# Antioxidants for Pancreatic Functions in Chronic Pancreatitis

## *A Double-blind Randomized Placebo-controlled Pilot Study*

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**Background:** Antioxidants (AO) supplementation in chronic pancreatitis (CP) has been evaluated for pain. But it is not clear whether AO in CP have an effect on pancreatic functions and other clinical outcomes. We evaluated effect of AO on endocrine function in CP.

**Materials and Methods:** Double-blind placebo (PL)-controlled randomized pilot study on 107 patients with CP assigned to receive daily combined AO or PL for 6 months. Primary outcome was: improvement in endocrine function (Homeostasis Model Assessment-Insulin Resistance). Secondary outcome measures were: improvement in C-peptide, Qualitative Insulin Sensitivity Check Index, exocrine pancreatic function (fecal elastase), surrogate markers of fibrosis (platelet-derived growth factor BB, transforming growth factor-β1, α-smooth muscle actin), quality of life (QOL), pain, nutritional status, markers of oxidative stress (OS), AO status, and inflammation.

**Results:** There was an increase in levels of serum selenium ($107.2 \pm 26.9$ to $109.7 \pm 26.9$ vs. $104.1 \pm 28.6$ to $124.0 \pm 33.6$ μg/L, $P = 0.022$) and serum vitamin E [0.58 (range, 0.27-3.22) to 0.66 (range, 0.34-1.98) vs. 0.63 (range, 0.28-1.73) to 1.09 (range, 0.25-2.91) mg/dL, $P = 0.001$] in the AO than the PL group. However, no significant differences were observed between groups in any of the primary or secondary outcome measures.

**Conclusions:** Supplementation with AO to patients with CP causes a sustained increase in blood levels of AO; however, it has no addition benefit over PL on endocrine and exocrine functions, markers of fibrosis, OS and inflammation, nutritional status, pain and QOL. Further larger studies with adequate sample size are required.

**Key Words:** oxidative stress, markers of fibrosis, quality of life, chronic pancreatitis, antioxidants

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Chronic pancreatitis (CP) is an inflammatory condition of the pancreas, in which development of fibrosis and loss of pancreatic parenchyma potentially leads to impaired endocrine and exocrine pancreatic function.[1] Oxidative stress (OS) is one of the mechanisms implicated in the etiopathogenesis of CP.[2] Clinically this leads to pain, pancreatic exocrine and endocrine insufficiency, malabsorption, and impaired quality of life (QOL). Various studies have shown that in patients with CP there is increased OS and deficiency of antioxidants (AO).[3–7] This imbalance in AO and pro-oxidants results in to change in morphology and function of β cell, stellate cells and trigger immune cells to secrete various cytokine affecting various signalling pathways of insulin secretion and fibrosis in both animal and clinical studies.[8–11] AO could reverse β cell dysfunction in animal model[9] and humans[12,13] and improve exocrine pancreatic secretion in CP.[12] Meta-analysis of AO supplementation in patients with CP has shown contradictory results on the pain relief.[14–18] But it is not clear whether AO supplementation in CP have an effect on pancreatic functions. We presume that by AO supplementation, probably the reversible component of β cell dysfunction and cytokine release may improve and progression of disease may be retarded to some extent.

Few studies were published later to the protocol finalization of our study, which evaluated the effect of AO supplementation on QOL,[19–21] endocrine and exocrine function,[12] and markers of fibrosis.[7] All these studies had small sample size and were not adequately powered to answer the research questions.

Thus, this double-blind PL-controlled randomized pilot study was conceptualized to evaluate the effect of AO

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supplementation compared with PL on endocrine and exocrine functions, markers of fibrosis, inflammation, OS, and AO capacity and clinical outcomes (pain, QOL, dietary intake, and weight) cohesively after 6 months of daily AO supplementation in patients with CP.

## MATERIALS AND METHODS

### Setting

Single tertiary care academic center.

### Study Design

Double-blind randomized PL-controlled, 2-arm parallel-group pilot study.

### Patients and Eligibility

All consecutive patients with CP attending the Pancreas Clinic, All India Institute of Medical Sciences, New Delhi, India were evaluated for inclusion. Diagnosis of CP was made based on clinical features and imaging evidence of CP based on ultrasonography or contrast-enhanced computed tomography or magnetic resonance imaging of abdomen or endoscopic ultrasonography. Diabetes and impaired glucose tolerance (IGT) was diagnosed as per World Health Organization[22] guidelines.

#### Inclusion Criteria

Diagnosed CP between 16 and 60 years of age, with disease duration of <3 years for nondiabetics and diabetics with any duration of disease.

#### Exclusion Criteria

Already taking AO or who had received AO in last 6 months, who had received decompressive therapy for CP (surgical or endoscopic), pregnant and lactating women, who had comorbid diseases such as chronic liver disease, chronic renal failure, or malignancy.

### Stratified Randomization

Stratified according to absence or presence of diabetes mellitus/IGT. Both stratums were randomly assigned in a 1:1 ratio to receive AO supplementation or PL. Block randomization with variable block size was done for each of the 2 strata using a computer-generated sequence by a pseudo random code. Generation of random numbers was done by a statistician not associated with the conduct of the study.

The packets containing AO and PL were prepared in advance according to the randomization numbers and the packets were numbered sequentially for each stratum. Each packet contained either the AO or the PL capsule supply for a period of 6 months. This was done by a person not associated with the study. The codes of intervention in the packets were kept with an investigator not involved in distribution of the packets. The packets were serially distributed (both stratum) to the patients according to their entry in the trial by the investigator.

