Digestive Diseases and Sciences (2023) 68:1500–1510
https://doi.org/10.1007/s10620-022-07676-5

ORIGINAL ARTICLE

Efficacy and Predictors of Pain Response to Combined Antioxidants in Children with Chronic Pancreatitis

Amrit Gopan[1] · Anshu Srivastava[1] · Amrita Mathias[1] · Surender Kumar Yachha[1] · Sunil Kumar Jain[2] · Prabhakar Mishra[3] · Moinak Sen Sarma[1] · Ujjal Poddar[1]

Received: 19 March 2022 / Accepted: 16 August 2022 / Published online: 28 August 2022

Abstract

Background Pain is a major problem in 90% of patients with chronic pancreatitis (CP). Studies evaluating response to antioxidants (AO) are conflicting and no pediatric studies are available.

Aims To evaluate markers of oxidative stress (OS), and efficacy and predictors of response to AO in improving pain in children with CP.

Methods Antioxidants were given to CP children for 6 months. Subjects were assessed at baseline and post-therapy for pain and markers of OS [serum thiobarbituric acid reactive substances (TBARS), superoxide dismutase (S-SOD)] and antioxidant levels [vitamin C, selenium, total antioxidant capacity-ferric reducing ability of plasma (FRAP)]. Matched healthy controls were assessed for OS and antioxidant levels. Good response was defined as ≥ 50% reduction in number of painful days/month.

Results 48 CP children (25 boys, age 13 years) and 14 controls were enrolled. 38/48 cases completed 6 months of therapy. CP cases had higher OS [TBARS (7.8 vs 5.2 nmol/mL; $p < 0.001$)] and lower antioxidant levels [FRAP (231 vs. 381.3 µmol/L; $p = 0.003$), vitamin C (0.646 vs. 0.780 mg/dL; $p < 0.001$)] than controls. Significant reduction in TBARS and S-SOD and increase in FRAP, vitamin C, and selenium occurred after 6 months. 10.5% cases had minor side effects. 26(68%) cases had a good response, with 9(24%) becoming pain-free. Subjects with severe ductal changes had lower median BMI (− 0.73 vs 0.10; $p = 0.04$) and responded less often than those with mild changes (17/29 vs 9/9; $p = 0.036$).

Conclusion CP children have higher OS than healthy controls. Antioxidant therapy is safe. Pain response is seen in 68% cases, less often in patients with severe ductal changes.

Keywords Antioxidants · Pancreatitis, Chronic · Oxidative stress · Pain · Pediatrics

Abbreviations

CP	Chronic pancreatitis
OS	Oxidative stress
AO	Antioxidants
MRCP	Magnetic resonance cholangiopancreatography
MPD	Main pancreatic duct
TBARS	Thiobarbituric acid reactive substances
FRAP	Ferric reducing ability of plasma
S-SOD	Serum superoxide dismutase
Se	Selenium
R^+	Patients with ≥ 50% response in pain after antioxidant therapy
R^-	Patients with < 50% response, no change or worsening of pain after antioxidant therapy
BMI	Body Mass Index
hpf	High power field

Introduction

Chronic pancreatitis (CP) is a progressive inflammatory disease of the pancreas resulting in slow destruction of pancreatic parenchyma and subsequent fibrosis. An epidemiological study showed a threefold rise in incidence of CP in adults, from 5.4 in 1994 to 14/100,000 population in 2011

✉ Anshu Srivastava
avanianshu@yahoo.com

[1] Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh 226014, India

[2] Department of Radiodiagnosis, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

[3] Department of Biostatistics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

[1]. A similar increase was witnessed in children with acute recurrent pancreatitis and CP over the last decade [2].

Pain is a major problem in 90% of patients with CP [3]. Oxidative stress (OS) and depletion of antioxidants (AO) and their precursors have been shown in adults with CP [4]. In 2014, a meta-analysis of 8 studies in adults with CP showed that antioxidant treatment leads to significant reduction of pain and analgesic requirement [5]. Another meta-analysis with overlapping studies concluded that overall there was no association of antioxidant therapy with pain reduction. However, studies using a combination of multiple AO showed significant pain reduction while those employing single AO did not improve pain [6]. Thus, there is still no consensus on role of AO therapy.

Children and adults with CP differ in terms of nutritional status, etiology (alcohol in adults), prevalence of smoking, and disease severity. Data from adults cannot be directly extrapolated to children. Although AO are often used in children with CP, there is no published study on its efficacy.

The objectives of our study were to evaluate (1) the markers of OS and antioxidants (AO) in children with CP as compared to healthy controls, (2) safety and efficacy of combination antioxidant supplementation in relieving pain, and (3) identify predictors of response to AO therapy.

Methods

Study Design

Prospective study was done from May 2018 to April 2020 in the pediatric gastroenterology services of a tertiary referral center.

Patients

Consecutive patients (age 6–18 years, newly diagnosed or in follow-up) with CP were evaluated. CP was defined as per International Study Group of Pediatric Pancreatitis: In Search for a Cure consortium (INSPPIRE) criteria [7]. All patients underwent a detailed evaluation including family history of pancreatitis, history of trauma, any smoking/alcohol intake, and co-morbidities. Biochemical work-up (lipid profile, liver enzymes, serum bilirubin, albumin, and calcium) was done in all cases. Ultrasonography and magnetic resonance cholangiopancreatography (MRCP) were done at 1st OPD visit *(visit 1, time "-1" month)* to diagnose CP and any structural/biliary abnormality. Modified MRCP Cambridge criteria were used to grade changes for CP as mild, moderate, and severe [8]. Diabetes mellitus was diagnosed as per American Diabetes Association criteria [9] and pancreatic exocrine insufficiency (PEI) by Sudan's stain of stool fat (> 10 globules/high power field) [10].

Patients on AO or pain modulatory medications (tricyclic antidepressants) in past 6 months, those with interventions for pain reduction (celiac ganglion block, pancreatic duct endotherapy, or biliopancreatic surgery), having infrequent pain (< 2 days in preceding 6 months) or refusing consent were excluded.

Controls

Age- and gender-matched healthy children were enrolled as controls ($n = 14$) for comparison of the markers of OS and AO levels. Subjects with chronic systemic illness, acute illness in last 6 months, intake of vitamin/micronutrient supplements, or any dietary restrictions were excluded.

