliferation and apoptosis, defense against free radicals, and DNA damage repair [6]. For instance, CuZn superoxide dismutase (SOD) is an important first-line defense enzyme against oxygen radical species and p53 is an important zinc-containing transcription factor that plays an essential role in the DNA damage response [7]. Zinc may modulate the oxido-reductive

Table 1 Demographic characteristics of study population

	Controls (n=113)	Alcoholic pancreatitis (n=84)	Tropical pancreatitis (n=91)
Age (mean [SD])	36 (11.5)	42 (11.8)	34 (13.7)
BMI (mean [SD])	20.5 (3.1)	19.6 (3.2)	19.2 (3.3)
Gender (male:female)	60:53	84:0	56:35
Diabetics	0	44 (52.3%)	53 (58.2%)
Smokers	0	67 (79.8%)	12 (13.1%)
Pain	0	59 (70.2%)	71 (78%)

environment in cells through modulation of thiol status [8]. Zinc antagonizes the activities of bivalent transition metals, including iron and copper, and prevents the deleterious free-radical reactions (e.g., Fenton reaction) stimulated by iron and copper [8]. Moreover, zinc is a component of metallothioneins, which are part of classic antioxidant defenses [8]. Therefore, it is conceivable that zinc depletion in vivo may cause oxidative stress [8]. Although these evidences suggest that zinc has antioxidant properties and protects tissue from oxidative damage, there is paucity of literature on the effects of zinc deficiency on oxidative stress and antioxidant status in CP.

Superoxide dismutases are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. In humans, three forms of SOD are present—SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reactive centre. Matsumura et al. showed that SOD inhibitor diethyldithiocarbamate can cause pancreatic fibrosis [9] and can be used as a rodent model for the development of pancreatic fibrosis from the viewpoint of oxidative stress.

Therefore, we estimated blood levels of enzymes that protect against oxidative damage, such as SOD, glutathionine peroxidase (GPx), and anti-oxidants, like reduced glutathione, vitamin C in TCP and ACP patients, and compared these with values healthy subjects. We also measured thiobarbituric acid reactive substance (TBARS) as an indicator of lipid peroxidation. Lastly, we assessed zinc status in ACP and TCP patients and examined its relationship to SOD activity.

Methods

Chronic pancreatitis patients were recruited for the study from the Pancreas Clinic of our hospital, and were diagnosed on the basis of presence of pancreatic calcification (US/CT) and/or parenchymal or ductal changes on imaging (CT/ERCP/EUS). Patients having CP with an alcohol consumption ≥80 g/day for at least five years were considered to have ACP while TCP was defined using previously reported criteria [10].

Diabetes mellitus was diagnosed if the fasting plasma glucose value was equal to or greater than 126 mg/dL, confirmed on two occasions, and/or a plasma glucose value equal to or greater than 200 mg/dL after a 2-hour glucose load confirmed on two occasions, and/or there are requirements for insulin or oral hypoglycemic drugs [11].

History of illness including presenting complaints, duration of illness, pain and diabetes mellitus, and risk factors, such as alcohol and smoking were recorded. Demographic parameters and anthropometric measurements were elicited and a detailed physical examination was conducted. BMI was calculated by the formula $\mathrm{BMI} = \mathrm{weight}/\mathrm{height}^2 (\mathrm{kg}/\mathrm{m}^2)$.

Patients with pancreatic cancer, CP patients who had undergone pancreatic surgery, CP patients with complications, like pseudocyst or common bile duct obstruction, or

Table 2 Blood antioxidant levels and lipid peroxidation product in chronic pancreatitis patients and controls

	Controls	Tropical pancreatitis	Alcoholic pancreatitis
Erythrocyte GSH (μmol/g Hb)	8.59 (0.21)	6.21 (0.25)[1]	5.07 (0.25)[2,3]
Erythrocyte GPx (nmol of NADPH oxidized/min/g Hb)	19.06 (0.33)	15.41 (0.54)[1]	15.83 (0.48)[2]
Erythrocyte SOD (IU/g Hb)	2984.87 (49.96)	2179.82 (74.36)[1]	2304.77 (86.66)[2]
Erythrocyte TBARS (nmol/g Hb)	5.62 (0.13)	10.1 (0.51)[1, 3]	7.44 (0.33)[2]
Plasma vitamin C (mg/dL)	0.82 (0.06)	0.29 (0.04)[1,3]	0.4 (0.06)[2]