### Blinding

The investigators and the clinicians attending to the patients were not involved in the randomization process and remained blinded to the type of treatment received by the patient throughout the study. Patients were blind to the identity of the intervention they were receiving. All study intervention was provided in identical packaging, appearance of intervention and PL capsules was identical and schedule of administration was identical. A double-blind procedure was followed to ensure minimum bias.

### Study Intervention

The AO supplementation included daily doses of 600 μg organic selenium, 0.54 g vitamin C, 9000 IU β-carotene, 270 IU vitamin E, and 2 g methionine as used by Braganza's group[23,24] and previous studies from our group.[6,7] Eight capsules per day of AO or PL (4 g/d starch) each were given in the dose of 2 capsules with breakfast and 3 capsules in lunch and dinner each.

Duration of intervention 6 months.

### Study Outcomes and Their Estimations

#### Endocrine Functions

Plasma glucose was estimated colorimetrically by the GOD-PAP (Randox, UK) method. Serum insulin and C-peptide were measured using chemiluminescent immunoassay technology by LIAISON analyzer (DiaSorin, Saluggia, VC, Italy). Homeostasis Model Assessment Method (HOMA-IR)[25] and Qualitative Insulin Sensitivity Check Index (QUICKI)[26] was calculated using fasting glucose and insulin levels.

#### Exocrine Function

Fecal elastase was measured with the Bioserv Diagnostics ELISA kit (Bioserv Diagnostics, Germany).

#### Surrogate Marker of Fibrosis

Serum platelet-derived growth factor (PDGF)-BB, transforming growth factor (TGF)-β1, and α-smooth muscle actin (SMA) were estimated. Quantikine ELISA kits were used to measure the concentration of serum TGF-β1 and PDGF-BB (R&D Systems Inc., for both kits). The concentration of serum α-SMA was measured with the Cusabio ELISA kit (Cusabio Biotech Co. Ltd, P.R. China).

### Other Outcomes

*OS* was measured with the urinary isoprostane ELISA kit (Product number: EA 85; Oxford Biomedical Research). Urine (spot) creatinine was measured using standard laboratory procedures on an automated analyzer (Hitachi 902 chemistry analyzer; Roche Diagnostics) using Dialab creatinine (modified Jaffe) kits. The results were expressed as mg/dL. Corrected urinary isoprostanes was calculated as urinary isoprostanes (ng/mL)/urinary creatinine (mg/dL)×100.

*Plasma total antioxidant capacity (TAC)* as a marker of *AO* defense was measured by the 2,2′-azino-bis-[3-ethyl-benzthiazoline sulphonate] method using kits from Randox (measuring range, 0.21 to 2.94 mmol/L) using the microplate method. High-performance liquid chromatography technique was used to analyze serum tocopherol[27] and retinol.[28] Roe and Kuether method[29] was modified as per our lab conditions for determination of plasma vitamin C (total ascorbic acid). Estimation of serum selenium was done by mass spectrometry (G. Toteja and S. Raj, personal written communication, 2011).

*Inflammatory markers* such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were measured by Quantikine ELISA kits (R&D Systems Inc. for all tests).

The *pain* assessment was done using a visual analogue score (VAS) scale (scale 0 to 10), where 0 was no pain, and 10 was the maximum (worst possible, unbearable excruciating pain). Pain was also assessed in terms of number of pain days per month, the requirement of oral/IV analgesic and/or

hospitalization. The severity of pain was measured in terms of usage of type of analgesic (mild—requiring oral analgesics, moderate—requiring IV analgesics, and severe—requiring hospitalization). The assessment of pain was done for preceding 3 months before the inclusion in the study.

A pretested, open-ended, semiquantitative food frequency proforma was used to collect *nutrient and dietary* information. Conversion of raw foodstuffs into nutrients was done by a software *Diet Cal* programmed as per Indian food composition tables.[30] *Bioimpedance* was measured using a Tanita TBF-215 leg-to-leg portable impedance analyzer (Tanita, Japan).

*QOL* was assessed using a formal, well-validated questionnaire-based European Organization for Research and Treatment of Cancer QOL Questionnaire Core questions 30 [EORTC QLQ C-30 version 3.0 (both in English and vernacular language)] and Pancreatic Modification (28 questions) [QLQ PAN-28 (in English)].[31]

## Therapeutic Management

Both groups also received standard care. Those with pain received analgesics on demand. Diabetics were treated by oral hypoglycemic agents or subcutaneous insulin. Patients with steatorrhea were treated with pancreatic enzyme therapy of around 25,000 to 40,000 EUP of lipase along with large meals. Alternate therapy in the form of endoscopic and/or surgical treatments was offered when the medical treatment failed.

## Study Time Points

Baseline (just before intervention), 3 and 6 months of intervention were study time points. During each visit clinical parameters, hematological and biochemical investigations along with study objective targeted assessment were done.

## Sample Collection

Venous blood sample were collected after overnight fast. The plasma and sera were separated, aliquoted, and frozen at −80°C. Plasma glucose and HbA1C were estimated within 4 hours of sample collection. All the samples were light protected to retard deterioration from oxidation of substrates during storage. Fresh fecal samples and spot urine were frozen at −80°C for later estimations.

