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Orginal article

Role of methionine containing antioxidant combination in the management of pain in chronic pancreatitis: A systematic review and meta-analysis☆

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ABSTRACT

Background: Pain in CP results from inflammation and neuroimmune alterations that are associated with oxidative stress, among other mechanisms. This is marked by depletion of antioxidant defenses including methionine, which is a donor of methyl moieties that maintains the acinar transsulfuration pathway. We performed a systematic review and meta-analysis of trials evaluating methionine-containing antioxidants in CP.

Patient and methods: Literature search was conducted in Medline/Pubmed, EMBASE, and Cochrane databases. Systematic review and meta-analysis was performed per PRISMA guidelines. Main study outcome was pain relief. GRADE system was used for quality assessment. Heterogeneity was assessed by the Q and I^2 measures; publication bias by Egger's test. Random-effect model (DerSimonian and Laird) was used if there was heterogeneity.

Results: Eight studies (n = 411) were identified that used methionine-containing antioxidants. The study duration ranged from 10 wks to 12 months. All studies used methionine, organic selenium, ascorbate, beta-carotene and alpha-tocoferol. Four studies (including two RCTs) that reported change in pain scores were metaanalyzed. Though overall effect [standardized difference in means (95% CI)] on pain score reduction was −0.95 (−1.738 to −0.160) (z = −2.36; p = 0.018), the significance was lost when the two RCTs were meta-analyzed. RCTs that reported the number of pain free patients had a statistically significant overall effect of −3.204 (p = 0.001). Though more patients on methionine containing antioxidants had adverse events, majority of them were mild.

Conclusion: Methionine containing antioxidants appear to result in pain reduction in a significant proportion of CP patients. Further randomized controlled trials with homogeneous outcome measures are needed.

Introduction

Pain in chronic pancreatitis (CP) is as enigmatic as the disease itself. Majority of the patients with CP presents with recurrent intractable abdominal pain that mandates aggressive management approaches. Several modalities of pain management in CP (eg. pancreatic enzymes, antioxidants, extracorporeal shock wave lithotripsy, pancreatic duct stenting, and surgical drainage and resection procedures) have evolved over the years. Unfortunately, none of these currently existing therapeutic modalities is completely efficient in providing permanent pain relief in a consistent and predictable manner. This testifies the complex and multifactorial mechanisms of genesis of pain in CP [1].

Abbreviations: CP, chronic pancreatitis; ROS, reactive oxygen species; PRISMA, preferred reporting items for systematic reviews and meta-analyses; EMBASE, excerpta medica database; CI, confidence interval; SD, standard deviation; VAS, visual analog scale; NRS, numeric pain rating scale; BPI, brief pain inventory scores; EORTC, European Organization for Research and Treatment of Cancer; SF, short form; RCT, randomized controlled trial.

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Several clinical and experimental studies have demonstrated generation of oxidative stress and depletion of antioxidant defenses in CP both within the gland in an organ-specific manner and in circulation [2–9]. In the pancreas, reactive oxygen species (ROS) are generated within the acinar and pancreatic stellate cells (PSCs) irrespective of the etiology. An important aspect of intra-acinar oxidative stress is the derangement of the transsulfuration pathway that is required for zymogen exocytosis [10–12]. This results in recurrent intraacinar zymogen activation consequent to pancreastasis. Methyl and thiol donors such as methionine and ascorbic acid are necessary for maintenance of the transsulfuration pathway. Depletion of these in the presence of recurrent ROS generation results in a pro-oxidant state that is known to trigger inflammatory cascades (Fig. 1) [13,14].

