A Randomized Controlled Trial of Antioxidant Supplementation for Pain Relief in Patients With Chronic Pancreatitis

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Background & Aims: **Oxidative stress has been implicated in the pathophysiology of chronic pancreatitis (CP). We evaluated the effects of antioxidant supplementation on pain relief, oxidative stress, and antioxidant status in patients with CP. *Methods:* In a placebo-controlled double blind trial, consecutive patients with CP were randomized to groups that were given placebo or antioxidants for 6 months. The primary outcome measure was pain relief, and secondary outcome measures were analgesic requirements, hospitalization, and markers of oxidative stress (thiobarbituric acid-reactive substances [TBARS]) and antioxidant status (ferric-reducing ability of plasma [FRAP]). *Results:* Patients (age 30.5 ± 10.5 years, 86 male, 35 alcoholic, and 92 with idiopathic CP) were assigned to the placebo (n = 56) or antioxidant groups (n = 71). After 6 months, the reduction in the number of painful days per month was significantly higher in the antioxidant group compared with the placebo group (7.4 ± 6.8 vs 3.2 ± 4, respectively; $P <$ .001; 95% CI, 2.07, 6.23). The reduction in the number of analgesic tablets per month was also higher in the antioxidant group (10.5 ± 11.8 vs 4.4 ± 5.8 respectively; $P =$ .001; 95% CI, 2.65, 9.65). Furthermore, 32% and 13% of patients became pain free in the antioxidant and placebo groups, respectively ($P =$ .009). The reduction in the level of TBARS and increase in FRAP were significantly higher in the antioxidant group compared with the placebo group (TBARS: placebo 1.2 ± 2.7 vs antioxidant 3.5 ± 3.4 nmol/mL; $P =$ .001; 95% CI 0.96, 3.55; FRAP: placebo −5.6 ± 154.9 vs antioxidant 97.8 ± 134.9 μMFe^{+2} liberated, $P =$ .001, 95% CI 44.98, 161.7). *Conclusions:* Antioxidant supplementation was effective in relieving pain and reducing levels of oxidative stress in patients with CP.**

Chronic pancreatitis (CP) is a progressive inflammatory disease of the pancreas resulting in slow destruction of pancreatic parenchyma and subsequent fibrosis.[1] Clinically, patients with CP present with abdominal pain in early stage and with diabetes and maldigestion in late stage due to endocrine and exocrine insufficiency, respectively.[2] Pain is the major problem in 90% of the patients with CP.[3] Although the mechanism of pain is not well understood, pancreatic ductal hypertension, pancreatic inflammation, and consequent pancreatic perineural infiltration by immune cells have been suggested to be important causes of pain in CP.[4] There is no effective medical therapy for relief from pain of chronic pancreatitis.[5] Endoscopic treatment and surgery are indicated in patients with dilated pancreatic duct, with the intent of decompressing the obstructed pancreatic ductal system that results from stones and/or stricture.[6,7] Both are invasive forms of therapy and their results are not satisfactory.

Oxidative stress has been implicated in the pathophysiology of CP.[8,9,10] Xenobiotics are detoxified in the body through phase I and phase II pathways, chiefly in the liver.[11] 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.[12,13] The pancreatic acinar cells are also exposed to oxidative stress.[14] 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.[15,16] Free radical peroxidation products may act as second messengers and block exocytosis in the pancreatic acinar cells, leading to increased autophagy and crinophagy and thus diverting the pancreatic enzymes into interstitium, causing degranulation of mast cells and resulting in inflammation mediated by chemotaxis and pain.[17]

A few reports have shown an increased oxidative stress in patients with alcoholic and idiopathic chronic pancre-

atitis.[18,19,20] Although 2 studies with a small sample size had reported some benefit of antioxidants in patients with CP,[21,22] data are insufficient to show whether supplementation with antioxidants will decrease oxidative stress and relieve pain in patients with CP. Convincing evidence is thus lacking to recommend antioxidants for the treatment of patients with CP. The objective of the present randomized controlled trial was to study the role of antioxidant supplementation for relief from pain and attenuation of oxidative stress in patients with CP.

Methods

Study Design

A double blind randomized placebo-controlled trial.

Setting

A tertiary care academic center.

Patients

All consecutive patients with chronic pancreatitis attending the pancreas clinic in our hospital were evaluated for inclusion in the study.

Inclusion Criteria

Patients with CP presenting with significant abdominal pain of pancreatic origin were included in the study. Pain was considered significant if there was at least 1 episode of pain every month requiring analgesics during the preceding 3 months, or at least 1 episode of severe pain requiring hospitalization in the preceding 3 months.

Exclusion Criteria

Patients with the following conditions were excluded: (a) having received earlier or taking at present antioxidant therapy, (b) having had an intervention earlier in the form of surgery or endoscopic therapy and/or lithotripsy for pancreatic calculi, (c) uncontrolled diabetes, (d) comorbid diseases such as liver disease, chronic renal failure, malignancy, and hypertension that might affect the antioxidant status and oxidative stress levels, (e) complications of CP such as pseudocyst, bile duct obstruction, or pancreatic cancer, (f) narcotic addicts (narcotic addiction defined according to the American Psychological Association's Diagnostic and Statistical Manual of Mental Disorders), (g) pregnant and lactating mothers, and (h) age <12 years.