### Assessment of Intervention Compliance

Adherence was assessed by return of empty packets of intervention and capsule count by the investigator. Overall compliance was enforced by telephonic call and cross-checking with the nearest relative. Telephonic communication was maintained with all patients, this helped to minimize the number of drop outs.

### Safety Evaluation

Adverse events for recorded at each visit. Patients were monitored for any side effects of the prescribed drug or PL and they were asked to report onset of any new symptom.

## Sample Size Calculation

No study was available on the effect of AO intervention on primary outcome measure of our study. Hence, sample size was to be computed on the interim analysis when primary outcome information became available for first 60 patients (ie, 30 patients in each group). The mean ± SD of HOMA-IR in the PL group was 2.8 ± 8.9 and in AO group 1.7 ± 1.5 with power of 80% and level of significance 5%, we require sample size of 511 in each group. As it was not possible to do a multicentric study, therefore, due to time constraints the doctoral committee (study supervising committee) suggested, stopping enrollment of subjects in this study at the end of 5 years since the commencement of the study. Stipulated maximum duration of the study was 6 years. It was also suggested that this study be treated as an exploratory study, results of which could be useful for future studies on this study question. In the sixth year enrolled patients were followed up till 6 months, laboratory estimations, decoding of study patients, statistical analysis, and writing of report was carried out.

## Statistical Analysis

Data were presented in frequency (%) and mean (SD)/median (min-max) as applicable. Data were analyzed by per protocol and intention-to-treat analysis for comparing the 2 groups with regard to outcome measures. If data were not following normal distribution, transformation was done.

Comparison of continuous variables between groups was done by Student *t* test/Wilcoxon rank-sum (Mann-Whitney *U*) test. Within group change in continuous variables was seen by paired-samples *t* test/repeated measures analysis of variance followed by post hoc multiple comparisons with Bonferroni test or signed ranks test/Friedman test followed by multiple comparison using Wilcoxon signed rank test. Overall between group was compared by generalized estimating equation.

Categorical variables were compared between groups by using $\chi^2$/Fischer exact and within group change was seen by McNemar test. All *P* values were 2 tailed and values $\leq 0.05$ were considered significant. The subgroup analyses should be considered as purely exploratory.

## Ethical Approval

Institutional ethical approval was obtained. The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines. Informed written consent was obtained from all the patients before enrollment in the study. The trial is registered with the Clinical Trials registry—India, CTRI/2011/05/001755.

# RESULTS

## Trial Participants and Follow-up

During a period of 36 months from April 2011, 447 patients with CP were screened for inclusion into the trial. Three hundred forty patients did not meet the inclusion criteria due to various reasons (Fig. 1). On the basis of the stratification 58 IGT/diabetics and 49 nondiabetic patients with CP fulfilled the inclusion criteria for trial. One hundred seven patients were randomized and received interventions in 2 groups: 53 in PL and 54 in AO. These patients were followed up at 3 and 6 months of intervention. During second visit (at 3 mo) 7 patients in PL group and 5 patients in the AO group did not come for follow-up but continued with the intervention and reported at 6 months. At the completion of RCT at 6 months, 46 and 44 patients in PL and AO group, respectively, reported to us. The follow-up of patients was 84% in the 6 months duration. No patient was shifted from one group to the other. The percentage of drop outs was 15.8% with 7/53 in PL group and 10/54 of the AO group.

## Baseline Comparability

The baseline characteristics (Table 1) are comparable in both groups.

## Intervention Compliance

The compliance as assessed by percentage consumed out of 100% expected intervention for the whole group was >94% and were comparable between groups at 3 and 6 months. The efficacy of AO supplementation was also reflected in the serum vitamins and mineral at 3 and 6 months. The serum selenium (124.02 ± 33.63 vs. 109.70 ± 26.94 μg/L, $P = 0.022$) and vitamin E [1.09 (range, 0.25 to 2.91) vs. 0.66 (range, 0.34 to 1.98) mg/dL, $P = 0.001$)] levels at 6 months was significantly higher in AO than in the PL group.