Even though several clinical studies have evaluated different antioxidant preparations (eg. methionine, ascorbic acid, selenium, dimethyl sulfoxide, alpha-tocoferol, beta-carotene, curcumin, S adenosyl methionine), results have been variable [15]. Even in actual clinical experience opinion regarding the efficacy of antioxidants in pain relief in patients with CP appears disparate. Few of the possible reasons for variability in results of antioxidant trials and non-efficacy of antioxidants in clinical practice could be the use of inappropriate antioxidant agents or antioxidant use at a suboptimal dose. Furthermore, in view of the complexity of pain mechanism, a single antioxidant agent is unlikely to show sufficient benefit. From a mechanistic perspective particularly in the context of CP, methionine appears to be an important component in maintaining the transsulfuration pathway and preventing pancreastasis and subsequent inflammation. We therefore conducted the current systematic review and meta-analysis to evaluate the role of methionine containing antioxidant combination in managing pain in CP.

Material and methods

Study selection

This systematic review and meta-analysis was conducted as per Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [16]. We conducted a systematic literature search in Medline/Pubmed, Excerpta Medica Database (EMBASE), Scopus, and Cochrane databases from January 1980 to August 2014 and proceedings of major conferences from January 2000 to August 2014, using the search words 'chronic pancreatitis', 'pain', and 'antioxidant'. Inclusion criteria included full-length manuscripts of trials that used antioxidant combinations containing methionine and reported effect on pain. There were no language restrictions in the selection. Articles in non-English language