Diagnosis of CP

The diagnosis of CP was made in the appropriate clinical setting if there was evidence of pancreatic duct dilatation and/or irregularity, and/or pancreatic calcification on imaging studies, for example, ultrasonography, endoscopic retrograde cholangiopancreatography, contrast enhanced computed tomography, and/or magnetic resonance imaging with magnetic resonance cholangiopancreatography.[23]

Etiology of CP

The etiology of CP was determined as follows:

(a) Alcoholic CP: If a patient was drinking more than 40 g alcohol per day for >5 years.
(b) Hereditary CP: If ≥1 first-degree relative was suffering from CP.
(c) Obstructive CP: If there was evidence of an obstructive pathology such as tumor in the proximal pancreatic duct and an upstream ductal dilatation.
(d) Hyperparathyroidism: If the serum level of parathyroid hormone was elevated.
(e) Traumatic: If there was a history of definite abdominal trauma with imaging evidence of pancreatic injury and subsequent ductal dilatation.
(f) Idiopathic: If no definite cause of CP was identified. Idiopathic CP was also called tropical pancreatitis.

Controls

One hundred and four healthy subjects were included in the study to compare the markers of oxidative stress and antioxidant levels at baseline between patients with CP and healthy controls. The healthy controls were recruited from among the patients' relatives and hospital staff. They were free from any chronic disease, and were non-alcoholic and non-smokers.

Work-Up of Patients

All the study patients underwent a detailed clinical evaluation and hematological and biochemical investigations that included liver function tests, renal function tests, serum calcium and phosphate, lipid profile at baseline, and serum amylase during acute exacerbation of pain. In addition, the following imaging studies were done: (a) transabdominal ultrasonography (b) magnetic resonance cholangiopancreatography or endoscopic retrograde cholangiopancreatography, and (c) contrast enhanced computed tomography of the abdomen.

Nutritional Assessment

Nutritional status was determined by body mass index (BMI). Patients were classified as either undernourished (BMI <18.5 kg/m^2), normally nourished (BMI 18.6–24.9 kg/m^2) or overweight (BMI 25–29.9 kg/m^2).[24]

Assessment of Complications of CP

Diabetes mellitus was diagnosed as per World Health Organization guidelines, if the fasting plasma glucose was ≥126 mg/dL or 2 h after glucose load the plasma glucose was ≥200 mg/dL.[25] Steatorrhea was diagnosed if the stool fat was greater than 7 g per 24-h as measured by Van de Kamer method.[26] Other complications such as pseudocysts, bile duct obstruction, and splenic vein thrombosis were diagnosed on imaging studies.

Management of CP

All the patients were treated in the standard manner with analgesics on demand and pancreatic enzyme replacement therapy in the dose of 4 capsules with meals, 3 times a day, each containing 8,000 units of lipase and 30,000 USP of proteases (Digestomen-P, Minarini Raunaq Pharma Ltd, India). Alternative therapy in the form of endoscopic and/ or surgical treatments was offered only when the medical treatment including the study intervention failed to relieve the pain of the patient.

Study Intervention

Randomization and blinding. The study subjects were randomized to receive either antioxidants or placebo. A block randomization process with concealed allocation of study medication was followed. The random number sequence was computer generated by a statistician not associated with the conduct of the study. The drug and the placebo were packed according to the code sheet and the boxes were numbered sequentially from 1 to 200. Each box contained either the drug or the placebo capsule for a period of 6 months. This was done by a person not associated with the study. The boxes were serially allocated as new patients were recruited into the study. Double blinding was done to ensure minimum bias. The clinicians attending to the patients were not involved in the randomization process and were blinded to the type of treatment received by the patient. Patients were blinded to the identity of the intervention that they were receiving because the placebo, an inert material (starch), was identical to the active drug in packaging, appearance, and schedule of administration. Separate individuals generated the allocation sequence, enrolled participants, and assigned participants to their groups.

Study medication. The antioxidant supplementation included daily doses of 600 μg organic selenium, 0.54 g ascorbic acid, 9000 IU β-carotene, 270 IU α-tocopherol and 2 g methionine (Betamore G, Osper Pharmanautics, India). The compliance and timing of the medication were monitored at each visit of the patient by questioning the patient and relatives, evidence of the empty boxes of the drug/placebo, and capsule count.

Assessment of Pain

The assessment of pain was done in terms of number of painful days per month, the requirement of oral/parenteral analgesics, and the need for hospitalization. The patients were provided with a pain diary to keep a detailed record of pain and consumption of analgesics. Assessment of pain was not done quantitatively such as on a visual analog scale or Likert scale because that is subject to bias and individual patient tolerance. Pain was assessed in terms of number of painful days requiring treatment such as analgesics or hospitalization. The assessment of pain, analgesic requirement, and hospitalization was done for 3 months prior to inclusion in the study and every month for 6 months during the period of intervention. The number of man-days lost per month due to pain was also recorded.

Follow-up assessment. All the patients were followed up monthly for 6 months with periodic clinical evaluation. Patients were monitored for the development of complications such as pseudocyst, portal hypertension, and/or biliary stricture during the follow-up. Any other symptom due to the primary disease was duly recorded. In addition, standard hematological and biochemical investigations were repeated at every visit. Imaging tests were repeated as indicated clinically.

Safety Evaluation

All the patients were carefully monitored for any adverse drug reactions of the prescribed intervention. Safety and tolerability assessments included the monitoring and recording of all adverse events and serious adverse events and of concomitant medications/significant non-drug therapies. Regular symptom assessment, physical examination, checks of routine blood chemistry, hematology, and urine analysis were carried out.

Primary Outcome Measure

Reduction in the number of painful days per month.

Secondary Outcome Measures

1. Decrease in the requirement of the numbers of oral analgesic tablets and parenteral analgesic injections per month.
2. Decrease in the number of attacks of severe pancreatitis requiring hospitalization.
3. Percentage of patients becoming pain-free during the study period.
4. Change in the markers of oxidative stress and antioxidant status following the intervention.