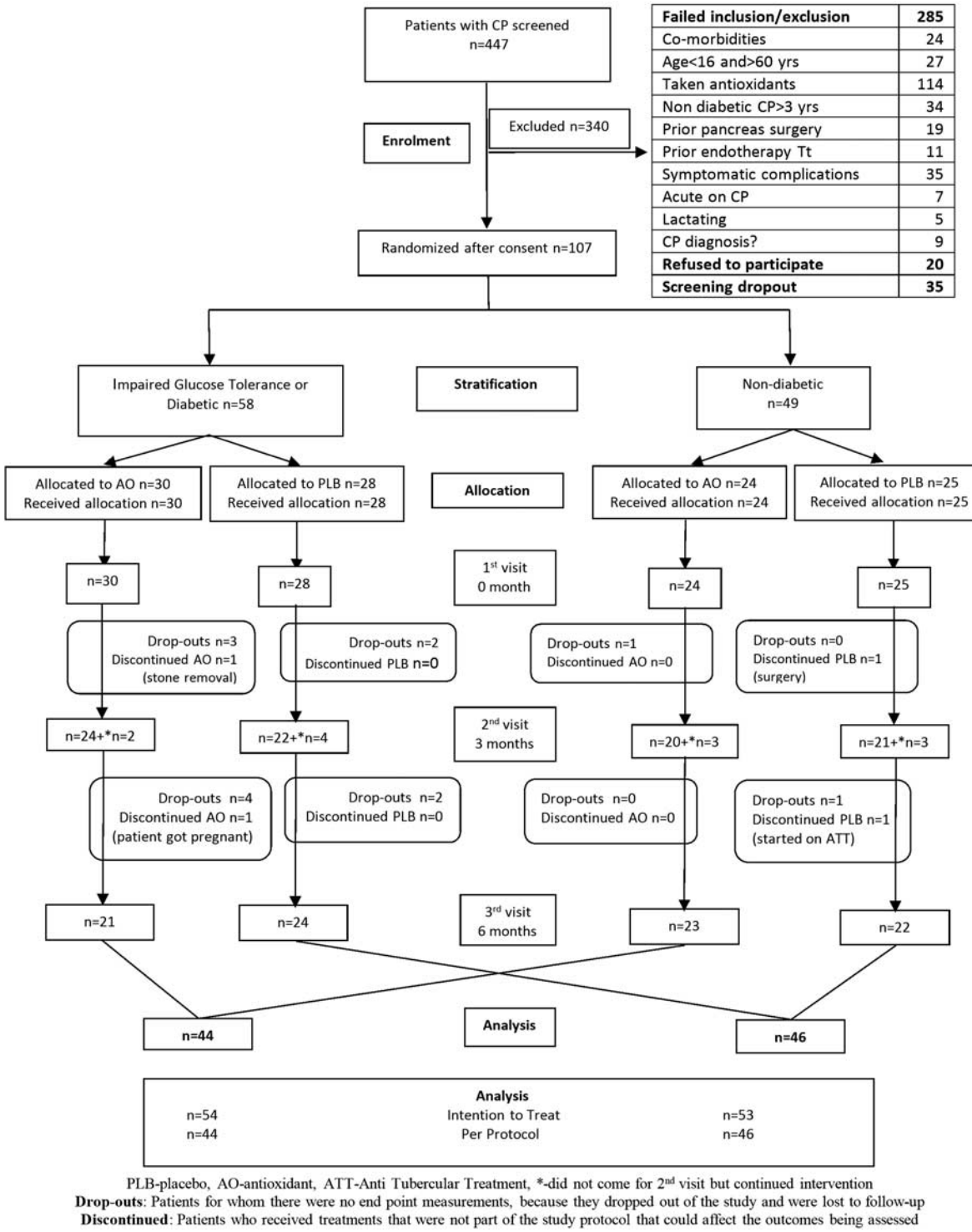


**FIGURE 1.** Consort flow chart.

**TABLE 1.** Patient Characteristics at Baseline According to Intervention

| Parameter | Placebo (n = 53) | Antioxidant (n = 54) | *P* |
|---|---|---|---|
| Age at enrollment (y) | 32.5 ± 12.3 | 30.4 ± 9.5 | 0.321 |
| Males [n (%)] | 44 (83) | 38 (70.4) | 0.093 |
| Duration of CP (y) | 3 (0.3-24) | 2 (0.2-25) | 0.312 |
| Body mass index (kg/m$^2$) | 19.4 ± 3.4 | 19.7 ± 3.8 | 0.649 |
| Etiology, alcoholic:idiopathic (n) | 16:37 | 8:46 | 0.046 |
| Dilated pancreatic duct [n (%)] | 45 (84.9) | 47 (87.0) | 0.484 |
| Calcifications [n (%)] | 45 (84.9) | 51 (94.4) | 0.095 |
| Steatorrhea [n (%)] | 8 (15.1) | 11 (20.4) | 0.475 |
| Diabetes [n (%)] | 14 (26.4) | 15 (27.8) | 0.524 |
| Duration of diabetes (y) | 2.75(0.1-14) | 4 (0.3-12) | 0.430 |
| Diabetic treatment [n (%)] | | | |
| Diet | 0 (0) | 1 (6.7) | 0.403 |
| Oral hypoglycemic agent | 4 (28.6) | 2 (13.3) | |
| Insulin | 10 (71.4) | 12 (80) | |
| Units/d | 21.4 ± 10.5 | 25.27 ± 10.3 | |
| Intake of alcohol | | | |
| Etiology, alcohol | n = 16 | n = 8 | |
| Amount of alcohol (g/d) | 102.9 (32-504) | 78.7 (30-147) | 0.046 |
| Duration of alcohol intake (y) | 16 (8-41) | 13 (7-24) | 0.231 |
| Ongoing alcohol use (n) | 9 | 3 | |
| Etiology, idiopathic | n = 4 | n = 6 | |
| Amount of alcohol (g/d) | 2.48 (2-8) | 2.48 (2-8) | 0.987 |
| Duration of alcohol intake (y) | 8.5 (8-9) | 11 (1-15) | 0.316 |
| Ongoing alcohol use (n) | 2 | 2 | |
| Tobacco use | | | |
| Tobacco with smoke [n (%)] | | | |
| Never | 34 (64.2) | 44 (81.5) | 0.129 |
| Current | 12 (22.6) | 6 (11.1) | 0.239 |
| Former | 7 (13.2) | 4 (7.4) | |
| Amount of cigarettes/ bidis smoked (number/d) | 10 (2-25) | 7 (2-15) | |
| Tobacco chewing [n (%)] | | | |
| Never | 38 (71.7) | 41 (75.9) | 0.696 |
| Current | 10 (18.9) | 7 (13) | |
| Former | 5 (9.4) | 6 (11.1) | |

Data are expressed as mean ± SD or median (min-max) or frequency (%).