Assessment of Pain and Response to Analgesics

At *Visit 2*, ("0"month) baseline pain profile over 6 months prior to enrollment (5 months of historic data from patient and one month run-in period between visit 1 and 2, Fig. 1) was ascertained in terms of number of painful days, analgesic tablets/injections taken, and days hospitalized by detailed assessment of the patient's previous records.

Patients were taught the Faces Pain scale [11] and asked to maintain a pain diary marking a painful day, type and number of analgesics taken, and days of hospitalization. A painful day was defined as one with pancreatic pain, with Faces scale score ≥ 4, lasting for > 30 min or any pain requiring analgesic or nocturnal pain disturbing sleep. Patients were assessed telephonically every month and followed in OPD at 3 (visit 3) and 6 months (visit 4, final follow-up) of starting AO. Pain frequency, compliance to medication, and any adverse effects were noted at each visit. Response to antioxidants was assessed at 6 months for those with full follow-up.

Study Medication

All patients were given a combination antioxidant preparation containing Selenium (75 µg), vitamin C (67.5 mg), Beta Carotene (1125 IU), vitamin E (33.75 IU), and L-Methionine (250 mg). In the absence of pediatric recommendations and adult dose being 8 capsules/day [12], an empirical dose of 1 capsule thrice daily (age < 10 years), 2 capsules twice daily (10–15 years), and 2 capsules thrice daily (> 15 years) was used.

Outcome Measures

Primary outcome was change in number of painful days per month from baseline. Secondary outcomes were change in consumption of analgesics (tablet or injectable), number of hospitalizations, days hospitalized, and

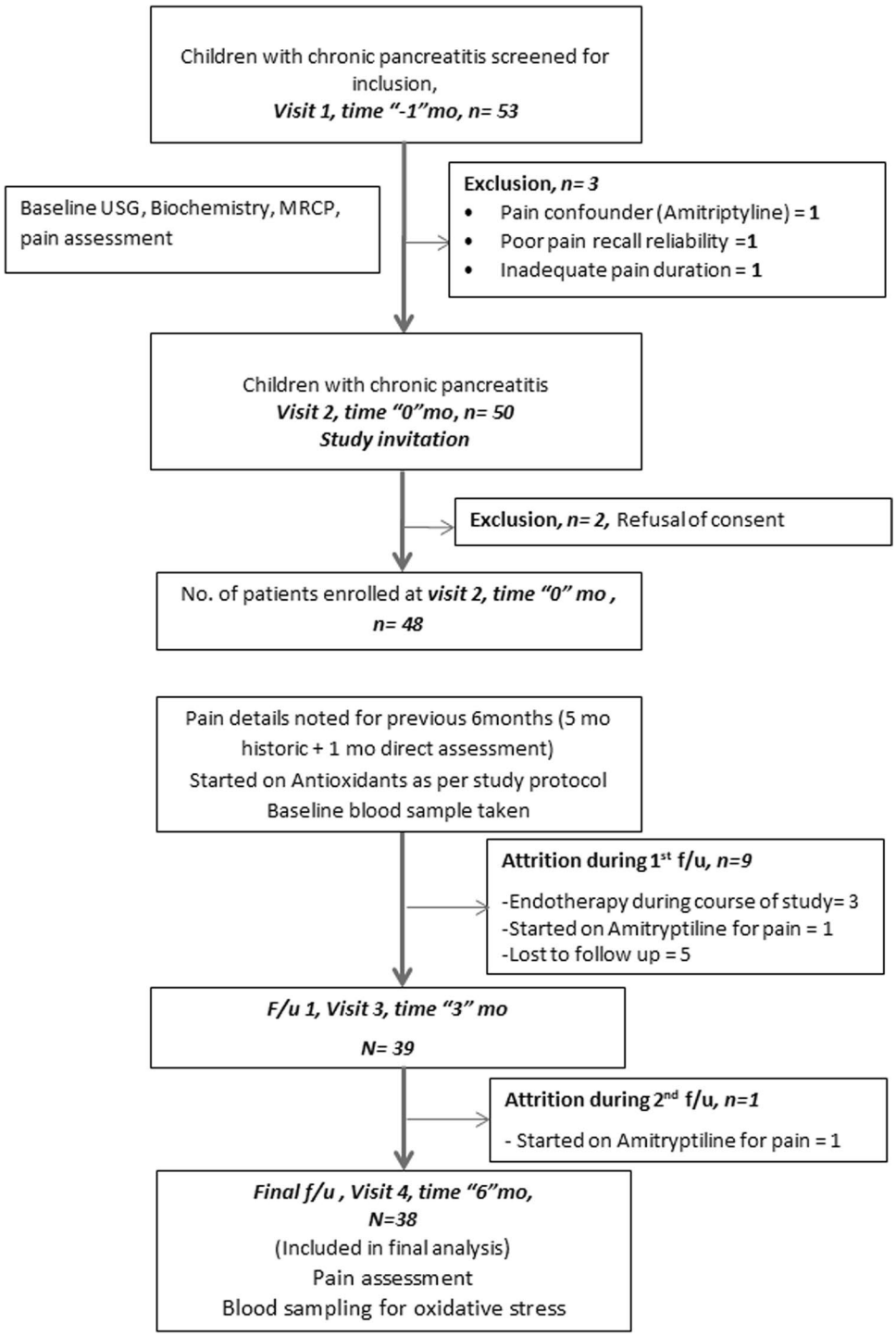

Fig. 1 Study flow depicting the steps of screening, baseline investigations, enrollment ($n=48$), pain assessment, estimation of oxidative stress (OS) and antioxidant (AO) levels and exclusions at various time points, and final follow-up ($n=38$)

markers of OS and AO capacity. Patients were classified as responders (pain-free [complete response] or $\geq 50\%$ reduction in the painful days) and non-responders ($< 50\%$ decrease [poor response] or no decrease or worsening of pain).

OS and AO

A 5-mL fasting blood sample (2 mL EDTA, 3 mL plain vial) was taken, at baseline and 6 months of therapy. Two aliquots of 200 µL of plasma and 4 aliquots of 200 µL of sera were stored

at − 80 °C till analysis. Serum superoxide dismutase (S-SOD, Marklund and Marklund [13]) and thiobarbituric acid reactive substances (TBARS, Buege and Aust [14]) which indicate the degree of lipid peroxidation were measured as markers of OS. AO measured included Vitamin C (Okamura [15]), Selenium (graphite furnace atomic absorption spectrometry [16]), and total antioxidant capacity (measured as ferric reducing ability of plasma, FRAP, Benzie, and Strain [17]).

