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Original Research Article

A Study to Estimate Oxidative Stress and Antioxidant Status in Tropical Chronic Pancreatitis (TCP) and Alcoholic Chronic Pancreatitis (ACP) and Correlate with Zinc Status

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Abstract

Aim: The aim of the present study was to estimate oxidative stress and antioxidant status in tropical chronic pancreatitis (TCP) and alcoholic chronic pancreatitis (ACP) and correlate with zinc status.

Methods: The present study was conducted in the Department of Gastroenterology, Big Apollo Spectra Hospital Patna, Bihar, India and Chronic pancreatitis patients were recruited for the study and was diagnosed on the basis of presence of pancreatic calcification (US/CT) and/or parenchymal or ductal changes on imaging. 100 patients were included in the study. This study was approved by the Institutional Ethics Committee and written informed consent was obtained from the subjects before enrollment.

Results: Of the 100 patients, there were 50 TCP patients and 50 ACP patients. 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. Plasma vitamin C was lower whereas, erythrocyte TBARS was higher in TCP patients as compared to ACP patients. Erythrocyte GSH level was significantly low in ACP as compared to TCP patients. We found lower values of erythrocyte GSH, GPx, SOD, and plasma vitamin C and higher erythrocyte TBARS in both diabetic and non-diabetic CP patients as compared to healthy controls. However, we did not find differences between diabetic and non-diabetic TCP patients or between diabetic and non-diabetic ACP patients.

Conclusion: The 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: estimate oxidative stress, antioxidant status, tropical chronic pancreatitis, alcoholic chronic pancreatitis (ACP), zinc status

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Introduction

The progressive and irreversible destruction to the pancreatic parenchyma and ductal anatomy in chronic pancreatitis (CP) patients is often accompanied by severe, intractable abdominal pain. Histologically, CP entails excessive deposition of collagen by pancreatic stellate cells (PSCs), loss of acinar and islet cells, and infiltration of the tissue by inflammatory cells and molecules. [1,2] Functional manifestations, usually occurring later in the disease after substantial damage is sustained to pancreatic tissues, include exocrine pancreatic insufficiency and loss of endocrine function, leading to diabetes.

Quality of life is reduced in CP patients, with 85% of patients experiencing pain. [3] CP-induced abdominal pain is deep and internal, ranging in severity from mild to unbearable. Such pain often leads to unemployment due to disability, frequent hospitalizations, excessive use of medications, including narcotics, and multiple medical and surgical interventions.[2]

Risk factors for CP are categorized by the TIGAR-O classification system as toxic-metabolic, idiopathic, genetic, autoimmune, recurrent severe acute pancreatitis, or obstructive. Toxic and metabolic factors include alcohol, tobacco, and some medications and toxins. Alcohol use is reported to precede 55–80% of CP cases; however, only 10% of heavy alcohol drinkers suffer from pancreatic disease. [4] This indicates the involvement of other risk factors in the development of alcohol-associated CP. In fact, most cases of CP probably result from a combination of risk factors. Up to 25% of CP cases are of unknown cause and are, thus, classified as idiopathic. [5]

Zinc is a key element in numerous proteins and plays an important role in essential cell functions such as defense against free radicals and DNA damage repair. [6] Approximately 10% to 40% of dietary zinc is absorbed in the small bowel and 0.5 to 1.0 mg/day is secreted into the biliary tract followed by passing the small and large bowel. [7] Pathophysiology of zinc deficiency in patients with chronic pancreatitis (CP) is not fully elucidated. It has been proposed that decreased secretion of binding proteins in the pancreatic juice explain compromised absorption of zinc in pancreatic exocrine insufficiency (PEI). [8] Zinc deficiency may be the effect of reduced absorption and can be a contributory factor in disease progression, via the reduction of free radicals. [9] It is known that zinc affects many aspects of the immune system, from the barrier of the skin to gene regulation in lymphocytes, and is crucial for development and function of neutrophils and natural killer cells. [10] There are conflicting published results of zinc levels in patients with CP. [11]

CP is a chronic inflammation of the pancreas triggered by various factors including alcohol misuse, smoking, autoimmunity, anatomical variants and genetic factors. Due to progressive fibrosis and destruction of the pancreas, both enzyme and insulin production ultimately become severely impaired, resulting in pancreatic exocrine and endocrine insufficiency. Deficiency of enzymes (exocrine insufficiency) leads to maldigestion and malnutrition which are associated with reduced absorption of fat-soluble vitamins. The essential role of zinc and its deficiency was described in 1963. [12] Quillot et al [13] have reported that diabetes worsens the antioxidant status in chronic pancreatitis patients.

The aim of the present study was to estimate oxidative stress and antioxidant status in tropical chronic pancreatitis (TCP) and alcoholic chronic pancreatitis (ACP) and correlate with zinc status.

Materials and methods

The present study was conducted in the Department of Gastroenterology, Big Apollo Spectra Hospital Patna, Bihar, India and Chronic pancreatitis patients were recruited for the study and was diagnosed on the basis of presence of pancreatic calcification (US/CT) and/or parenchymal or ductal changes on imaging. 100 patients were included in the study. This study was approved by the Institutional Ethics Committee and written informed consent was obtained from the subjects before enrollment. 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. [14]

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. [15]

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 were calculated by the formula BMI = weight / height2(kg/m2).

Patients with pancreatic cancer, CP patients who had undergone pancreatic surgery, CP patients with complications, like pseudocyst or common bile duct obstruction, or CP patients consuming protein, vitamin, and mineral supplements were excluded.