Estimation of Markers of Oxidative Stress and Antioxidant Capacity

The markers of oxidative stress estimated in the present study included serum superoxide dismutase (S-SOD) and thiobarbituric acid reactive substances (TBARS), which indicate the degree of lipid peroxidation. The markers of antioxidant status studied were vitamins A, C, and E, total antioxidant capacity (measured as ferric reducing ability of plasma; FRAP), total glutathione (T-GSH), and erythrocyte SOD (e-SOD). The methods to measure these markers were standardized in our laboratory and included quality control assays.[27] The blood samples were collected in the morning after an overnight fast. Ten milliliters of blood was drawn into vacutainers. The plasma and sera were separated within 2 hours of blood collection and stored at −80°C till analyzed. TBARS was measured as a marker of lipid peroxidation by the method of Buege and Aust.[28] Superoxide dismutase (se-

rum and erythrocyte) was assessed by the method given by Marklund and Marklund.[29] Total glutathione was measured by Griffith's method.[30] FRAP was assessed by the method described by Benzie and Strain.[31] Vitamin A (all-trans-retinol) was measured by trifluoroacetic acid method.[32] Vitamin E (α-tocopherol) was measured by the method of Emmerie and Engel.[33] Vitamin C (total ascorbic acid) was measured by the method described by Okamura.[34]

Statistical Analysis

Sample size calculation. Sample size was calculated on the basis of probability sampling method. Data from our own preliminary study[35] were used, hypothesizing that minimal meaningful reduction in the number of painful days per month should be at least 50% in the intervention group. For the baseline value of 5.8 ± 7.34 painful days per month, a sample size of 100 patients in each arm was calculated using the standard formula:

$$n = \frac{[Z_\alpha + Z_\beta]^2 \, 2(SD)^2}{(\mu_2 - \mu_1)^2}$$

The power of the study was kept at 80% and significance level at 5% ($\alpha = 0.05$). However, the doctoral committee (study supervising committee) suggested an interim analysis at the end of 4.5 years since the commencement of the study because the stipulated maximum duration of the study was 5 years and the recruitment rate of patients into the study was slower than expected because of various inclusion and exclusion criteria. Further recruitment of patients was stopped after October 2006 because the results were found to be significant in favor of antioxidant therapy.

Descriptive statistics (mean, standard deviation, and frequency distribution) were calculated for each variable in the study. Data are presented as mean ± SD. To compare the 2 groups, Student t test or analysis of variance for quantitative variables and chi square test for qualitative variables were applied as appropriate. Linear regression was used to adjust for baseline values. The effect of the etiology of chronic pancreatitis (alcoholic or idiopathic) on pain response was analyzed by regression analysis. Intention to treat analysis was done to compare the 2 groups with regard to the outcome measures. Statistical software STATA 9.0 (Statacorp, Texas, US) was used for statistical analysis.

Ethical Clearance

The study was approved by the Ethics Committee of our institute. The purpose of the study was explained clearly to the patients and their informed written consent was obtained. The trial had been registered at clinicaltrials.gov (NCT00319358). We followed the CONSORT guidelines for the conduct of a randomized study (Figure 1).
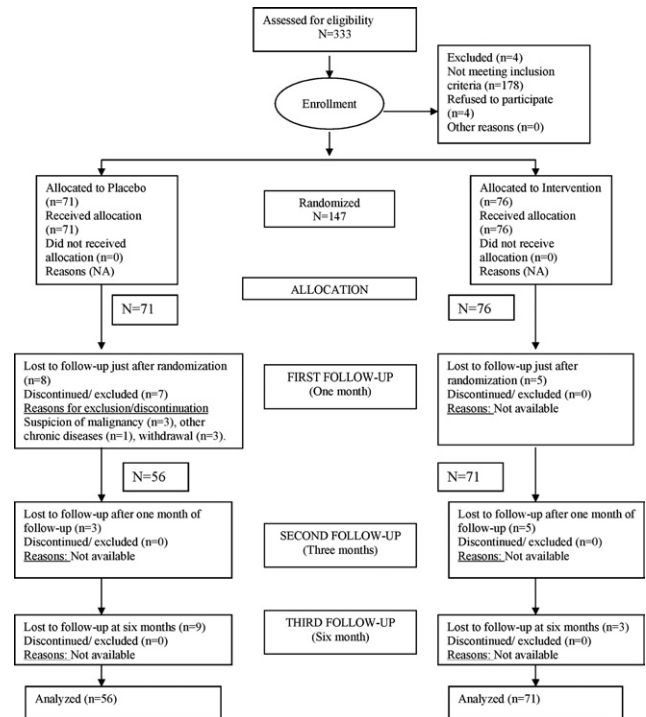

Figure 1. CONSORT flow chart.

Results

A total of 333 patients with CP were assessed during the study period. Of them, 151 patients fulfilled the inclusion criteria, but 4 refused to participate in the study. The remaining 147 patients were randomized: 71 to the placebo and 76 to the antioxidant arm. One hundred twenty seven patients reported at the time of first follow-up at one month and were included in the final analysis (CONSORT flow chart).

Clinical, Demographic, and Biochemical Parameters

The mean age of the patients, gender distribution, duration of disease, alcohol consumption and smoking status, clinical features, and body mass index were comparable in the 2 groups at baseline (Table 1). The hematological and biochemical investigations were also similar at baseline between the groups (Supplementary Table 1).