Ethics

Institute's ethics committee (IEC code 2018-72-DM-103) approved the study before initiation. Informed consent was taken from parents of all participating subjects prior to enrollment.

Statistical Analysis

Continuous data are represented as median (interquartile range "IQR") and categorical as frequency (%). Continuous variables between 2 groups were compared with Mann Whitney *U* test and paired continuous data of the same group by Wilcoxon signed rank test. Categorical variables were compared by χ^2 test/Fisher exact test. Univariate and multivariate binary logistic regression analysis was done to identify the predictors of pain response. A p value < 0.05 was taken as significant. Statistical package for social sciences version-23 (SPSS-23, IBM Chicago, USA) was used.

Results

Patient Characteristics and Study Flow

Patient enrollment and assessment are shown in Fig. 1. Age at onset of symptoms and enrollment (n-48) was 10.5 (8.3, 12) and 13 (12, 15.8) years, respectively. Consanguinity was present in 3 (6.2%) and family history of CP in 3 (6.2%, paternal uncle, aunt, and 1st cousin) cases. Only 6.2% (3/48), 4.2% (2/48), and 8.3% (4/48) patients had a weight, height, and BMI < − 2SD, respectively [18]. Three patients (3/48, 6.2%) were obese (BMI > 27 adult equivalent). No patient had clinical steatorrhea. 5/48 (10.4%) cases had > 10 fat globules/hpf; however, only 1 patient was on pancreatic enzyme replacement therapy (PERT) both before and during AO therapy.

According to Cambridge classification, 10 (20.8%) were mild, 11 (23%) moderate, and 27 (56%) were severe CP. Overall, 28/48 (58.3%) cases had pancreatic calcifications, ductal calculi in 20/48 (41%), and parenchymal calcifications in 8/48 (16.7%).

4/48 (8.3%) cases had a small pseudocyst on imaging at enrollment. Of them, 1 patient was lost to follow-up and there was no change in size of pseudocyst or need of drainage in the remaining 3 cases, over the study period. Lipid profile and calcium levels were normal in all. The etiology of CP was post-traumatic in 2 (4.1%) patients and idiopathic (no other discernible cause) short of genetic studies in remaining.

38 patients (age < 10 years ($n = 6$), 10–15 years ($n = 23$), > 15 years ($n = 9$)) completed the study and final follow-up. Of the remaining 10 cases, 5 (10.4%) were lost to follow-up at 3-month visit and 5 required additional treatment for pain (endotherapy-3, amytriptyline-2) after starting antioxidants (Fig. 1).

Comparison of OS and AO Markers Between CP and Controls

Age, gender, anthropometry, and markers of OS in CP cases and controls are shown in Table 1. Among measures of OS,

Table 1 Comparison of baseline demography, oxidative stress, and antioxidant markers between children with CP ($n = 48$) and controls ($n = 14$)

Parameter[a]	CP ($n = 48$)	Controls ($n = 14$)	p value
Age in years	13 (12, 15.75)	11.8 (9.87, 16.0)	0.46
Gender [Male-*n*(%)]	25 (52.1%)	10 (71.4%)	0.19
Weight z score	− 0.64 (− 1.31, 0.27)	− 0.08 (− 0.94, 0.67)	0.17
Height z score	− 0.57 (− 0.88, 0.44)	− 0.22 (− 0.76, 1.05)	0.27
Body mass index z score	− 0.56 (− 1.17, 0.18)	− 0.22 (− 1.14, 0.74)	0.40
Thiobarbituric acid reactive substances (nmol/mL)	7.79 (6.64, 8.9)	5.20 (4.15, 6.0)	**< 0.001**
Serum superoxide dismutase (U/mL)	3.66 (2.15, 4.32)	3.36 (2.27, 3.86)	0.44
Ferric reducing ability of plasma (µmol/L)	230.9 (177.3, 321.2)	381.3 (245.7, 436.0)	**0.003**
Vitamin C (mg/dL)	0.65 (0.59, 0.70)	0.78 (0.72, 0.97)	**< 0.001**
Selenium (µg/L)	30.3 (25, 37.7)	34.4 (32, 41.6)	0.07

Bold values are statistically significant ($p < 0.05$)

[a]Data represented as median (IQR 25,75) or *n* (%)

TBARS was significantly higher in cases than controls. Among the antioxidant markers, FRAP and vitamin C levels were significantly lower in CP.

Response in Pain, OS, and AO Markers After 6 Months of Therapy

The pain frequency, need of analgesics and hospitalization, OS and AO markers at baseline and at 6 months of antioxidant therapy in the 38 cases with full follow-up are shown in Table 2.

All patients were compliant to therapy barring 2 cases who took lesser dose inadvertently for 1 month and one patient who missed the medication for 2 weeks in between. These 3 cases were re-instructed about proper dose and compliance during telephonic assessment. There were no major adverse effects and none required dose modification or drug discontinuation. However, 4/38 (10.5%) cases had minor side effects: constipation ($n=2$, resolved with oral polyethylene glycol), reflux symptoms ($n=1$, responsive to proton pump inhibitor), and bad taste ($n=1$).

After antioxidant supplementation, pain reduced significantly to ≤ 50% of baseline in 68.4% cases (26/38, Responders, ***R +***) with 9 cases (23.7%) being pain-free. In the remaining 12 (31.6%, non-responders, ***R-***), pain reduced by < 50% in 4 (10.53%) and worsened in 8 (21.05%) patients (Fig. 2). We separately looked at response rate in patients with frequent pain (≥ 1 day per month, $n=25$) and infrequent pain (< 1 day/month, $n=13$) and found no difference in proportion of responders (17/25[68%] vs 9/13[69%]; $p=0.94$). Two out of 3 patients with family history of CP completed the study, both were responders and there was no significant difference in

Table 2 Pain attributes, oxidative stress, and antioxidant status before and after Antioxidants