Values are as (mean [SE]) [1, 2] Comparison with the controls (p<0.001)

[3] Comparison between TCP and ACP patients (p<0.001)

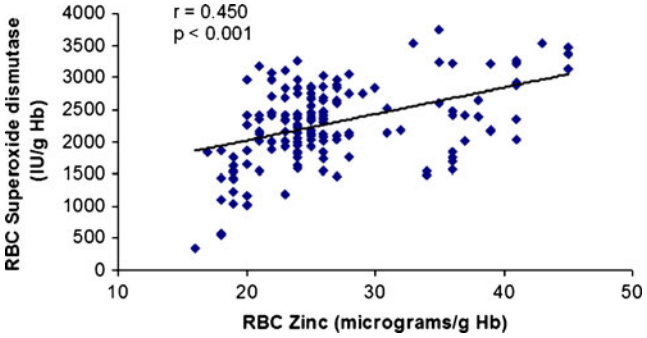

Fig. 1 Correlation between erythrocyte superoxide dismutase and zinc levels in CP patients

CP patients consuming protein, vitamin, and mineral supplements were excluded.

This study was approved by the Institutional Ethics Committee and written informed consent was obtained from the subjects before enrollment.

The study protocol conforms to the ethical guidelines of the "World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects" adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, as revised in Tokyo 2004.

Fasting blood samples were collected in EDTA tubes and immediately placed in an icebox. Blood samples were centrifuged at 1,000 g for 10 minutes at 4°C. The erythrocytes were carefully sampled from the bottom of the tubes to minimize contamination from leucocytes; they were washed three times with ice-cold isotonic saline solution (1/10 vol/vol) and resuspended in a washing solution to give a 50% solution. Hemolysate obtained was divided into aliquots and stored at −20°C for subsequent enzyme assay. The biochemical tests were performed in the Metabolic Laboratory of the institute. Standard reactions were used to measure the levels of erythrocyte glutathione (GSH) [12], (GPx) [13] SOD [14], TBARS) [15], hemoglobin [16], and plasma vitamin C [17] using a UV-visible double beam spectrophotometer (Systronics 2201, Ahmedabad, India).

Erythrocyte zinc was estimated as it provides an assessment of zinc status over a longer period of time as compared to that of the rapidly turning over plasma pool [18]. Erythrocyte lysate was diluted 10-fold with milli-Q water; zinc concentration was determined by flame atomic absorption spectrophotometry (3110, Perkin Elmer, Waltham, MA, USA) [19].

Statistical analysis was done by using SPSS version 11 software (SPSS Inc, Chicago, USA). Differences in mean were calculated using one-way analysis of variance with Scheffe post hoc test. Nonparametric Mann-Whitney *U* test and Kruskal-Wallis test, as appropriate, were used to compare variables without a normal distribution. Biochemical values were expressed as the mean (SE) for comparison.

Results

The demographic characteristics of study population are given in Table 1. Of the 175 patients, there were 91 TCP patients (35 men) and 84 ACP patients (all men). The mean age of all CP patients was comparable with the age of controls; however, TCP patients were younger than ACP patients. The mean body mass index was comparable in all three groups.

The erythrocyte GSH, GPx, SOD, and plasma vitamin C levels were lower, and erythrocyte TBARS was higher in both TCP and ACP patients as compared to healthy controls (Table 2).

Plasma vitamin C was lower whereas, erythrocyte TBARS was higher in TCP patients as compared to ACP patients (Table 2). Erythrocyte GSH level was significantly lower in ACP as compared to TCP patients (Table 2).

A positive correlation (Fig. 1) was found between erythrocyte zinc and erythrocyte SOD activity (r=0.450, p<0.001).