Response to Treatment

Primary outcome measure. *Number of painful days per month.* The 2 groups were comparable at baseline with regard to the number of painful days per month. Following therapy, the number of painful days per month was significantly lower in the antioxidant group compared with that in the placebo group at 6 months ($P = .012$) (Table 2, Figure 2). Furthermore, the reduction in the number of painful days per month was significantly higher in the antioxidant group compared with that in the placebo group (Table 2). This significance was re-

Table 1. Baseline Demographic and Clinical Parameters in the Antioxidant and Placebo Groups

Parameter	Placebo group (n = 56)	Antioxidants group (n = 71)	P value
Age (y)	29.6 ± 9.3	31.3 ± 11.4	.345
Male:females (n)	39:17	47:24	.414
Duration of disease (y)	4.8 ± 5.4	4.5 ± 4.2	.731
Etiology, alcoholic:idiopathic (n)	15:41	25:46	.206
If alcoholic			
Amount of alcohol (g/d)	103.5 ± 71.1	102 ± 81.5	.954
Duration of alcohol intake (y)	10.7 ± 5.1	9.7 ± 5.9	.584
Smokers: nonsmokers (n)	14:42	22:49	.553
If smokers			
Number of cigarettes per day	8 (1–20)	8 (1–80)	.490
Duration of smoking (y)	15.8 ± 7.6	15.7 ± 9.8	.874
Diabetes (n)	8	15	.335
Duration of diabetes (y)	1.5 (0.1–10)	3 (0.1–15)	.336
Steatorrhea (n)	12	14	1.000
Dilatation of main pancreatic duct	45 (80.4%)	55 (77.5%)	.535
Pancreatic calcifications	46 (82.1%)	59 (83.1%)	.535
BMI (kg/m^2)	20.2 ± 3.1	19.7 ± 3.5	.372
Undernourished (BMI <18.5)	18	28	.547
Normal nourished (BMI = 18.6–24.9)	36	39	
Overweight (BMI >25)	2	4	

tained after adjustment for the number of painful days at baseline. The reduction in the number of painful days was noted at 3 months of follow up (placebo 3.84 ± 5.52 vs antioxidant 1.96 ± 4.05; P = .03) (Supplementary Figure 1). The mean difference of the reductions in painful days/month between the placebo and the antioxidant groups among patients with alcoholic pancreatitis was 3.73 ± 2.56, which was statistically not different compared with 4.22 ± 1.24 among patients with idiopathic pancreatitis (P = .61).

    Secondary outcome measures. *Analgesic requirement.* The requirement for the number of oral analgesic tablets per month was similar between the antioxidant and the placebo groups at baseline. The reduction in the number of oral analgesic tablets per month was significantly higher in the antioxidant group following therapy compared with that in the placebo group (placebo 4.4 ± 5.8 vs antioxidants 10.5 ± 11.8; P = .001, 95% CI 2.65, 9.65). Ten patients in the antioxidants group and 7 in the placebo group were taking opioid analgesics on an as-

required and when-required basis. The requirement of parenteral analgesic injections, which was comparable at baseline, also decreased significantly at 6 months in the antioxidants group compared with that in the placebo group (P = .026). The reduction in parenteral analgesic requirement was significantly higher in the antioxidant group after adjusting for baseline values (Table 3).

    Need for hospitalization. The need for hospitalization, which was similar at baseline (placebo 0.19 ± 0.23 vs antioxidants 0.23 ± 0.29; P = .411) decreased at 6 months (placebo 0.05 ± 0.12 vs antioxidants 0.02 ± 0.06; P = .062). Although the reduction in the need for hospitalization was not different between the 2 groups (placebo 0.14 ± 0.23 vs antioxidants 0.2 ± 0.28; P = .220, 95% CI 0.036, 1.57), the difference between the 2 groups became significant after adjustment for the baseline values (mean difference 0.034, 95% CI 0.002, 0.069, P = .049).

    Pain-free patients. One third (23/71) of the patients in the antioxidant group became pain free during

Table 2. Number of Painful Days in the Antioxidant and Placebo Groups Following Intervention

	Placebo group	Antioxidant group	Mean difference (95% CI lower, upper)	P value
Number of painful days				
Prior to intervention	7.21 ± 5.34 range 1–30 (n = 56)	9.14 ± 7.60 range 1.67–30 (n = 71)	1.92 (0.44, 4.29)	.111
At 6 months after intervention	3.36 ± 4.35 range (0–20.33) (n = 53)	1.68 ± 2.80 range (0–17.17) (n = 66)	1.68 (0.37, 2.98)	.012
Reduction in painful days per month	3.21 ± 3.99 (n = 53)	7.37 ± 6.75 (n = 66)	4.15 (2.07, 6.23)	<.001
			2.33* (1.15, 3.51)	<.001

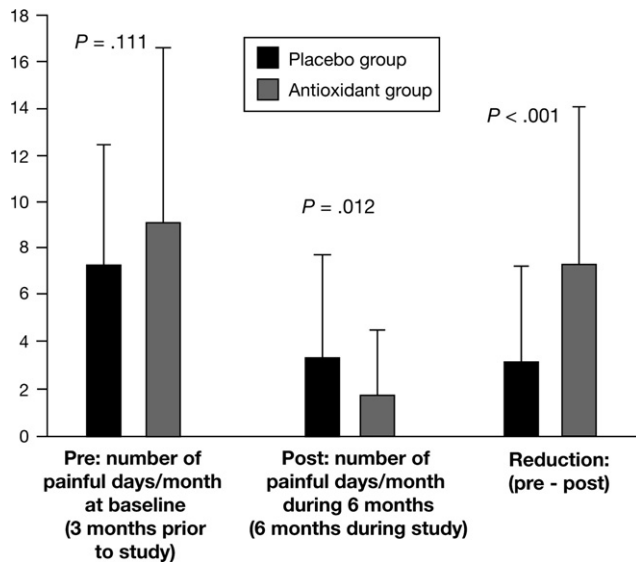*Adjusted for number of painful days per month prior to intervention.

Figure 2. Effect of intervention on primary outcome measure: number of painful days per month.

6 months of therapy compared with only 7 of 56 patients in the placebo group ($P = .009$).