Characteristic[a]	Before antioxidants ($N=38$)	After 6 mo of antioxidants ($N=38$)	p value
No. of painful days/month	1.41 (0.66, 1.74)	0.5 (0.12, 1.16)	0.001
Oral analgesic requirement/month	1 (0.33, 2.41)	0.5 (0, 1.37)	0.008
Parenteral analgesic requirement/month	0.5 (0, 1.37)	0 (0, 0.16)	< 0.001
No. of hospitalizations/month	0.167 (0, 0.17)	0 (0, 0)	< 0.001
No. of days of hospital stay/month	0.583 (0, 1.21)	0(0, 0)	< 0.001
Thiobarbituric acid reactive substances (nmol/mL)	7.99 (6.09, 9.07)	6.65 (5.2, 7.42)	0.004
Serum superoxide dismutase (U/mL)	3.83 (2.13, 4.34)	2.49 (1.65, 3.98)	0.001
Ferric reducing ability of plasma (μmol/L)	229.65 (173.04, 317.95)	457.36 (341.71, 595.47)	< 0.001
Vitamin C (mg/dL)	0.646 (0.594, 0.713)	0.859 (0.694, 0.972)	< 0.001
Selenium (μg/L)	28.15 (23.85, 39.06)	32.62 (25.82, 38.39)	0.04

[a]Data represented as median(IQR)

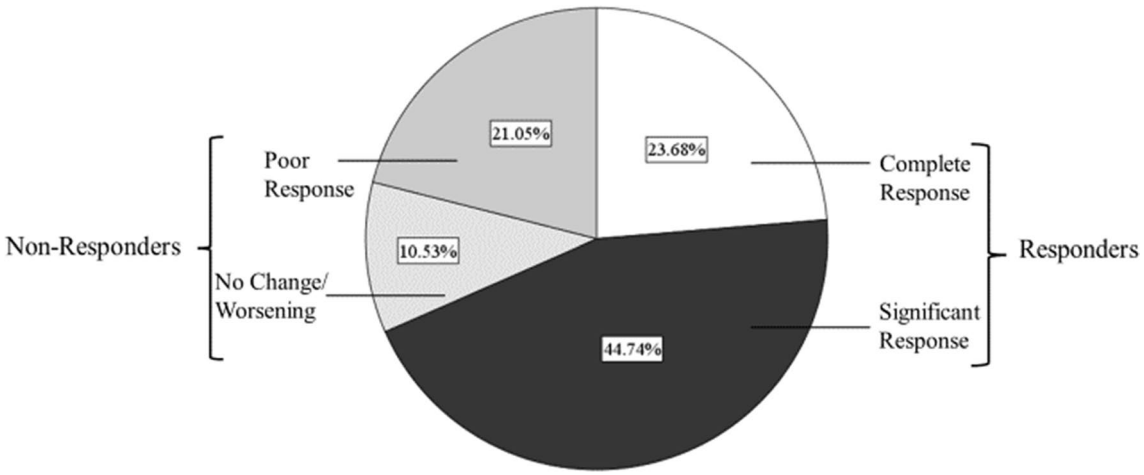

Fig. 2 Pie chart showing proportion of patients with complete, significant, poor, and no response to antioxidants at 6 months of therapy. Complete response: 100% response (no painful days); Significant response: ≥ 50% decrease in painful days; Poor response: < 50% decrease in painful days; No response/Increase: no change in no. of painful days or an increase in no. of painful days

outcome of pain response between those with or without a family history of CP ($p=0.324$).

Comparison Between Responders and Non-responders

Table 3 shows the comparative profile of responders ($\boldsymbol{R^+}$) and non-responders ($\boldsymbol{R^-}$). The groups were similar in terms of age at disease onset, anthropometry, and pain characteristics. Baseline OS and AO markers were similar, except serum selenium which was significantly lower in $\boldsymbol{R^-}$ (25.8 [17.79, 27.92] vs. 33.1 [25.35, 41.67] µg/L; $\boldsymbol{p=0.03}$). Although serum selenium increased (8.02 [2.99, 14.70] vs 2.35 [−4.66, 5.75] µg/L; $p=0.01$) significantly more in the $\boldsymbol{R^-}$ group than the $\boldsymbol{R^+}$ group, the final levels were similar in $\boldsymbol{R^+}$ and $\boldsymbol{R^-}$ (Table 3). Patients with mild changes as per Cambridge responded more often than those with moderate–severe disease [100% (9/9) vs. 58% (17/29); $p=0.036$]. On univariate analysis, higher hemoglobin (OR 2.13, 95%CI 1.15–3.94, $p=0.045$) and higher serum selenium (OR 1.099, 95% CI 1.02–1.18, $p=0.03$) at baseline were significant predictors of response. However, on multivariate analysis, none were significant.

Table 4 shows the comparison of the pain characteristics, anthropometry, and markers of oxidative cases in patients with mild vs moderate–severe disease. Subjects with mod–severe disease had lower BMI and higher proportion of calcific disease including MPD calculi. There was no difference in markers of OS between the groups.

Discussion

Pain was the predominant complaint at hospital visit in our children, similar to others [19]. The reasons for pain in CP include OS, pancreatic neuropathy, ductal hypertension,

Table 3 Comparison of clinico-laboratory profile, Oxidative stress, and Antioxidant markers between responders and non-responders ($N=38$)

Characteristic[a]	Responders ($N=26$)	Non-responders ($N=12$)	p value
Age at onset (years)	11 (9, 14)	10.5 (7, 12)	0.23
Age at enrollment (years)	13.3 (12, 16.3)	13 (8.75, 13)	0.1
Gender (Male)	16 (61.5%)	5 (41.2%)	0.25
Baseline characteristics			
Weight z score	−0.52 (−1.20, 0.16)	−0.75 (−1.30, 0.04)	0.43
Height z score	−0.57 (−0.82, 0.37)	−0.63 (−1.20, 0.24)	0.50
Body Mass Index z score	−0.38 (−1.11, 0.23)	−0.69 (−1.23, 0.32)	0.31
Cambridge grade (n, %)			**0.036**
Mild (9, 23.6%)	9 (100%)	0 (0%)	
Moderate-severe (29, 76.4%)	17 (58.6%)	12 (41.4%)	
Calcific disease	13 (50%)	9 (75%)	0.15
Exocrine insufficiency (abnormal fecal fat), n(%)	4 (15.4%)	1 (8.3%)	0.55
Endocrine insufficiency (Diabetes, HbA1c > 6.5)	0	0	NA
Transient non-compliance to medication	1(3.8%)	2(17%)	0.17
Baseline			
TBARS-A (nmol/mL)	7.75 (6.20, 8.97)	7.99 (5.68, 9.16)	0.91
sSOD-A (U/mL)	4.03 (2.39, 4.92)	2.91 (1.97, 3.95)	0.08
FRAP-A (µM/L)	214.77 (153.31, 299.52)	258.11 (190.18, 322.48)	0.31
Vitamin C—A (mg/dL)	0.646 (0.598, 0.703)	0.643 (0.594, 0.715)	0.82
Selenium-A (µg/L)	33.1 (25.35, 41.67)	25.8 (17.79, 27.92)	**0.02**
After 6 months of antioxidant therapy			
TBARS-B (nmol/mL)	6.75 (5.43, 7.60)	5.45 (4.67, 6.85)	0.13
sSOD-B (U/mL)	3.16 (2.01, 4.48)	1.76 (1.61, 2.34)	**0.003**
FRAP-B (µM/L)	444.42 (294.73, 643.30)	474.82 (378, 561.51)	1.0
Vitamin C—B (mg/dL)	0.841 (0.714, 1.02)	0.874 (0.651, 0.920)	0.52
Selenium-B (µg/L)	32.62 (24.24, 38.4)	32.15 (26.71, 39.30)	0.82

