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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 its Correlation with Zinc Status

Manish Kumar Bhaskar

Senior Consultant, Department of Gastroenterology, PARAS, HMRI Hospital, Patna, Bihar, India

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Corresponding Author: Dr. Manish Kumar Bhaskar
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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 for two years and Chronic pancreatitis patients were recruited for the study and were 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.

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 lower 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: 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

Introduction

Chronic pancreatitis (CP) is inflammation of the pancreas that does not heal or improve-it gets worse over time and leads to permanent damage. Chronic pancreatitis eventually impairs a patient's ability to digest food and make pancreatic hormones. Chronic pancreatitis often develops in patients between the ages of 30 and 40, and is more common in men than women. [1] Chronic pancreatitis is a long-standing inflammation of the pancreas that alters the organ's normal structure and functions. [2] 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.

Chronic pancreatitis is possibly a result of a complex interaction between multiple factors. Within the framework of the latter, there is a tacit acknowledgement that we have not yet identified all the risk factors, genetic or otherwise, that predispose an individual to developing chronic pancreatitis. This is especially relevant for the vast majority of patients suffering from Idiopathic Chronic Pancreatitis (ICP), including its variant Tropical Calcific Pancreatitis (TCP), which is more prevalent in India. The pathophysiologic mechanisms of chronic pancreatitis are not fully understood. Most available data indicate that the primary site for the development of CP is the pancreatic acinar cell. [3] The role of reactive oxygen species (ROS) has also been postulated in perpetuating the pancreatic inflammation and the development of extra pancreatic complications. [4] ROS represent oxidative state of cell. In cell, there is defense enzyme which are active against oxygen radical species.

Zinc (Zn) are essential dietary trace metals. Over 300 metalloenzymes require Zn as a catalyst, and almost 2500 human transcription factors require Zn to maintain their structural integrity. Cu-Zn

superoxide dismutase (SOD) is a first-line defense enzyme against oxygen radical species and p53 is a zinc-containing transcription factor which is important in the DNA damage response. [5] Zinc modulate the oxidoreductive environment in cells through modulation of thiol status and antagonizing the activities of iron and copper. Zinc is a component of metallothioneins, which are part of antioxidant defenses. [6] In what may be considered over-simplification, it can be propounded that, within the paradigm of chronic pancreatitis, Zn has considerable anti-oxidant properties.

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 ,PARAS, HMRI Hospital, Patna, Bihar, India for two years and Chronic pancreatitis patients were recruited for the study and were 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. 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. [7]

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. [8]

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 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 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) [9], (GPx) [10] SOD [11], TBARS) [12], haemoglobin [13] and plasma vitamin C [14] 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. [15] 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). [16]

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

Table 1: Demographic characteristics of study population

	Controls (*n*=50)	Alcoholic pancreatitis (*n*=50)	Tropical pancreatitis (*n*=50)
Age (mean [SD])	37 (12.8)	43 (12.8)	36 (14.6)
BMI (mean [SD])	20.5 (3.1)	19.6 (3.2)	19.2 (3.3)
Gender (male:female)	30:20	50:0	38:22
Diabetics	0	26 (52%)	29 (58.2%)
Smokers	0	40 (80%)	6 (12%)
Pain	0	36 (72%)	40 (80%)

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)	8.59 (0.21)	6.21 (0.25)	5.07 (0.25)
Erythrocyte GPx (nmol of NADPH oxidized/min/g Hb)	19.06 (0.33)	15.41 (0.54)	15.83 (0.48)
Erythrocyte SOD (IU/g Hb)	2984.87 (49.96)	2179.82 (74.36)	2304.77 (86.66)
Erythrocyte TBARS (nmol/g Hb)	5.62 (0.13)	10.1 (0.51)	7.44 (0.33)
Plasma vitamin C (mg/dL)	0.82 (0.06)	0.29 (0.04)	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 lower 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])

	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	Yes	5.38 (0.2)	14.33 (0.37)	2266.55 (64.72)	9.8 (0.39)	0.38(0.05)
pancreatitis	No	5.93 (0.24)	14.95 (0.5)	2281.73 (59.02)	9.02 (0.35)	0.3(0.04)
Tropical	Yes	6.02 (0.32)	14.11 (0.57)	2265.72 (78.31)	11.05 (0.6)	0.28(0.05)
pancreatitis	No	6.51 (0.41)	15.99 (0.82)	2185.01 (92.26)	9.52 (0.6)	0.31(0.06)
Alcoholic	Yes	4.61 (0.15)	14.6 (0.45)	2267.54(108.08)	8.29 (0.34)	0.52(0.09)
pancreatitis	No	5.39 (0.22)	13.95 (0.55)	2373.62 (72.76)	8.55 (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

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. [17] The role of reactive oxygen species (ROS) has been studied in both experimental and human CP. [18,19] ROS play a role in perpetuating the pancreatic inflammation and the development of extra pancreatic complications. [20] 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. [21]

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 lower in ACP as compared to TCP patients. 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. [22] 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. [23] Braganza et al[24] have suggested the need for region specific antioxidant supplementation 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

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.

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