Man-days lost. The number of man-days lost per month, which was similar at baseline (placebo 10.7 ± 8.4 vs antioxidants 12.9 ± 9.3, $P = .161$) decreased at 6 months (placebo 3.1 ± 4.5 vs antioxidants 1.5 ± 3, $P = .029$). The reduction was significantly higher in the antioxidant group (placebo 7.6 ± 7.2 vs antioxidants 11.4 ± 9.1, $P = .014$, 95% CI 0.86, 7.4) and the significance was retained after adjustment for the baseline values (mean difference 1.98, 95% CI 0.59, 3.36, $P = .005$).

Markers of oxidative stress and antioxidant defense. Oxidative stress and antioxidant status were measured in patients with CP and healthy controls at baseline. The mean age of the healthy control subjects was 33.04 ± 11.1 years and there were 73 men and 31 women. At baseline, the parameters of oxidative stress were higher in patients with CP as compared with those in controls (Table 4). The antioxidant status was lower in CP patients as compared with that in controls (Table 4).

Markers of oxidative stress. Lipid peroxidation products (TBARS), the marker of oxidative stress, decreased significantly ($P = .001$) in the antioxidant group compared with that in the placebo group at 6 months following the intervention. The reduction in TBARS was significantly higher in the antioxidant group compared with that in the placebo group ($P = .001$) (Table 5, Supplementary Figure 2). Serum SOD decreased significantly at 6 months in the antioxidant group as compared with that in the placebo group ($P < .001$) (Table 5).

Markers of antioxidant status. The markers of antioxidant status, vitamins A, C, and E, which also acted as markers of compliance, increased significantly at 6 months in the antioxidant group when compared with those in the placebo group. The increments in these markers were also significant in the antioxidant group as compared with those in the placebo group ($P < .001$) (Table 6). The total antioxidant capacity, as measured by FRAP, increased significantly in the antioxidant group at 6 months as compared with that in the placebo group ($P = .038$). The increase in FRAP was significantly higher in the antioxidant group compared with that in the placebo group, in which FRAP actually decreased ($P = .001$) (Table 6, Supplementary Figure 3).

Adverse drug reactions. A total of 15 adverse drug reactions were reported, 3 in the placebo group and 12 in the antioxidant group, all during the first month of treatment. The most commonly reported adverse event was headache (3 in the placebo group and 8 in the antioxidant group). The other adverse reaction was constipation in 4 patients in the antioxidant group. None of the patients experienced any significant adverse drug reaction requiring discontinuation of the therapy. None of the patients died during the study.

Need for Alternative Treatment

None of the patients required endotherapy or surgery during the study period. One patient each in the antioxidant and the placebo groups required surgery, and 3 patients in the antioxidant group and 8 patients in the placebo group required endotherapy during the follow up.

Table 3. Analgesic Requirement in the Antioxidant and Placebo Groups Following Intervention

	Placebo group	Antioxidant group	Mean difference (95% CI lower, upper)	P value
Number of oral analgesic tablets required per month				
Prior to intervention	9.30 ± 9.04	13.34 ± 15.03	4.04 (0.46, 8.55)	.078
At 6 months after intervention	4.02 ± 5.10	2.28 ± 4.83	1.73 (0.07, 3.54)	.06
Reduction in number of oral analgesic tablets required/month	4.36 ± 5.78	10.51 ± 11.77	6.15 (2.65, 9.65)	.001
			2.86 (1.39, 4.33)*	<.001
Number of parenteral analgesic injections required per month				
Prior to intervention	2.65 ± 2.77	3.14 ± 4.30	0.48 (0.82, 1.80)	.465
At 6 months after intervention	0.75 ± 1.41	0.32 ± 0.53	0.43 (0.05, 0.80)	.026
Reduction in the number of parenteral analgesic injections required/month	1.89 ± 3.01	2.59 ± 3.88	0.70 (0.59, 1.99)	.288
			0.44 (0.07, 0.81)*	.019

*Adjusted for number of analgesics required per month prior to intervention.

Table 4. Oxidative Stress and Antioxidant Status at Baseline: Healthy Controls Versus Patients with CP

	Healthy controls (n = 104)	Patients with CP (n = 125)	P value
TBARS (nmoles/mL)	1.34 ± 0.6	7.1 ± 3.5	<.001
S-SOD (U/mL)	1.2 ± 0.6	3.5 ± 3	<.001
Vitamin A (μg/dL)	44.6 ± 9.3	22.4 ± 7.5	<.001
Vitamin C (mg/dL)	1.2 ± 0.5	1.2 ± 0.7	.476
Vitamin E (mg/dL)	1.6 ± 0.7	0.74 ± 0.3	<.001
FRAP ($\mu molFe^{+2}$ liberated)	454 ± 151	340 ± 127	<.001
E-SOD (U/mg Hb)	88.2 ± 38	24 ± 18	<.001
E-TGSH (mmol/g Hb)	1.9 ± 0.8	0.89 ± 0.8	<.001

Discussion

Abdominal pain, the predominant symptom in patients with CP, is difficult to treat. Analgesics provide only temporary benefit and opioid analgesics have an added risk of addiction. Initial clinical trials suggested that pancreatic enzymes supplementation in high doses might provide some relief from pain in patients with CP. However, randomized controlled trials (RCTs) and a meta-analysis of 6 RCTs subsequently failed to show any substantial benefit of pancreatic enzymes for pain relief in patients with CP.[36] The main reason for a largely ineffective medical treatment is that the mechanism of pain in CP is not well understood. Pancreatic inflammation is considered to be the major determinant of pain in CP. There are histological,[37] biochemical,[38] and imaging evidences[6] to show pancreatic inflammation in CP. Pancreatic inflammation is associated with perineural invasion by inflammatory cells that may exacerbate pain by exposing the nerves directly to cytokines and other nociceptive mediators.[39] Indeed, in advanced end stage CP, when inflammation is largely replaced by fibrosis and pancreatic atrophy, there remains minimal pain—the so-called "burnt out" CP.[40,41]