Bold values are statistically significant ($p < 0.05$)

OS oxidative stress, *AO* antioxidants, *TBARS* thiobarbituric acid reactive substances, *sSOD* serum superoxide dismutase, *FRAP* ferric reducing ability of plasma;

A—baseline values, B—after 6 months of antioxidants

[a]*Data represented as median (IQR 25, 75) or n(%)*

Table 4 Comparison of clinico-laboratory profile and markers of oxidative stress between patients with mild vs moderate–severe ductal changes at enrollment ($N=38$)

Characteristic[a]	Mild ($N=9/38$)	Mod–severe ($N=29/38$)	p value
Age at onset (years)	11 (9, 16)	11 (2,16)	0.4
Age at enrollment (years)	13.5 (12, 17)	13 (6,17)	0.2
Symptom duration (months)	24 (9, 96)	24 (6, 120)	0.9
Baseline characteristics			
Weight z score	−0.06 (−2.0, 2.0)	−0.8 (−1.9, 1.2)	0.06
Height z score	−0.1 (−1.1, 2.3)	−0.5 (−2.0, 3.4)	0.2
Body Mass Index z score	0.10 (−2.0, 2.0)	−0.73 (−2.3, 1.6)	**0.04**
MPD calculi	0/9	15/29	**0.006**
Only parenchymal calcification	1/9 (11.1%)	6/29 (20.7%)	0.51
Calcific disease	1/9(50%)	21/29(75%)	**0.002**
Painful days/month	1 (0.6, 3.5)	1.5 (0.3, 13)	0.9
TBARS-A (nmol/mL)	8.2 (4.8, 25)	7.9 (3.5, 19.2)	0.3
sSOD-A (U/mL)	3.9 (1.6, 5.1)	3.8 (1.03, 11.1)	0.7
FRAP-A (µM/L)	227.7 (111.27, 477.4)	231.5(98.3, 840.9)	0.8
Vitamin C—A (mg/dL)	0.69 (0.57, 1.08)	0.62 (0.51, 1.3)	*0.06*
Selenium-A (µg/L)	32.9 (14.8, 43.8)	27.1 (13.1–55.1)	0.4

Bold values are statistically significant ($p < 0.05$)

After 6 months of antioxidant therapy: Both the groups showed a similar change (delta) in TBARS, sSOD, FRAP, Vitamin C, or selenium values (post therapy- pretherapy)

TBARS thiobarbituric acid reactive substances, *sSOD* serum superoxide dismutase, *FRAP* ferric reducing ability of plasma

[a]Data represented as median (range) or n (%)

and local complications (pseudocysts) which may be operating simultaneously [20]. Braganza et al. suggested that pathologic exposure of the pancreatic acinar cells to short-lived oxygen-free radicals leads to "OS" causing cellular injury [21]. Depletion of antioxidant factors with an excess of OS both within the pancreas and in circulation occurs in CP [22]. Our study is in line with these observations. Both markers of OS i.e., TBARS and sSOD were higher in children with CP than controls, but the difference was significant only for TBARS. This lack of significance could be due to a small sample size or because higher SOD is mainly a feature of alcoholic pancreatitis [23, 24]. None of our subjects consumed alcohol.

Among AO, vitamin C and FRAP were significantly lower in CP compared to controls, consistent with studies in adults with CP [25, 26]. Uden et al. showed significantly lower serum Se levels in CP [27]. In our study, Se levels were lower in CP patients, but not significantly. Vaona et al. [28] suggested an association of selenium with PEI, being significantly lower in patients with moderate to severe PEI than in controls. We also found lower (though not significant) Se levels in our patients with PEI ($n=5$) vs. those without PEI ($n=43$, 24.9 [17.2, 36.8] µg/L vs. 31.4 [25.5, 38] µg/L; $p=0.28$). Se levels were significantly lower ($p=0.05$) in CP with PEI compared to healthy controls, similar to the above study.

Oxidative stress may also be influenced by malnutrition, with higher OS in patients with severe malnutrition compared to healthy controls [29–31]. Four (8.3%) CP children had a low BMI (< − 2SD), but the OS markers were not different from those without malnutrition (TBARS 6.95 [4.44, 8.15] vs. 7.99 [6.65, 8.99] nmol/mL, $p=0.25$; sSOD 3.60 [2.62, 7.04] vs. 3.66 [2.12, 4.32] U/mL, $p=0.65$). Among AO, FRAP (237.4 [180.5, 329.3] vs. 230.9 [177.2, 321.2] µmol/L, $p=0.84$) and Se (23.02 [10.07, 35.95]) vs. 30.30 [25.5, 38.52] µg/L; $p=0.27$) were comparable, while Vit C (0.572 [0.543, 0.627] vs. 0.658 [0.605, 0.715] mg/dL, $p=0.03$) was lower in those with malnutrition. This supports that the increased OS is likely to be due to CP and not malnutrition. Lower Vit C concentrations may be independently found in malnutrition [32, 33]. The etiology of CP was idiopathic in a majority, after excluding metabolic and structural causes with < 5% being post traumatic. There was no alcohol intake or smoking (active or passive), which can affect the OS and common in adults [12, 34–36].