## Response to Treatment

### Primary Outcome Measures

Results were similar by both analysis: intention to treat and per protocol. The tables are based on intention-to-treat analysis and involved all patients who were randomly assigned. Between the intervention and PL groups there was no change in the endocrine (Table 2): HOMA-IR ($P = 0.74$) markers during the 6 months period. No patient became diabetic during the RCT.

### Secondary Outcome Measures

*Exocrine marker and surrogate markers of fibrosis*. Between the intervention and PL groups there was no change in exocrine function (Table 3): fecal elastase ($P = 0.66$) and (Table 4): PDGF-BB ($P = 0.44$), TGF-β1 ($P = 0.99$), and α-SMA ($P = 0.35$) during the 6 months period.

*Markers of OS, AO defense, and inflammation*. Corrected urinary isoprostane, remained same in both the groups ($P = 0.88$) (Table 5). Total AO capacity showed a trend toward increase ($P = 0.06$) in the AO group whereas it reduced in the PL group. The markers of AO status, vitamin E ($P = 0.001$) and serum selenium ($P = 0.022$) which also acted as markers of compliance, increased at 6 months in the AO group when compared with PL group. There was no change in the inflammatory markers studied in the 2 groups (Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/JCG/A484).

*QOL*. Detailed data of QOL subscales and items from both groups are summarized in Table 6. An increase in global QOL of 1.07 points was observed in the AO group ($P = 0.001$) compared with an increase of 0.74 points in the PL ($P = 0.07$) group. However, overall global QOL was similar in both groups ($P = 0.54$). Analysis of individual questions and subdomains of QOL questionnaire at 6 months identified no statistically significant differences in role function, emotional function, cognitive function, pain, pancreatic pain but physical function and social function improved statistically but with doubtful clinical relevance.

*Pain*. Table 7 gives the pain pattern of the patients included in the study. Number of patients having pain at baseline, 3, and 6 months were comparable in the 2 groups. The 2 groups were comparable at baseline with regard to the number of painful days per month. After therapy, the number of painful days per month reduced in both the groups at 6 months ($P = 0.006$ and 0.001). Furthermore, the reduction in number of painful days per month was equal in both the AO and PL group. The reduction in the number of painful days per month was noted at 3 months of follow-up (PL $P = 0.001$ vs. AO $P = 0.001$) and the reduction continued till 6 months. The intensity of pain (VAS) was significantly reduced at 3 months of intervention in both groups ($P = 0.001$ each in both groups) and this reduction

**TABLE 2.** Comparison of Endocrine Markers in the 2 Groups

| Groups | Baseline ($t_1$) | 3 mo ($t_2$) | 6 mo ($t_3$) | *P* Overall Within Group | *P* Overall b/w Group |
|---|---|---|---|---|---|
| HOMA-IR | | | | | |
| Placebo, n = 53 | 0.99 (0.17-4.57) | 0.96 (0.13-5.69) | 0.96 (0.16-16.1) | 0.791 | 0.740 |
| AO, n = 54 | 1.13 (0.06-8.08) | 1.21 (0.04-8.80) | 1.12 (0.04-7.13) | 0.895 | |
| C-peptide (ng/mL) | | | | | |
| Placebo, n = 53 | 0.87 (0.01-4.98) | 0.94 (0.04-4.98) | 0.85 (0.02-2.43) | 0.987 | 0.502 |
| AO, n = 54 | 1.27 (0.01-3.1) | 1.04 (0.01-4.1) | 0.87 (0.01-3.28) | 0.228 | |
| QUICKI | | | | | |
| Placebo, n = 53 | 0.39 ± 0.06 | 0.39 ± 0.06 | 0.39 ± 0.06 | 0.835 | 0.939 |
| AO, n = 54 | 0.39 ± 0.09 | 0.40 ± 0.11 | 0.39 ± 0.09 | 0.468 | |

Data are expressed as mean ± SD or median (min-max).
AO indicates antioxidant; HOMA-IR, homeostasis model assessment method; QUICKI, Qualitative Insulin Sensitivity Check Index.



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**TABLE 3.** Comparison of Exocrine Marker in the 2 Groups

| Groups | Baseline ($t_1$) | 3 mo ($t_2$) | 6 mo ($t_3$) | *P* Overall Within Group | *P* Overall b/w Group |
|---|---|---|---|---|---|
| Fecal elastase (μg/g) | | | | | |
| Placebo, n = 53 | 20 (0.01-600) | 20 (0.01-436) | 18 (0.001-440) | 0.351 | 0.660 |
| AO, n = 54 | 20 (0.01-410) | 13.5 (0.01-600) | 19.5 (0.01-600) | 0.529 | |

Data are expressed as median (min-max).
AO indicates antioxidant.

continued till 6 months in both groups (PL $P = 0.009$ vs. AO $P = 0.016$). The severity of pain of the patients at 3 time points was similar in both the groups.

*Dietary assessment and body composition*. Macronutrient intake in patients was similar in the 2 groups (Supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/JCG/A484). There was a significant increase in body weight in both the groups ($P = 0.009$ and 0.001). Weight gain of 2.62 kg in PL and 2.88 kg in AO group was seen in 3 months. This increase was maintained till 6 months. The increase in weight was due to both components—fat mass and fat-free mass in the body. Increase in fat-free mass was similar in the AO (1.02 times) and PL (1.03 times) group.