The present RCT has shown that medical therapy with antioxidants significantly reduced abdominal pain in patients with CP. Since pain is a subjective symptom, its assessment is prone to bias. Therefore, we studied the most objective measures of pain; that is, number of painful days per month, requirement of analgesics, need for hospitalization, and the percentage of patients who became pain free. Significant improvement was noted with antioxidants in respect to all the parameters of pain in the present study. Reduction in pain also resulted in fewer man-days lost, thus providing functional employment gain to the patients. The beneficial effect of antioxidants on pain relief was noted early—at 3 months. Our study included patients with alcoholism as well as those with idiopathic chronic pancreatitis. Idiopathic chronic pancreatitis in India is also known as tropical pancreatitis. Its etiology is not clearly known; both genetic mutations and dietary factors have been postulated as causal factors. Serine protease inhibitor Kazal type 1 (SPINK1) gene mutation has been shown in up to 40% of patients with tropical pancreatitis but the causal association is unproven.[42] SPINK1 enzyme provides protection against only 20% of the activated trypsin within the pancreas, the main culprit in the pathogenesis of pancreatitis, and hence the SPINK1 gene mutation is likely to have only a modifier effect.[43] We have recently shown that any specific dietary factor, such as ingestion of cassava, is not causally related to idiopathic chronic pancreatitis in India.[44] Recent observations suggest that the earlier description of tropical pancreatitis from India may not be true anymore, and the disease resembles idiopathic chronic pancreatitis that is seen in western countries except that it often affects younger persons and is generally an advanced form of the disease.[3,23,45] In the present study, we did not find any effect of possible confounders, such as etiology of chronic pancreatitis or abstinence from alcohol on the treatment response.

Pain also was reduced in the placebo group, which could have several reasons: (a) effect of high dose of pancreatic enzyme supplementation, which was prescribed to patients in both the groups, (b) dietary advice regarding intake of adequate macro- and micronutrients, (c) abstinence from alcohol in a subset of patients, (d) Hawthorne (protocol) effect,[46] and (e) natural course of the disease. Loss to follow-up is an important issue and

Table 5. Oxidative Stress in the Antioxidant and Placebo Groups Following Intervention

	Placebo group		Antioxidant group		P value	Mean difference (95% CI)
	Values	n	Values	n		
TBARS (nmol/mL)						
Baseline	7.07 ± 3.57	54	7.06 ± 3.52	71	.979	0.02 (1.25, 1.28)
One month	6.16 ± 3.61	53	5.87 ± 3.21	68	.641	0.29 (0.94, 1.52)
Six months	5.43 ± 2.69	38	3.61 ± 2.37	62	.001	1.82 (0.79, 2.84)
Reduction	1.20 ± 2.73	38	3.46 ± 3.40	62	.001	2.25 (0.96, 3.55)
Serum SOD (U/mL)						
Baseline	3.61 ± 2.97	54	3.43 ± 3.09	71	.748	0.17 (0.91, 1.26)
One month	2.60 ± 1.95	52	2.73 ± 2.19	69	.729	0.13 (0.62, 0.89)
Six months	3.52 ± 2.64	38	1.97 ± 1.55	62	<.001	1.55 (0.72, 2.39)
Reduction	0.56 ± 2.94	38	1.48 ± 3.07	62	.142	0.92 (0.31, 2.15)

Table 6. Antioxidant Status in the Antioxidant and Placebo Groups Following Intervention

	Placebo group		Antioxidant group			
	Values	n	Values	n	P value	Mean difference (95% CI)
Vitamin A ($\mu g/dL$)						
Baseline	21.92 ± 7.36	54	22.75 ± 7.56	71	.529	0.85 (1.82, 3.52)
One month	23.72 ± 8.09	53	26.95 ± 8.21	69	.032	3.23 (0.28, 6.18)
Six months	25.24 ± 8.14	39	35.54 ± 9.38	63	<.001	10.30 (6.69, 13.91)
Increment	3.16 ± 5.52	39	12.07 ± 6.25	63	<.001	8.91 (6.49, 11.33)
Vitamin C (mg/dL)						
Baseline	1.09 ± 0.56	54	1.33 ± 0.73	71	.05	0.23 (0.0002, 0.547)
One month	1.14 ± 0.62	53	1.66 ± 0.86	68	<.001	0.51 (0.23, 0.79)
Six months	1.19 ± 0.54	38	2.08 ± 0.82	62	<.001	0.88 (0.58, 1.18)
Increment	0.02 ± 0.52	38	0.76 ± 0.78	62	<.001	0.73 (0.44, 1.02)
Vitamin E (mg/dL)						
Baseline	0.72 ± 0.24	53	0.76 ± 0.36	71	.509	0.03 (0.07, 0.15)
One month	0.78 ± 0.25	52	1.10 ± 0.50	69	<.001	0.31 (0.16, 0.46)
Six months	0.81 ± 0.24	38	1.44 ± 0.65	62	<.001	0.62 (0.40, 0.84)
Increment	0.07 ± 0.20	38	0.68 ± 0.47	62	<.001	0.62 (0.46, 0.78)
FRAP ($\mu M\ Fe^{+2}\ liberated$)						
Baseline	352.6 ± 138	54	326.8 ± 117	71	.322	22.80 (22.59, 68.19)
One month	332.1 ± 97	53	370.0 ± 123	68	.069	37.92 (2.97, 78.83)
Six months	371.0 ± 120	38	425.2 ± 128	62	.038	54.15 (2.96, 105.33)
Increment	−5.56 ± 154	38	97.8 ± 134	62	.001	103.37 (44.98, 161.76)
E-SOD ($U/mg\ Hb$)						
Baseline	27.31 ± 19.55	53	21.89 ± 16.40	71	.097	5.41 (0.98, 11.81)
One month	27.50 ± 18.70	52	30.11 ± 28.81	68	.572	2.60 (6.49, 11.71)
Six months	27.30 ± 18.81	38	51.07 ± 20.75	62	<.001	23.77 (15.57, 31.96)
Increment	−1.05 ± 19.35	38	29.99 ± 15.97	62	<.001	31.04 (23.96, 38.13)
E-TGSH ($mmol/g\ Hb$)						
Baseline	0.92 ± 0.72	52	0.81 ± 0.60	70	.373	0.10 (0.13, 0.34)
One month	0.89 ± 0.54	51	1.27 ± 0.96	69	.014	0.37 (0.07, 0.67)
Six months	1.00 ± 0.79	38	1.83 ± 1.01	62	<.001	0.83 (0.45, 1.21)
Increment	−0.01 ± 1.05	38	1.00 ± 0.75	62	<.001	1.02 (0.66, 1.38)