We found a significant reduction in markers of OS and improvement in AO levels after 6 months of supplementation which is expected and similar to others [12, 36]. The composition and dosage of the antioxidant supplementation varies in adult studies, but most had selenium, ascorbic acid, beta carotene, and methionine [22]. The most important component is thought to be methionine, a methyl group donor with potential to restore signal transduction for

zymogen exocytosis in the pancreatic acinar cells. Its deficiency leads to intra-acinar zymogen release and activation of the inflammatory cascade [37, 38]. A dose of 2–4 g/day is effective in reducing pain in adults with CP [22]. There is no recommendation about methionine dose in children with CP. We used a dose of 0.75 to 1.5 g/day as per age and found it to be equally effective across 6–18y of age (response seen in 3/6, 15/23 and 8/9 cases in < 10y, 10–15 years and > 15 years old subjects; $p = 0.3$). The medication was well tolerated with minor side effects in 10.5% cases. The meta-analysis of studies with methionine containing AO showed side effects in 19.2% cases vs. 4.3% ($p = 0.005$) in placebo. Common side effects included constipation, nausea, heart burn, bad taste, and headache, similar to our observation [22].

One meta-analysis [5] and a Cochrane review [39] in adults have shown the efficacy of AO in reducing pain in CP, while another meta-analysis [6] showed efficacy of combined AO and refuted efficacy of single AO. The included studies showed pain relief with AO in 32–100% cases. We showed a significant response in pain using combined antioxidant therapy in 2/3rd of our cases with 23.7% becoming pain-free. 32% cases became pain-free in the study of Bharadwaj et al., pain improvement being present around ~ 3 months [12]. Table 5 summarizes studies which included pediatric patients albeit in limited numbers, and compares it with the present exclusive pediatric prospective study.

Non-responders had lower selenium, hemoglobin, and more severe Cambridge grade with changes in the main pancreatic duct (MPD) than responders. Studies in adults implicate alcohol as etiology, continued smoking, and high addiction to opiates to be poorly responsive to antioxidant therapy [40, 41]. None of these were applicable to our children. Lower selenium in R^- suggests higher OS, and excessive consumption of Se for neutralizing the release of free radicals locally [42]. We feel that in patients with dilated MPD with/without stenosis, calculi, an additional factor of ductal hypertension may be contributing to pain and poorer response. Talukdar et al. evaluated CP patients with recurrence of pain after clearance of ductal calculi. Patients were treated with a combination of AO and pregabalin and showed superior pain response compared to placebo [43]. This supports that pain in CP is a result of multiple independent but overlapping mechanisms, and a combination of treatments is required to achieve response [44].

The post-therapy sSOD in our study was higher in the responders as compared to non-responders. However, it was not higher than the value in healthy controls. Possibly, overall collective change in OS is more important than change in one marker and needs to be studied further.

Our study has certain limitations. We have not done genetic analysis in our cohort, hence we could not determine the proportion of hereditary pancreatitis cases. However, in a previous study of our idiopathic CP cases, none had mutation in PRSS-1gene [45]. Fecal elastase rather than Sudan stain should have been used for diagnosis of PEI. As there was only one case on PERT and he was taking the enzymes both before and during antioxidant therapy, it is unlikely to confound the assessment of response. A small pseudocyst was present in 3 cases with response to AO. However, the pseudocyst did not reduce in size during therapy and thus unlikely to have contributed to pain response. On exclusion of these 3 cases, the proportion of responders (23/35, 65.7%) and other results did not change.

We have evaluated OS at baseline and 6 months, so time-line of improvement in OS cannot be assessed. Pain assessment has limitations including a recall bias for baseline pain. To minimize this, we used one month of run-in period and five months of recall (total 6mo). All children were neuro-developmentally normal, and did not have any other cause of pain. We interviewed both parents, reviewed the hospital records and medical prescriptions of the preceding 6 months, and used a calendar to aid and enhance recall and then note the actual "number of painful days" at baseline. During the antioxidant phase, for younger children (< 10 years), parents maintained the pain chart, while older children maintained the charts themselves under parental supervision. Regular phone calls from the investigators and a well-designed pain diary using vernacular language in addition to English increased the accuracy of pain measurement. Even the R^- group of patients had a reduction in the number of injectable analgesics and hospitalization, attributable to disease counseling, step-wise pain management with optimal utilization of oral analgesics, and alleviation of patient/parental anxiety. Our study is not a randomized controlled trial (RCT) which would have provided the best evidence for efficacy of AO therapy. This being a pilot study in children is limited by a relatively small sample size and a shorter duration of follow-up. However, this is the first study to prospectively establish the safety and efficacy of AO in CP children and sets the stage for future RCTs.

We conclude that children with CP have higher OS and lower antioxidant capacity compared to healthy controls. Methionine containing AO are well tolerated and 68% children show a good pain response with improvement in the markers of OS. A step-up pain management strategy and counseling leads to reduced hospitalization and injectable analgesic use in all cases.

Key Messages

- There is higher OS in children with CP compared to healthy controls.
- Antioxidant therapy is safe and well tolerated in children. Six months of therapy reduces the number of painful

Table 5 Summary of studies on pain relief with antioxidants in patients with acute recurrent/chronic pancreatitis incorporating children with or without adults

Author, ref, year, type of study	Exclusive pediatric/ combined adult + pediatric	No. of cases	Details of pediatric data available separately	Age cut off	ARP/CP	Primary clinical (pain) response assessment criteria	Pain response results
Bhardwaj et al. [12], 2009 RCT—Parallel groups	Combined	147 Enrolled (127 assessed)	No	> 12 years	CP	Comparison of reduction in no. of painful days/month between AO and PL	Mean 7.37 (AO) vs 3.21(PL) days ($p < 0.01$)32.3%
						-Proportion of patients "pain free" on antioxidants	
Dhingra et al. [36], 2012 RCT— Parallel groups	Combined	61 Enrolled (61 completed)	No	> 12 years	CP	AO vs PL: Frequency of association between pain reduction and reduction in surrogate marker of fibrosis	54.8% in AO vs 30% PL ($p = 0.05$)
Kirk et al. [35], 2006 Randomized DB cross over study	Combined	36 enrolled (19 completed)	No	> 16 years	CP	Comparison of change in *SF-36 QoL dimensional scores* including pain and 8 other dimensions between AO and PL	+ 17 in AO vs − 7 in PL in pain dimension ($p < 0.05$)
Uden et al. [27], 1990 DB cross over study	Combined	28 enrolled (23 completed)	1 patient; 17 years old	N/A	ARP + CP	Comparison of proportion with attack of pancreatitis while on AO or placebo at any point	ITT analysis- 1/23 pain during AO vs 8/23 during PL ($p = 0.04$)
						Comparison of pain scores as per *McGill Standard Pain Questionnaire*	18.3 in AO vs 21.3 in PL ($p = 0.1$)
Present study-Prospective Observational study	Pediatric	48 enrolled (38 completed)	Yes	< 18 years	CP	Proportion with > 50% reduction in no. of painful days/month assessed by *Faces Pain Scale* after 6mo of AO	26/38 (68.4%)