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 were 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) [16], (GPx) [17], SOD [18], TBARS) [19], haemoglobin [20] and plasma vitamin C [21] using a UV-visible double beam spectrophotometer (Systronics 2201, Ahmedabad, India). Erythrocyte zinc were

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. [22] Erythrocyte lysate was diluted 10-fold with milli-Q water; zinc concentration were determined by flame atomic absorption spectrophotometry (3110, Perkin Elmer, Waltham, MA, USA). [23]

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, was used to compare variables without a normal distribution. Biochemical values were expressed as the mean (SE) for comparison.

Results

Table 1: Demographic characteristics of study population

	Controls (*n*=50)	Alcoholic pancreatitis (*n*=50)	Tropical pancreatitis (*n*=50)
Age (mean [SD])	38 (14.6)	44 (13.7)	36 (14.6)
BMI (mean [SD])	21.6 (3.2)	19.7 (3.4)	19.3 (3.4)
Gender (male:female)	32:18	50:0	36:14
Diabetics	0	27 (54%)	29 (58%)
Smokers	0	41 (82%)	7 (14%)
Pain	0	37 (74%)	41 (82%)

Of the 100 patients, there were 50 TCP patients and 50 ACP patients. 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.

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

	Controls	**Alcoholic pancreatitis**	**Tropical pancreatitis**
Erythrocyte GSH (μmol/g Hb)	8.52 (0.24)	6.34 (0.22)	5.06 (0.22)
Erythrocyte GPx (nmol of NADPH oxi-dized/min/g Hb)	19.07 (0.36)	15.44 (0.56)	15.75 (0.46)
Erythrocyte SOD (IU/g Hb)	2974.82 (48.92)	2180.85 (75.35)	2307.63 (85.65)
Erythrocyte TBARS (nmol/g Hb)	5.65 (0.15)	10.2 (0.52)	7.43 (0.32)
Plasma vitamin C (mg/dL)	0.84 (0.07)	0.28 (0.05)	0.4 (0.06)

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

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

	Diabe-tes	**Erythrocyte GSH (μmol/g Hb)**	**Erythrocyte GPx (nmol of NADPHoxidized/min/g Hb)**	**Erythrocyte SOD (IU/g Hb)**	**Erythrocyte TBARS (nmol/g Hb)**	**Plasma vitamin C (mg/dL)**
Controls		8.58 (0.22)	19.08 (0.36)	2988.82 (49.96)	5.65 (0.15)	0.84(0.06)
Chronic pancreatitis	Yes	5.40 (0.2)	14.28 (0.37)	2266.44 (64.78)	9.8 (0.39)	0.36(0.04)
	No	5.91 (0.24)	13.97 (0.5)	2278.73 (59.02)	9.02 (0.35)	0.3(0.04)
Tropical pancreatitis	Yes	6.04 (0.36)	14.12 (0.56)	2264.76 (78.31)	11.05 (0.6)	0.28(0.04)
	No	6.51 (0.41)	15.99 (0.82)	2185.01 (92.26)	9.52 (0.6)	0.31(0.06)
Alcoholic	Yes	4.64 (0.16)	14.7 (0.44)	2268.52(108.0	8.32	0.52(0.09

pancreatitis				8)	(0.34)	)
	No	5.38 (0.22)	12.98 (0.55)	2378.62 (72.76)	8.52 (0.37)	0.28(0.06)

We found lower values of erythrocyte GSH, GPx, SOD, and plasma vitamin C and higher erythrocyte TBARS in both diabetic and non-diabetic CP patients as compared to healthy controls. However, we did not find differences between diabetic and non-diabetic TCP patients or between diabetic and non-diabetic ACP patients.

Discussion

Zinc (Zn) is one of the most common trace elements in the human body and plays a substantial role in growth and development, acting as a signaling factor. [24] This metal takes part in the regulation of chronic inflammatory status through the reduction of inflammatory cytokines. Zinc also reduces oxidative stress by participating in the synthesis of antioxidant enzymes and acts as a catalyzer of enzymes, taking part in lipid, carbohydrate, and protein metabolism. It is involved in the synthesis, storage, and release of insulin, which suggests the critical role of this microelement in the progression of type-2 diabetes mellitus, atherosclerosis, and metabolic syndrome (MS). [25-28]

Chronic pancreatitis is a long-standing inflammation of the pancreas that alters the organ's normal structure and functions. [29] It can present as episodes of acute inflammation in a previously injured pancreas, or as chronic damage with persistent pain or malabsorption. It is a disease process characterized by irreversible damage to the pancreas as distinct from reversible changes in acute pancreatitis. Of the 100 patients, there were 50 TCP patients and 50 ACP patients. 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. Most available data indicate that the primary site for the development of CP is the pancreatic acinar cell. [30] The role of reactive oxygen species (ROS) has been studied in both experimental and human CP. [31,32] ROS play a role in perpetuating the pancreatic inflammation and the development of extra pancreatic complications. [33] 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. [34]

Plasma vitamin C was lower whereas, erythrocyte TBARS was higher in TCP patients as compared to ACP patients. Erythrocyte GSH level was significantly low in ACP as compared to TCP patients. We found lower values of erythrocyte GSH, GPx, SOD, and plasma vitamin C and higher erythrocyte TBARS in both diabetic and non-diabetic CP patients as compared to healthy controls. However, we did not find differences between diabetic and non-diabetic TCP patients or between diabetic and non-diabetic ACP patients. Quillot et al. have reported that diabetes worsens the antioxidant status in CP patients. [35] Braganza et al [36] have suggested the need for region specific antioxidant supplementation.

Conclusion

The 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.

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