might affect the analysis in a randomized controlled trial. The unavailability of complete outcomes of some of the lost-to-follow-up patients, although there were more in the placebo group, was a concern in the present study too.

The efficacy of antioxidants to reduce oxidative stress and relieve abdominal pain in patients with CP has been studied previously as well. Uden et al first showed the benefit of antioxidants in a crossover trial of 20 patients with CP.[21] However, this study comprised a heterogeneous mix of patients and the duration of intervention was short. An observational study published as an abstract only showed pain relief with antioxidants in patients with alcoholic pancreatitis.[47] A recent randomized study involving 36 patients also showed the benefit of antioxidants in ameliorating pain in patients with CP.[22] Allopurinol, a xanthine oxidase inhibitor, was not found to be beneficial.[48] These studies were, however, limited in their impact and generalizability because of many reasons: (a) small sample size, (b) less robust study design, (c) study of predominantly alcoholic pancreatitis, (d) subjective and variable assessment of pain and its response to therapy, and (e) short duration of therapy and its response.

Commensurate with the clinical benefit in terms of pain relief there was a reduction in oxidative stress and an increase in antioxidant capacity in patients receiving antioxidants compared with those on placebo in the present study. In the present study, the measures of oxidative stress were increased in patients with CP as compared with healthy controls at baseline. At the end of the trial, the markers of oxidative stress reduced significantly only in patients taking antioxidants compared with those taking placebo.

Oxidative stress has been implicated as one of the dominant mechanisms of pancreatic inflammation irrespective of etiology.[49] Oxidative stress results from exposure to xenobiotics, which are detoxified by phase I and phase II pathways. These detoxification pathways also operate in the pancreas. An immunological study of drug metabolizing enzymes in surgical biopsies of the pancreas confirmed induction of the phase I enzymes CYP1A2, CYP3A, and NADPH-CYP oxido-reductase, but not the phase II enzyme glutathione-S-transferase, which facilitates the removal of toxic metabolites by conjugation with glutathione.[50] It has been suggested that oxidative stress is enhanced significantly in patients with CP. Patients with alcoholism

as well as idiopathic CP have elevated markers of oxidative stress such as lipid peroxidation and serum superoxide dismutase.[18,19]

Antioxidant capacity was found to be lower in patients with CP compared with that in healthy controls at baseline. This is likely to be multi-factorial. First, the dietary intake of macro- and micronutrients in patients with CP is lower because of low intake due to pain and alcoholism. Second, the absorption of fat-soluble vitamins, the natural antioxidants, is low in patients with CP due to exocrine insufficiency. Third, an increased exposure to xenobiotics causes consumption of antioxidants. We assessed the antioxidant capacity by measuring FRAP and other individual antioxidants such as vitamins A, E, C, erythrocyte total glutathione, and erythrocyte SOD. FRAP is a sensitive marker of global antioxidant capacity.[30] In the present study, the antioxidant capacity increased significantly in patients taking antioxidant supplementation compared with those taking placebo. The antioxidant capacity of patients with CP has been shown to be low in earlier studies as well.[10,51] Markers of antioxidant capacity such as serum levels of vitamin, E, A, C, and glutathione have been found to be decreased in patients with CP.[10,47]

There are 2 important implications of our study—one with regard to the pathophysiology of CP and the other for management of pain in CP. The fact that measures of oxidative stress were increased initially and decreased subsequently after supplementation with antioxidants suggests that there is a state of heightened free radical mediated injury in CP, and that injury is reversible. Even if oxidative stress is not the sole factor or the initiating factor for pancreatic inflammation, it seems to be playing an important role in either precipitating or perpetuating pancreatic inflammation.

With regard to the management, the present randomized trial has shown that antioxidant therapy is effective for pain relief in patients with CP. This assumes significance since no effective medical therapy exists for pain relief for such patients. The limitation of our study is that it is not known for how long the effect of antioxidants will last and for how long the antioxidant supplementation should be continued.

The mechanism of pain relief by antioxidants is likely to be mediated through a reduction in oxidative stress and pancreatic inflammation. Recent work has shown that transient receptor potential vanilloid 1 (TRPV1), a cation channel present on the sensory pancreatic neurons, is up-regulated and mediates hyperalgesia in experimental chronic pancreatitis.[52] Transient receptor potential A1 (TRPA1) is another excitatory ion channel present on the primary afferent somato-sensory neurons that contain substance P and calcitonin gene-related peptide.[53] It is involved in peripheral

mechanisms controlling pain hypersensitivity. 4-hydroxy-2-nonenal (HNE) is an endogenous product of membrane phospholipids peroxidation in response to tissue injury, inflammation, and oxidative stress. It has recently been shown that HNE provokes release of substance P and calcitonin gene-related peptide from central (spinal cord) and peripheral nerve endings, and injection of HNE into rodent hind paw elicits pain-related behaviors that are inhibited by TRPA1 antagonist.[54] It is thus likely that a reduction in oxidative stress by antioxidants might decrease perineural inflammation and attenuate stimulation of the nociceptive receptors, and relieve pain. Further work is warranted to document such a mechanism of decreased neural inflammation and pain relief by antioxidants.