RCT randomized controlled trial, *y* year, *AO* antioxidants, *OS* oxidative stress, *PL* placebo, *ITT* intention to treat, *DB* double blind, *ARP* acute recurrent pancreatitis, *CP* chronic pancreatitis, *N/A* not available

The first 3 studies do not give number or subgroup analysis of pediatric (< 18 year) cases

days, requirement of analgesics, and hospitalization in two-thirds of cases.
- Patients with milder ductal changes are more likely to respond to antioxidant therapy.

Author's contribution AG: Collection of data, analysis, and interpretation of data and drafting the article. AS: Conception and design of study, analysis of data, and co-drafting the manuscript. AM: Conducting the tests, collection of data, and revising the manuscript. SKY: Concept of study, interpretation of data, and revising it critically for important intellectual content. SKJ: Conducting the tests, data analysis, and revising it critically for important intellectual content. PM: Analysis and interpretation of data, revising it critically for important intellectual content. MSS: Interpretation of data and revising it for important intellectual content. UP: Interpretation of data and revising it for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding None.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Machicado JD, Rebours V, Yadav D. Epidemiology of chronic pancreatitis. *Pancreapedia Exocrine Pancreas Knowl Base*. 2016. https://doi.org/10.3998/panc.2016.13.
2. Gariepy CE, Heyman MB, Lowe ME et al. Causal evaluation of acute recurrent and chronic pancreatitis in children: consensus from the INSPPIRE group. *J Pediatr Gastroenterol Nutr.* 2017;64:95–103. https://doi.org/10.1097/MPG.0000000000001446.
3. Tandon RK, Sato N, Garg PK. Chronic pancreatitis: Asia-Pacific consensus report. *J Gastroenterol Hepatol.* 2002;17:508–518. https://doi.org/10.1046/j.1440-1746.2002.02762.x.
4. Uden S, Acheson D, Reeves J et al. Antioxidants, enzyme induction, and chronic pancreatitis: a reappraisal following studies in patients on anticonvulsants. *Eur J Clin Nutr.* 1988;42:561–569.
5. Zhou D, Wang W, Cheng X, Wei J, Zheng S. Antioxidant therapy for patients with chronic pancreatitis: a systematic review and meta-analysis. *Clin Nutr.* 2015;34:627–634. https://doi.org/10.1016/j.clnu.2014.07.003.
6. Cai GH, Huang J, Zhao Y et al. Antioxidant therapy for pain relief in patients with chronic pancreatitis: systematic review and meta-analysis. *Pain Phys.* 2013;16:521–532.
7. Morinville VD, Husain SZ, Bai H et al. Definitions of pediatric pancreatitis and survey of current clinical practices: report from INSPPIRE (International Study Group of Pediatric Pancreatitis : in Search for a Cure). *J Pediatr Gastroenterol Nutr.* 2012;55:261–265. https://doi.org/10.1097/MPG.0b013e31824f1516.DEFINITIONS.
8. Balci NC, Bieneman BK, Bilgin M, Akduman IE, Fattahi R, Burton FR. Magnetic resonance imaging in pancreatitis. *Top Magn Reson Imaging* 2009;20:25–30. https://doi.org/10.1097/RMR.0b013e3181b483c2.
9. American Diabetes Association. Standards in medical care of diabetes-2017. *Diabetes Care* 2017;40:11–24.
10. Masamune O, Takahashi T, Nagasaki A, Iwabuchi J, Ishikawa M. Diagnostic significance of the Sudan III staining for fecal fat. *Tohoku J Exp Med.* 1977;122:397–402. https://doi.org/10.1620/tjem.122.397.
11. International Association for the study of Pain. Faces Pain Scale – Revised (FPS-R). Published online 2001:7858. http://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1519
12. Bhardwaj P, Garg PK, Maulik SK, Saraya A, Tandon RK, Acharya SK. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. *Gastroenterology* 2009;136:149-159.e2. https://doi.org/10.1053/j.gastro.2008.09.028.
13. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem.* 1974;47:469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x.
14. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol.* 1978;52:302–310. https://doi.org/10.1016/S0076-6879(78)52032-6.
15. Masato O. An improved method for determination of l-ascorbic acid and l-dehydroascorbic acid in blood plasma. *Clin Chim Acta* 1980;103:259–268. https://doi.org/10.1016/0009-8981(80)90144-8.
16. Gardiner PHE, Littlejohn D, Halls DJ, Fell GS. Direct determination of selenium in human blood serum and plasma by electrothermal atomic absorption spectrometry. *Top Catal.* 1995;9:74–81. https://doi.org/10.1016/S0946-672X(11)80014-3.
17. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem.* 1996;239:70–76. https://doi.org/10.1006/abio.1996.0292.
18. Khadilkar V, Yadav S, Agrawal KK et al. Revised IAP growth charts for height, weight and body mass index for 5- to 18-year-old Indian children. *Indian Pediatr.* 2015;52:47–55. https://doi.org/10.1007/s13312-015-0566-5.
19. Pasricha PJ. Unraveling the mystery of pain in chronic pancreatitis. *Nat Rev Gastroenterol Hepatol.* 2012;9:140–151. https://doi.org/10.1038/nrgastro.2011.274.
20. Talukdar R, Reddy DN. Pain in chronic pancreatitis: managing beyond the pancreatic duct. *World J Gastroenterol.* 2013;19:6319–6328. https://doi.org/10.3748/wjg.v19.i38.6319.
21. Braganza JM. The pathogenesis of chronic pancreatitis. *QJM Mon J Assoc Phys.* 1996;89:243–250. https://doi.org/10.1007/978-1-4471-3356-8_17.
22. Talukdar R, Murthy HVV, Reddy DN. Role of methionine containing antioxidant combination in the management of pain in chronic pancreatitis: a systematic review and meta-analysis. *Pancreatology* 2015;15:136–144. https://doi.org/10.1016/j.pan.2015.01.003.
23. Thome J, Foley P, Gsell W et al. Increased concentrations of manganese superoxide dismutase in serum of alcohol dependent patients. *Alcohol Alcohol.* 1997;32:65–69. https://doi.org/10.1093/oxfordjournals.alcalc.a008235.
24. Szuster-Ciesielska A, Daniluk J, Kandefer-Szersze M. Oxidative stress in blood of patients with alcohol-related pancreatitis. *Pancreas* 2001;22:261–266. https://doi.org/10.1097/00006676-200104000-00006.
25. Girish BN, Rajesh G, Vaidyanathan K, Balakrishnan V. Assessment of oxidative status in chronic pancreatitis and its relation with zinc status. *Indian J Gastroenterol.* 2011;30:84–88. https://doi.org/10.1007/s12664-011-0094-8.