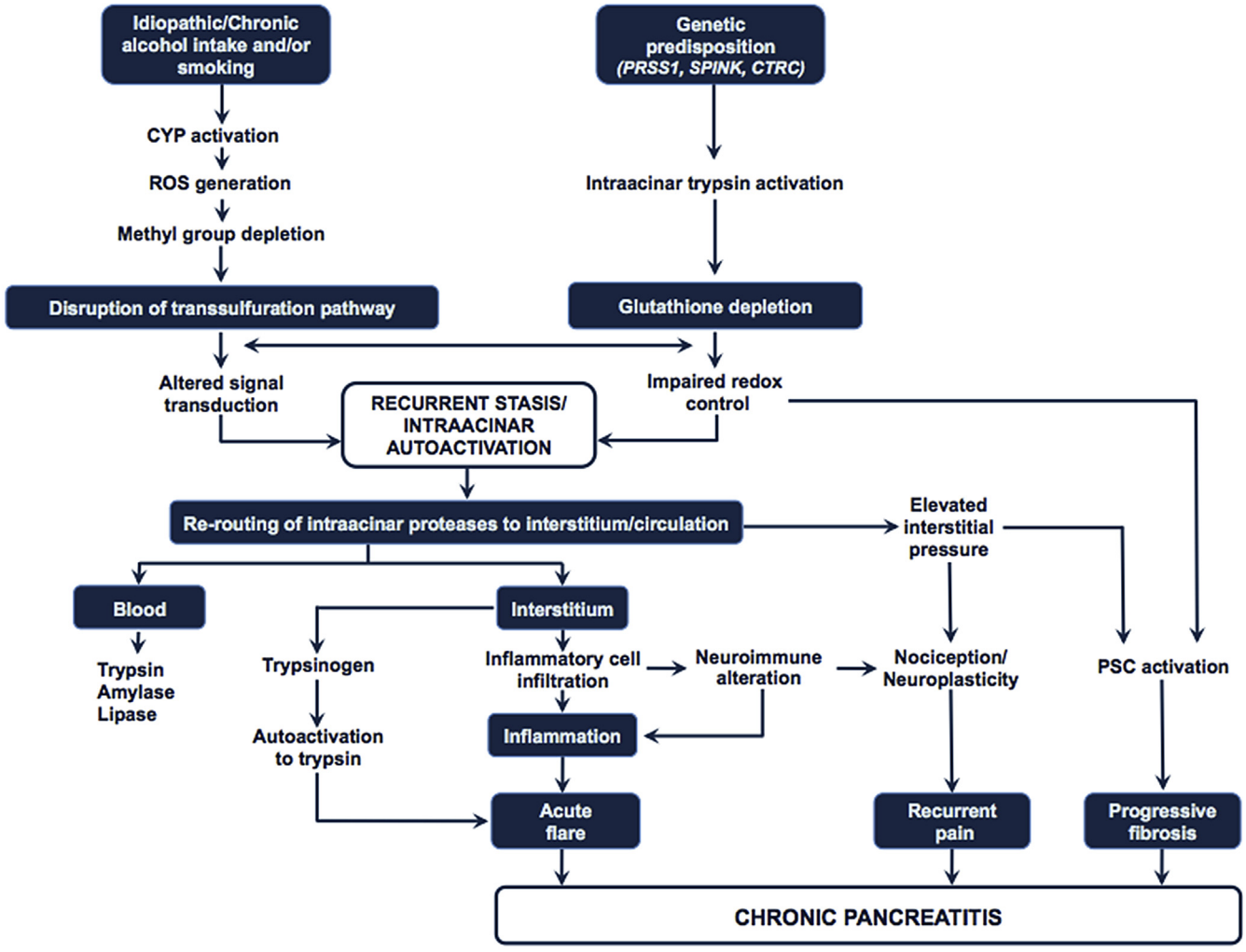

Fig. 1. Schematic diagram representing the rationale for studying methionine containing antioxidants (adapted from Refs. [10,11,13,14]). *CYP indicates cytochrome; ROX reactive oxygen species; PSC pancreatic stellate cells.*

were translated to English using online translation services (Google Translate). Exclusion criteria were: experimental studies; observational and mechanistic (association) studies; studies using single antioxidant agent; case report/series; abstracts; editorials, commentary and review articles.

Data abstraction

Two of the investigators (RT and HVVM) abstracted data independently using a standard proforma; and any discrepancies were mutually resolved with consensus. Following parameters were recorded: first author and year of publication; country of origin; sample size in treated and placebo groups; study duration; outcome measures and tools used; adverse event reporting; markers of oxidative stress; circulating antioxidant levels; and antioxidant capacity. Attempts were made to contact the corresponding authors of studies for any missing data points.

Outcome measures

The study outcome measures were: change in pain score; number of patients who were completely pain free; number of hospital visits/hospitalization; analgesic requirement; change in antioxidant levels in blood; change in markers of oxidative stress in blood; change in antioxidant capacity; and adverse events related to antioxidant treatment.

Assessment of study quality

Quality of individual studies was assessed by the Cochrane GRADE system [17]. Individual studies were assessed for random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other potential biases. The risk of bias was expressed as low, intermediate or high grade for each component in individual studies. The grades of risk were defined as per the Cochrane Collaboration definitions.

We also used the GRADE working group definitions [18] to rate the quality of evidence from individual studies. These included: *high quality*-further research is very unlikely to change confidence in the estimate of effect; *moderate quality*-further research is likely to have an important impact on confidence in the estimate and may change the estimate; *low quality*-further research is very likely to have an important impact on confidence in the estimate and is likely to change the estimate; *very low quality*-uncertainty about the estimate.

Statistical analysis

A database was generated in Excel for Mac (Microsoft Corp., Redmont, WA) and meta-analysis was performed using the Comprehensive Meta-analysis package (Ver. 2.2.064; 2011). Effect sizes for numerical variables were expressed as standardized difference in means with 95% confidence interval (CI); while that of categorical data were expressed as odd's ratio with 95% CI. Whenever data in individual studies were expressed as a range, they were converted to standard deviation (SD) before analysis. Between-study heterogeneity was assessed by the I^2 measure, and was considered to be important if it was greater than 25%. Q measure was used to evaluate significance of heterogeneity and was considered statistically significant when $p < 0.1$. Random effect model (DerSimonian and Laird) [19] was used when there was heterogeneity, while a fixed effect model (Mantel–Haenszel method) [20] was used in the absence of heterogeneity. Publication bias was evaluated and quantified by the Egger's test.