We found lower values of erythrocyte GSH, GPx, SOD, and plasma vitamin C and higher erythrocyte TBARS in

Table 3 Blood antioxidant levels and lipid peroxidation product in diabetic and non-diabetic CP patients and controls (mean [SE])

	Diabetes	Erythrocyte GSH (μmol/g Hb)	Erythrocyte GPx (nmol of NADPH oxidized/min/g Hb)	Erythrocyte SOD (IU/g Hb)	Erythrocyte TBARS (nmol/g Hb)	Plasma vitamin C (mg/dL)
Controls		8.59 (0.21)	19.06 (0.33)	2984.87 (49.96)	5.62 (0.13)	0.82 (0.06)
Chronic pancreatitis	Yes	5.38 (0.2)[1]	14.33 (0.37)[1]	2266.55 (64.72)[1]	9.8 (0.39)[1]	0.38 (0.05)[1]
	No	5.93 (0.24)[1]	14.95 (0.5)[1]	2281.73 (59.02)[1]	9.02 (0.35)[1]	0.3 (0.04)[1]
Tropical pancreatitis	Yes	6.02 (0.32)[1]	14.11 (0.57)[1]	2265.72 (78.31)[1]	11.05 (0.6)[1]	0.28 (0.05)[1]
	No	6.51 (0.41)[1]	15.99 (0.82)[1]	2185.01 (92.26)[1]	9.52 (0.6)[1]	0.31 (0.06)[1]
Alcoholic pancreatitis	Yes	4.61 (0.15)[1]	14.6 (0.45)[1]	2267.54 (108.08)[1]	8.29 (0.34)[1]	0.52 (0.09)[2]
	No	5.39 (0.22)[1]	13.95 (0.55)[1]	2373.62 (72.76)[1]	8.55 (0.37)[1]	0.28 (0.06)[1]

[1] Comparison with controls (p<0.001)

[2] Comparison with controls (p<0.05)

both diabetic and non-diabetic CP patients as compared to healthy controls (Table 3). However, we did not find differences between diabetic and non-diabetic TCP patients or between diabetic and non-diabetic ACP patients.

Discussion

Oxidative stress can be increased in a system where the rate of free radical production is increased and/or the antioxidant mechanisms are impaired. In recent years, the oxidative stress-induced free radicals have been implicated in the pathology of CP and antioxidant therapy has been considered as one of the modalities of medical management [2, 20].

The present study examined both antioxidant and oxidant status in CP patients. Significant reduction in levels of free-radical scavengers, such as glutathione, GPx, SOD, and vitamin C, and elevation in levels of TBARS was noted in CP patients as compared to controls. Our findings are consistent with previously reported studies [3, 21].

In a previous study, we found that CP patients had hypozincemia, which correlated with exocrine and endocrine insufficiency [22]. A key finding in this study was a positive correlation between erythrocyte zinc and erythrocyte SOD activity. This suggests that zinc deficiency may play a role in aggravating oxidative stress in CP and is another possible mechanism by which zinc deficiency impacts the pathogenesis of CP and its complications.

While vitamin C levels were lower in both TCP and ACP patients as compared to controls, we found that vitamin C level was lower in TCP as compared to ACP patients, a finding not reported previously. Early age of onset and more rapid course of TCP as compared to ACP is probably one of the reasons for drastic decrease in vitamin C levels and higher TBARS in TCP patients that could precipitate oxidative stress. It is also possible that greater levels of oxidative stress result in earlier onset of endocrine and exocrine insufficiency and also hastens their progress in TCP as compared to ACP.

Quillot et al. have reported that diabetes worsens the antioxidant status in CP patients [23]. However, we did not find any difference in antioxidant status between diabetic and non-diabetic CP patients. This finding was seen in both ACP and TCP patients.

The findings of the present study indicate a need for studying further the benefits of zinc supplementation in chronic pancreatitis. Furthermore, vitamin C appears to be a significant deficiency in TCP. In addition, we have recently reported deficiency of other micronutrients such as methionine and folate in chronic pancreatitis [24]. Braganza et al. have suggested the need for region specific antioxidant supplementation [2, 21].

Our findings provide additional evidence that the approach to antioxidant therapy may need to be tailored to meet specific requirements in different patient groups in the future. These issues can be addressed through suitably designed trials to determine the ideal antioxidant and micronutrient combination.

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