## Adverse Events

The mean overall duration of exposure to AO/PL was 99.9 ± 14.7 days at 3 months and 204 ± 24 days at 6 months visit. None of the patients experienced any significant adverse event requiring discontinuation of the therapy (Supplemental Table 3, Supplemental Digital Content 1, http://links.lww.com/JCG/A484). There were no deaths reported during the study.

## Exploratory Subgroup Analysis

### Diabetics

There were 29 known diabetics on treatment for diabetes. No patient became diabetic during the trial period. A subgroup analysis on diabetics observed no difference in the 2 groups after 6 months AO supplementation on outcome measures and clinical parameters (Supplemental Table 4, Supplemental Digital Content 1, http://links.lww.com/JCG/A484).

### IGT

Subgroup analysis of the 29 patients with IGT is shown in Supplemental Table 5, Supplemental Digital Content 1, http://links.lww.com/JCG/A484.

### Steatorrhea

Patients with steatorrhea and/or abnormal fecal fat (n = 30) showed no difference in the 2 groups in any parameter over a period of 6 months supplementation, except that there was a significant weight gain from 51.3 to 54.1 kg ($P = 0.007$) and 50.9 to 57.6 kg ($P = 0.013$) in PL and AO group, respectively.

## DISCUSSION

Systematic reviews and meta-analyses on the effect of AO on pain in CP are available in the literature.[15–18,32,33] However, their role on pancreatic functions and other clinical outcomes has not been widely studied. This study found that AO supplementation in CP along with standard care showed no beneficial effect on any of the parameters studied including the endocrine and exocrine functions, markers of fibrosis, OS, inflammation and QOL, pain relief and nutritional status.

Not much has been reported in the literature on the effects of AO supplementation in CP diabetics. There is nearly 47% reduction in insulin secretion in response to glucose stimulation in patients CP. A study from India showed reduced expression of PDX-1which plays an important role in β-cell function.[34] Interferon-γ inhibit translocation of PDX-1 to nucleus and inhibit insulin gene expression.[34] It is postulated that OS-induced β cell dysfunction occurs in early stage and once β-cell dysfunction occurs, probably AO may not help in reversal of the process as possible in early stages. A study showed an improvement in endocrine pancreatic secretion (carbohydrate metabolism) and pain in CP patients who received vitamin C and E for 6 months.[12] Possibly, AO supplementation may be of limited help once β-cell destruction has taken place. It has been shown[35] that proportions of β cells in CP is reduced to about 60% of the control values. In our study, C-peptide levels were similar to those reported from another Indian study on CP patients.[36] Baseline HOMA-IR values [cutoff

**TABLE 4.** Comparison of Surrogate Markers of Fibrosis in the 2 Groups

| Groups | Baseline ($t_1$) | 3 mo ($t_2$) | 6 mo ($t_3$) | *P* Overall Within Group | *P* Overall b/w Group |
|---|---|---|---|---|---|
| Platelet-derived growth factor BB (pg/mL) | | | | | |
| Placebo, n = 53 | 4600 (624-16,000) | 3600 (740-21,000) | 3000 (700-12,800) | 0.004 | 0.442 |
| AO, n = 54 | 4000 (800-22,000) | 3900 (960-16,000) | 3200 (700-16,000) | 0.048 | |
| Transforming growth factor-β1 (pg/mL) | | | | | |
| Placebo, n = 53 | 33,264 ± 12,270 | 34,406 ± 13,834 | 34,323 ± 12,204 | 0.805 | 0.990 |
| AO, n = 54 | 35,074 ± 12,990 | 33,540 ± 10,492 | 33,314 ± 9497 | 0.575 | |
| α-Smooth muscle actin (ng/mL) | | | | | |
| Placebo, n = 53 | 1.6 (1.6-319) | 3.66 (1.6-416) | 1.6 (0.66-113) | 0.022 | 0.351 |
| AO, n = 54 | 1.6 (0.47-449) | 1.6 (0.17-1286) | 1.6 (0.33-291) | 0.963 | |

Data are expressed as mean ± SD or median (min-max).
AO indicates antioxidant.

**TABLE 5.** Comparison of Oxidative Stress and Antioxidant Status by Groups

| Groups | Baseline ($t_1$) | n | 3 mo ($t_2$) | n | 6 mo ($t_3$) | n | *P* Overall Within Group | *P* Overall b/w Group |
|---|---|---|---|---|---|---|---|---|
| Total antioxidant capacity (mmol/L) | | | | | | | | |
| Placebo | 1.67 ± 0.63 | 51 | 1.81 ± 0.84 | 43 | 1.57 ± 0.49 | 46 | 0.130 | 0.064 |
| AO | 1.47 ± 0.52 | 54 | 1.54 ± 0.29 | 44 | 1.67 ± 0.53 | 44 | 0.160 | |
| Urinary isoprostane (ng/mL) | | | | | | | | |
| Placebo | 0.98 (0.03-26) | 50 | 0.58 (0.03-20) | 42 | 0.42 (0.03-26) | 46 | 0.575 | 0.773 |
| AO | 0.98 (0.03-20) | 54 | 0.8 (0.03-10) | 42 | 0.76 (0.03-18) | 44 | 0.360 | |
| Corrected urinary isoprostane (ng/mg Cr excretion) | | | | | | | | |
| Placebo | 2.35 (0.04-57.1) | 46 | 2.64 (0.10-311) | 42 | 2.35 (0.04-57.14) | 46 | 0.793 | 0.876 |
| AO | 2.25 (0.02-47.61) | 42 | 3.54 (0.04-181.8) | 42 | 2.25 (0.02-47.61) | 42 | 0.327 | |

Data are expressed as mean ± SD or median (min-max).
AO indicates antioxidant.