Results

Characteristics of individual studies

As shown in Fig. 2 (PRISMA flow chart), a total of 369 citations were identified on initial database search. After screening, 306 of these were excluded and the remaining 63 were thoroughly assessed for eligibility criteria. Fifty-five of these met exclusion criteria and were thus excluded. The remaining eight studies [21–28] were included for qualitative and quantitative analysis. Two of these studies were the same in terms of design and patient cohort [27,28], but the clinical outcomes and biochemical parameters were published separately as two manuscripts. Only one sample size was considered from these two studies.

Table 1 shows the study characteristics. There were a total of 452 enrolled patients, of which 34 (7.5%) patients did not complete follow-up, withdrew participation or had to be excluded due to various reasons after enrollment. Of the 418 analyzed patients 241 were male and mean (SD) age was 36.3 (11.4) yrs. Six studies [21,22,24,25,27,28] were double blinded randomized controlled trials (three parallel group and three crossover); while one each had a non-randomized prospective [23] and a prospective descriptive pre-post open design [26] respectively. All patients had documented CP in four studies, while the studies by Castano et al. [26] and Uden et al. [27,28] included three and five patients with recurrent acute pancreatitis respectively. The study by Kirk et al. [25] did not report the etiology of chronic pancreatitis. In the remaining studies, 155 patients had alcoholic CP; and 140 patients smoked cigarettes. Adverse outcomes were reported in three [22,24,25] studies. All of the included studies used organic selenium, methionine, alpha-tocoferol, beta-carotene and ascorbic acid as the antioxidant combination, in doses as shown in Table 1.

Study outcomes

Table 2 shows the tools that were used to measure the reported study outcomes. Pain score was reported in 5 studies [22,23,25,26,28]; however the tools utilized were variable. Three studies used the visual analog scale (VAS) [23,25,26], one study used a numeric pain rating scale (NRS) and brief pain inventory scores (BPI) [22], and one used a pain vocabulary score sheet [28]. Three studies [24,26,28] reported the number of patients with pain relief. Three studies [24,26,28] reported the number of patients who were completely pain free, while one study [26] reported the number of admissions during the study period and two [22,24] reported the number of hospital visits (in days). Four studies [21–24] reported the analgesic requirement. Quality of life was measured in three studies [22,23,25], two [22,23] of which used the EORTC QLQ C 30 with PAN 28 while one [25] used the SF36 questionnaire. Plasma antioxidant levels and oxidative stress markers were measured in four [22,24,25,27] and three [21,24,27] studies respectively; but there were variability in the parameters reported. Antioxidant capacity was measured in three studies [21,24,25].

Study quality assessment

Table 3 shows the GRADE analysis of risk of bias across the individual studies; while Supplementary Fig. 1 depicts the composite risk of bias graph across all the included studies. Overall, the risk of bias was low in four [21,22,24,27,28] of the randomized studies. The two non-randomized studies [23,26] had high risks of selection, performance and detection biases. The risk of detection bias was