26. Verlaan M, Roelofs HMJ, van Schaik A et al. Assessment of oxidative stress in chronic pancreatitis patients. *World J Gastroenterol.* 2006;12:5705–5710. https://doi.org/10.3748/wjg.v12.i35.5705.
27. Uden S, Bilton D, Nathan L, Hunt LP, Main C, Braganza JM. Antioxidant therapy for recurrent pancreatitis: placebo-controlled trial. *Aliment Pharmacol Ther.* 1990;4:357–371. https://doi.org/10.1111/j.1365-2036.1990.tb00482.x.
28. Vaona B, Stanzial AM, Talamini G, Bovo P, Corrocher R, Cavallini G. Serum selenium concentrations in chronic pancreatitis and controls. *Dig Liver Dis.* 2005;37:522–525. https://doi.org/10.1016/j.dld.2005.01.013.
29. Ghone RA, Suryakar AN, Kulhalli PM et al. A study of oxidative stress biomarkers and effect of oral antioxidant supplementation in severe acute malnutrition. *J Clin Diagnos Res.* 2013;7:2146.
30. Golden MHN, Ramdath D. Free radicals in the pathogenesis of kwashiorkor. *Proc Nutr Soc.* 1987;46:53–68. https://doi.org/10.1079/pns19870008.
31. Shrivastava K, Gaur A, Ambey R. Oxidative stress in children with severe acute malnutrition between 6 months to 5 years of age. *Int J Contemp Pediatr.* 2018;5:1327. https://doi.org/10.18203/2349-3291.ijcp20182434.
32. Osaro E, Abdullahi S, Jiya N, Van Dyke K, Erahbor T. Ascorbate deficiency among Children of African descent with protein energy ascorbate deficiency among childrens of African descent with protein energy malnutrition in Sokoto, North Western Nigeria. *COJ Nurse Healthc.* 2018;3:1–8. https://doi.org/10.31031/COJNH.2018.03.000558.
33. Khare M, Mohanty C, Das B, Jyoti A, Mukhopadhyay B, Mishra S. Free radicals and antioxidant status in protein energy malnutrition. *Int J Pediatr.* 2014;1:1–6. https://doi.org/10.1155/2014/254396.
34. Siriwardena AK, Mason JM, Sheen AJ, Makin AJ, Shah NS. Antioxidant therapy does not reduce pain in patients with chronic pancreatitis: the ANTICIPATE study. *Gastroenterology* 2012;143:655–663. https://doi.org/10.1053/j.gastro.2012.05.046.
35. Kirk GR, White JS, McKie L et al. Combined antioxidant therapy reduces pain and improves quality of life in chronic pancreatitis. *J Gastrointest Surg.* 2006;10:499–503. https://doi.org/10.1016/j.gassur.2005.08.035.
36. Dhingra R, Singh N, Sachdev V, Upadhyay AD, Saraya A. Effect of antioxidant supplementation on surrogate markers of fibrosis in chronic pancreatitis: a randomized, placebo-controlled trial. *Pancreas* 2013;42:589–595. https://doi.org/10.1097/MPA.0b013e31826dc2d7.
37. Bhardwaj P, Yadav RK. Chronic pancreatitis: role of oxidative stress and antioxidants. *Free Radic Res.* 2013;47:941–949. https://doi.org/10.3109/10715762.2013.804624.
38. Tandon RK, Garg PK. Oxidative stress in chronic pancreatitis: pathophysiological relevance and management. *Antioxidants Redox Signal.* 2011;15:2757–2766. https://doi.org/10.1089/ars.2011.4115.
39. Ahmed Ali U, Jens S, Busch ORC et al. Antioxidants for pain in chronic pancreatitis. *Cochrane Database Syst Rev.* 2014. https://doi.org/10.1002/14651858.CD008945.pub2.
40. Garg PK. Antioxidants for chronic pancreatitis: reasons for disappointing results despite sound principles. *Gastroenterology* 2013;144:e19–e20. https://doi.org/10.1053/j.gastro.2012.12.031.
41. Siriwardena A, Mason J. Reply. *Gastroenterology* 2013;144:20–21. https://doi.org/10.1053/j.gastro.2013.01.050.
42. Bowrey D, Morris-Stiff G, Puntis M. Selenium deficiency and chronic pancreatitis: disease mechanism and potential for therapy. *HPB Surg.* 1999. https://doi.org/10.1155/1999/97140.
43. Talukdar R, Lakhtakia S, Reddy DN et al. Ameliorating effect of antioxidants and pregabalin combination in pain recurrence after ductal clearance in chronic pancreatitis: results of a randomized, double blind, placebo-controlled trial. *J Gastroenterol Hepatol.* 2016;31:1654–1662. https://doi.org/10.1111/jgh.13332.
44. Anderson MA, Akshintala V, Albers KM et al. Mechanism, assessment and management of pain in chronic pancreatitis: Recommendations of a multidisciplinary study group. *Pancreatology* 2016;16:83–94. https://doi.org/10.1016/j.pan.2015.10.015.
45. Poddar U, Yachha SK, Borkar V, Srivastava A, Saraswat VA. Clinical profile and treatment outcome of chronic pancreatitis in children: a long-term follow-up study of 156 cases. *Scand J Gastroenterol.* 2017;52:773–778. https://doi.org/10.1080/00365521.2017.1295465.