≥ 2.6 insulin resistance[37] and QUICKI (cutoff ≤ 0.33[37])] showed no insulin resistance in our patients. Adrych et al[38] showed HOMA values which were higher than our study. Patients in their study were older and had higher body mass index compared with our patients.

Severe elastase insufficiency (normal > 100 U/g stool) was seen in 92.5% patients, suggesting an advanced exocrine dysfunction. Studies have reported varying values of fecal elastase ranging from 47.5 (range, 15 to 500),[39] to 206.5 ± 198 μg/g stool.[20] An Indian study showed low elastase in two thirds of their CP patients.[40] Compared with all these studies our patients had greater exocrine insufficiency. Exocrine insufficiency is a late manifestation in natural history of CP and cannot be reversed.

Our own previous study[7] and other studies[41,42] on patients with CP showed higher values of PDGF-AA and TGF-β1 compared with healthy controls. Recent in vitro studies on collagen-producing human pancreatic stellate cells have shown that inflammatory cytokines and increased OS activate pancreatic stellate cells leading to increased fibrosis.[43] PDGF-BB and TGF-β1 are significantly raised in patients with CP and is a surrogate marker of fibrosis.[38] No significant change in the surrogate markers have been noticed.

Increased levels of OS and decreased AO status in CP compared with healthy controls has been reported around the globe[3–5,44] including India[45] and our own group.[6,7] Similarly pancreatic tissue damage by OS has also been documented in various studies.[46,47] Our findings on OS are similar to the ANTICIPATE study[20] but in contrast to other studies which showed a decrease in patients receiving AO supplementation.[6,7]

In our study baseline values of TAC were lower than that reported in healthy Indian industrial population.[48] The

**TABLE 6.** Comparison of Quality of Life in the 2 Groups

| Groups | Baseline ($t_1$) | n | 3 mo ($t_2$) | n | 6 mo ($t_3$) | n | *P* Overall Within Group | *P* Overall b/w Group |
|---|---|---|---|---|---|---|---|---|
| Global health status/QOL (raw scores 1-7) high score represents high QOL | | | | | | | | |
| Placebo | 4.54 ± 1.51 | 53 | 5.02 ± 1.40 | 43 | 5.28 ± 0.83 | 46 | 0.071 | 0.541 |
| AO | 4.43 ± 1.64 | 54 | 5.21 ± 1.40 | 44 | 5.5 ± 1.30 | 44 | 0.001 | |
| Functional scale—physical function (raw scores 1-4) | | | | | | | | |
| Placebo | 1.13 ± 0.29 | 53 | 1.03 ± 0.1 | 43 | 1.02 ± 0.09 | 46 | 0.125 | 0.030 |
| AO | 1.21 ± 0.37 | 54 | 1.10 ± 0.19 | 44 | 1.06 ± 0.12 | 44 | 0.013 | |
| Functional scale—role function (raw scores 1-4) | | | | | | | | |
| Placebo | 1.09 ± 0.29 | 53 | 1.01 ± 0.07 | 43 | 1.02 ± 0.10 | 46 | 0.175 | 0.179 |
| AO | 1.18 ± 0.39 | 54 | 1.03 ± 0.16 | 44 | 1.02 ± 0.10 | 44 | 0.009 | |
| Functional scale—emotional function (raw scores 1-4) | | | | | | | | |
| Placebo | 1.50 ± 0.50 | 53 | 1.30 ± 0.46 | 43 | 1.22 ± 0.34 | 46 | 0.006 | 0.886 |
| AO | 1.54 ± 0.66 | 54 | 1.24 ± 0.44 | 44 | 1.21 ± 0.38 | 44 | 0.001 | |
| Functional scale—cognitive function (raw scores 1-4) | | | | | | | | |
| Placebo | 1.35 ± 0.44 | 53 | 1.19 ± 0.47 | 43 | 1.18 ± 0.32 | 46 | 0.001 | 0.191 |
| AO | 1.29 ± 0.46 | 54 | 1.07 ± 0.18 | 44 | 1.12 ± 0.26 | 44 | 0.002 | |
| Functional scale—social function (raw scores 1-4) | | | | | | | | |
| Placebo | 1.28 ± 0.53 | 53 | 1.11 ± 0.35 | 43 | 1.08 ± 0.24 | 46 | 0.672 | 0.029 |
| AO | 1.54 ± 0.70 | 54 | 1.21 ± 0.39 | 44 | 1.11 ± 0.23 | 44 | 0.001 | |
| Symptom scale—pain (raw scores 1-4) | | | | | | | | |
| Placebo | 1.34 ± 0.67 | 53 | 1.17 ± 0.49 | 43 | 1.09 ± 0.32 | 46 | 0.016 | 0.449 |
| AO | 1.48 ± 0.72 | 54 | 1.19 ± 0.43 | 44 | 1.15 ± 0.33 | 44 | 0.009 | |
| Symptom scale—pancreatic pain (raw scores 1-4) | | | | | | | | |
| Placebo | 1.37 ± 0.49 | 53 | 1.31 ± 0.50 | 43 | 1.16 ± 0.25 | 46 | 0.053 | 0.886 |
| AO | 1.42 ± 0.49 | 54 | 1.28 ± 0.34 | 44 | 1.17 ± 0.28 | 44 | 0.002 | |

Data are expressed as mean ± SD.
Except for global health status low score represents high QOL.
AO indicates antioxidant; QOL, quality of life.