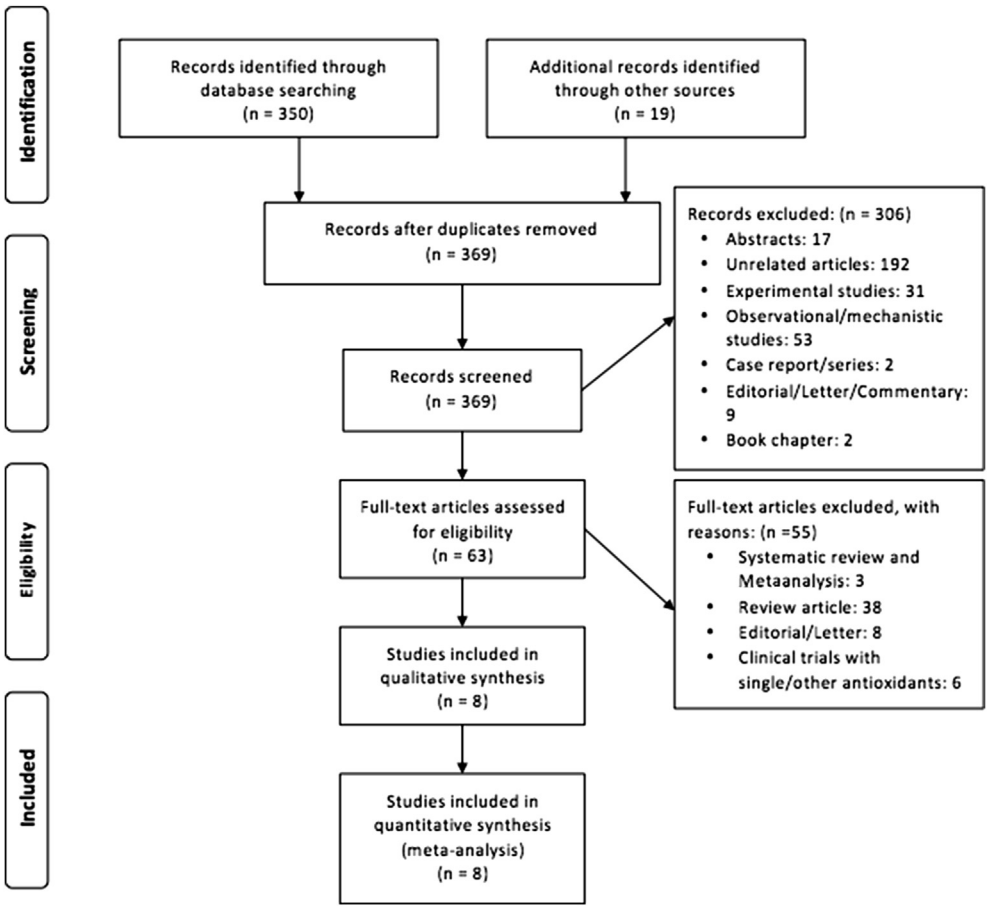

Fig. 2. PRISMA flow diagram depicting the selection of studies for systematic review and meta-analysis.

unclear in three studies [21,25,27,28]. High attrition and reporting biases were observed in the study by Kirk et al. [25], which did not have a wash out period and had a high dropout rate. Furthermore, VAS was recorded erratically and retrospectively in few cases in this study, and was therefore not analyzed by the authors.

Effect of antioxidant combination on pain

Four [22,23,26,28] studies reported the effect of antioxidant combination on pain scores. Even though the study by Kirk et al. [25] did measure pain score, it did not analyze the data. As shown in Fig. 3a, antioxidant combination resulted in a significant reduction in pain score, with an overall effect size (Z) of −2.36 ($p = 0.018$). However, there was significant heterogeneity among the studies ($I^2 = 83.38\%$; $p < 0.0001$). Furthermore, when the non-randomized studies by Shah et al. [23] and Castano et al. [26] were removed from the meta-analysis, the efficacy of antioxidant combination in reduction of pain scores lost its statistical significance ($Z = -1.11$; $p = 0.27$). There was heterogeneity between the two studies ($I^2 = 64.84\%$; $p = 0.09$). The quality of evidence from these two RCTs was of moderate level.

Three studies [24,26,28] (two RCTs) reported on the proportion of pain free patients after treatment with antioxidant combination; of which the two RCTs [24,28] were meta-analyzed. The grade of evidence of these two RCTs was moderate. 47.9% of patients receiving antioxidants remained completely pain free compared to 27.8% in the placebo group during treatment. Meta-analysis revealed a statistically significant lower Odd's of having pain in favor of antioxidant combination, with an effect size (Z) of −3.204 ($p = 0.001$). The number needed treat (NNT) for pain relief was 5.