Concerns have been raised regarding the long-term use of antioxidants. A recent meta-analysis showed an increased mortality due to preventive use of antioxidants.[55] However, antioxidants were used for a long period of time (mean 2.7 y) for either primary or secondary prevention of a variety of diseases including cancers, coronary artery disease, or infections in the trials analyzed in that meta-analysis. The meta-analysis excluded tertiary prevention trials for treatment of specific diseases. A recent study found a trend toward greater adverse effects of antioxidants in patients with acute pancreatitis.[56] However, the clinical setting of acute pancreatitis is much different, with marked systemic inflammation that is the cause of most mortality. Furthermore, the combination of antioxidants, the dose, and the intravenous route used in that study were different from what we have used. The results of that study may not apply to oral supplementation of antioxidants in an appropriate dose for reducing long-standing oxidative stress in chronic pancreatitis. We did not observe any significant adverse drug reactions due to antioxidants in our study. This could be due to the fact that the patients were antioxidant deficient. We believe that antioxidant therapy can be prescribed in an appropriate clinical setting of CP.

We conclude that antioxidant supplementation was effective in relieving abdominal pain in patients with CP and that it led to a significant decrease in oxidative stress in these patients supporting the oxidative stress hypothesis in the etiopathogenesis of CP.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2008.09.028.

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CLINICAL ADVANCES IN LIVER, PANCREAS, AND BILIARY TRACT

Supplementary Table 1. Supplementary: Baseline Laboratory Parameters in the Antioxidant and Placebo Groups

Parameter	Placebo group	Antioxidant group	P value
Hemoglobin (*g/dl*)	12.57 ± 2.17	13.21 ± 2.05	0.145
Fasting blood glucose (*mg/dl*)	109.68 ± 45.69	120.84 ± 61.68	0.343
Serum cholesterol (*mg/dl*)	148.35 ± 44.82	161.46 ± 49.94	0.308
Serum triglycerides (*mg/dl*)	111.36 ± 50.1	134.19 ± 57.69	0.298
Serum Calcium (*mg/dl*)	10.1 ± 0.57	9.9 ± 0.57	0.158
Serum Bilirubin (*mg/dl*)	0.8 ± 0.68	0.69 ± 0.47	0.426
Serum Alkaline phosphatase (*IU*)	160 ± 83.94	173.5 ± 91.69	0.443
Serum Aspartate transaminase (*IU*)	32.87 ± 21.75	36.79 ± 22.71	0.336
Serum Alanine transaminase (*IU*)	29.6 ± 19.3	36.1 ± 31.8	0.223
Total serum protein (*g/dl*)	8.02 ± 0.62	7.91 ± 0.76	0.467
Serum Albumin (*g/dl*)	4.9 ± 2.3	4.54 ± 0.49	0.203
24-h Fecal fat (*g/24-h*)	7.3 ± 10.49	8.78 ± 10.24	0.552
Fecal chymotrypsin (*U/g of stool*)	6.03 ± 4.36	7.78 ± 7.89	0.407
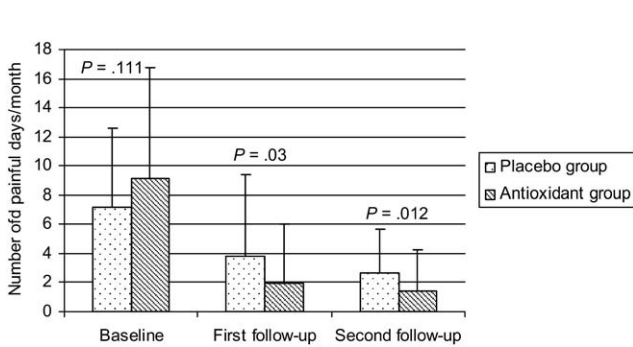
Supplementary Figure 1. The trend in the reduction in the number of painful days/month at baseline, 3 months, and 6 months.
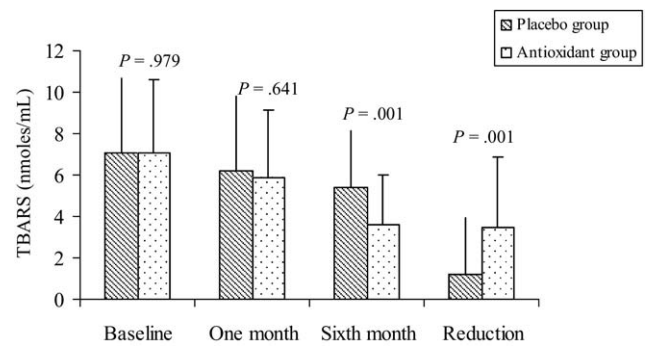
Supplementary Figure 2. Effect of intervention on lipid peroxidation products (TBARS).
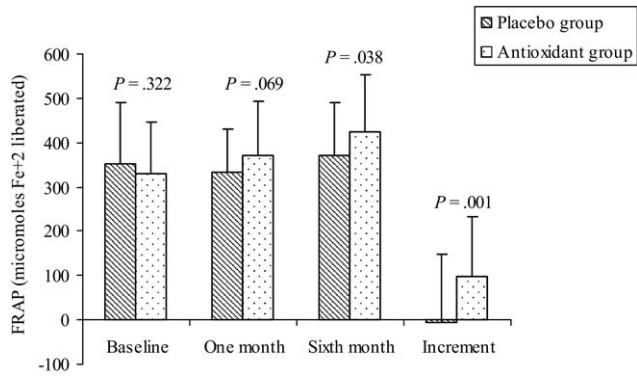
Supplementary Figure 3. Effect of intervention on ferric reducing ability of plasma (FRAP).