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**TABLE 7.** Comparison of Duration and Intensity of Pain in All the Patients

| Groups | Baseline ($t_1$) | Pain [n (%)] | 3 mo ($t_2$) | Pain [n (%)] | 6 mo ($t_3$) | Pain [n (%)] | *P* Overall Within Group | *P* Overall b/w Group |
|---|---|---|---|---|---|---|---|---|
| Painful days per month | | | | | | | | |
| Placebo (n = 53) | 1.33 (0-30) | 38 (71.7) | 0 (0-18.6) | 17 (39.5) | 0.02 (0-30) | 23 (50.0) | 0.006 | 0.699 |
| AO (n = 54) | 1.0 (0-30) | 35 (64.8) | 0 (0-30) | 18 (40.9) | 0.03 (0-30) | 23 (52.3) | 0.001 | |
| Visual analogue score (0-10) | | | | | | | | |
| Placebo | 4 (0-8) | 53 | 0 (0-6) | 43 | 0.50 (0-6) | 46 | 0.001 | 0.407 |
| AO | 3 (0-9) | 54 | 0 (0-5) | 44 | 1 (0-6) | 44 | 0.001 | |

Data are expressed as median (min-max).
AO indicates antioxidant.

individual increase in micronutrient AO level is in agreement with studies from United Kingdom,[19,20,24,39] Poland,[12] and India.[6] Total AO capacity increased after supplementation in various studies[6,7] and our study. Despite increasing levels of AO the OS showed no signs of improvement.

Studies have shown higher CRP,[38,41,42,49] TNF-α, and IL-6[42,49] values in patients with CP as compared with controls. Contrary to this Bamba et al[50] showed IL-6 levels similar to normal controls. One reason for this could be due to the fact that normal values of these markers are difficult to define as they are not done routinely. Our results are similar to ANTICIPATE study[39] in which there was no elevation in the broad array of proinflammatory and anti-inflammatory cytokines in the AO group compared with PL either at baseline or after 6 months of AO therapy.

Our results on pain are in contrast to studies which have shown a significant reduction in number of pain days per month,[6,7] and intensity of pain as assessed by VAS[23,24,39] in patients receiving AO. But, our results are in consonance with the study by Siriwardena et al,[20] which showed no improvement in pain control. Recent Cochrane review on AO for pain in CP concluded that AO can reduce pain slightly in patients with CP but the clinical relevance of such a small reduction is uncertain.[15]

Various studies have shown that QOL is impaired in patients with CP.[31,51] The findings of our study are in contrast with those of studies assessing QOL in patients with CP receiving AO.[19,21] Shah et al[21] showed improved QOL and decreased requirement of pain killers in patients taking AO. This study was criticized for its methodological problems and the conclusions were disqualified.[52] A subsequent study by the same group[20] showed no improvement in QOL by AO supplementation.

The energy intake of our patients was deficient by approximately 17% of the "Recommended Dietary Allowances" (RDA) for Indians. This has improved compared to a 37% deficit seen by us in a similar group of patients almost 15 years back.[53]

Results of this study are *generalizable* to the patients who fulfill the study inclusion and exclusion criteria. In this study we have taken all precautions to minimize bias at different stages of the study and control for the effect of confounders (if any). Therefore, the results obtained in this study are scientifically valid. Since the power of the study is <80% (usually accepted), the reproducibility of the study is less than desired. Hence there is a need to confirm the findings of this study by taking appropriate sample size, that is by ensuring adequate power of study to confirm the reliability of the effect of intervention as compared with usual care.

The *strength* of our study is that to our knowledge it is the first randomized, PL-controlled trial conducted in a relevant and well characterized patient population that has comprehensively assessed the effect of AO supplementation on all aspects of CP. In our study only 15.8% lost to analysis, with similar withdrawals in both treatment allocations. Compliance with study intervention was formally verified and was good. Blood AO levels in patients showed steady and considerable treatment effect consistent with compliance.

Our study has a few potential *limitations*. Our study is not adequately powered. One of the reasons the sample size being small is that 285 patients were excluded of 447 patients with CP screened. We think that having some information (in the context of small sample size) is preferable than having no information. This study alone is not sufficient enough to be conclusive. The results based on this exploratory study can be only treated as the basis for future studies and it could contribute to a meta-analysis on this issue.

The large variations observed in the results indicate a highly variable effect on the markers studied this could be due to the disease duration ranging from 0.03 to 25 years. Surrogate markers studied should have been shown in the pancreatic tissue instead of blood. Pancreas is a deep-seated organ, and biopsy is not indicated in routine management, and so, we had to study surrogate markers in blood.

The results of our study could impact on clinical practise in the foreseeable future. In a heterogeneous population of patients with CP with long standing disease, the effect of AO on endocrine and exocrine functions and other clinical outcomes is not discernable. AO therapy may have some impact in patients with CP at an early stage or in patients with recurrent acute pancreatitis. The exploratory study results of this double-blind randomized PL-controlled study suggests that 6 months of daily AO supplementation in pharmacologic concentrations in patients with CP leads to an increase in circulating individual AO without affecting the endocrine and exocrine function, markers of fibrosis, OS, inflammation, with no benefit on QOL, pain and nutritional status. However, a larger adequately powered study will be required.

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