We attempted to perform a subgroup analyses on treatment response based on etiology (alcohol related vs idiopathic CP). However, none of the individual studies conducted systematic analyses of response according to etiology, even though the study by Bhardwaj et al. [24] mentioned that there were no confounding effects by factors such as the disease etiology or abstinence from alcohol. Furthermore, the study by Uden et al. (1990) [28] did report a time series analysis of pain dairies in which significant change in mean pain scores was observed in 2 patients each in the alcoholic and idiopathic CP group. Interestingly, seven patients in the idiopathic group in this study smoked cigarettes; and there was no statistically significant reduction in pain scores in six of these seven patients.

The effect of the duration of the study period (3 months vs 6 months) was also not formally evaluated by any of the individual studies. However, the recent RCTs by Siriwerdena et al. and Bhardwaj et al. evaluated patients at different time intervals within the study period; and there was a statistically significant trend in the reduction in number of painful days/month at 3 and 6 months in the study by Bhardwaj et al.

Need for hospital admissions/outpatient care

Two RCTs [22,24] that reported the number of hospital visits and admissions were meta-analyzed. An effect size (z) of −1.856 for reduction of hospital visits and admission was observed in favor of

Table 1
Characteristics of included studies.

Author; year; Country	Design	Sample size (analyzed)		Durn. of study	Individual antioxidant components and daily doses	Outcomes measures	Adverse event report
		Antioxidant group	Placebo group				
Dhingra et al.; 2013 [21] India	Randomized double-blinded placebo controlled trial; Parallel group	31	30	3 months	Methionine 2 g; Selenium 600 μg; Beta-carotene 54 mg; Alpha-tocopherol 280 mg; Ascorbic acid 540 mg.	- Change in surrogate markers of fibrosis. - Change in blood markers of oxidative stress. - Number of painful days and analgesic requirement	No
Siriwerdena et al.; 2012 [22] UK	Randomized double-blinded placebo controlled trial.	33	37	6 months	Methionine 2.88 g; Selenium 300 μg; Beta-carotene 25.2 mg; Alpha-tocopherol 228 mg; Ascorbic acid 720 mg.	- Change in pain score. - Quality of life. - Opiate use. - Pancreatitis related hospital visits or admission. - Change in antioxidant levels.	Yes
Shah et al.; 2010 [23] UK	Non-randomized prospective study	28	28	6 months		- Change in pain score. - Quality of life.	No
Bhardwaj et al.; 2009 [24] India	Randomized double-blinded placebo controlled trial.	71	56	6 months	Methionine 2 g; Selenium 600 μg; Beta-carotene 54 mg; Alpha-tocopherol 280 mg; Ascorbic acid 540 mg.	- Change number of painful days. - Change in analgesic requirement. - Change in severe attacks of pancreatitis. - Need for hospitalization. - Complete pain relief. - Change in markers of oxidative stress and antioxidant levels.	Yes
Kirk et al.; 2006 [25] UK	Randomized double-blinded placebo controlled cross over	19	19	10 weeks in each arm	Methionine 1.6 g; Selenium 300 μg; Beta-carotene 12 mg; Alpha-tocopherol 188 mg; Ascorbic acid 600 mg.	- Change in pain score. - Quality of life. - Additional analgesic requirement - Change in antioxidant levels.	Yes
Castano et al.; 2000 [26] Spain	Prospective descriptive pre-post open design	10	10	12 months		- Change in pain score. - Number of hospital admissions.	No
Uden et al.; 1992 [27] UK Uden et al., ; 1990 [28] UK	Randomized double-blinded placebo controlled cross over	23	23	10 weeks in each arm	Methionine 2 g; Selenium 600 μg; Beta-carotene 54 mg; Alpha-tocopherol 280 mg; Ascorbic acid 540 mg.	- Antioxidant and free radical markers in blood. - Pain score - Pain frequency. - Psychological assessment.	No

antioxidant combination; with a trend towards statistical significance ($p = 0.063$).

Adverse events

Adverse events were reported in 3 RCTs [22,24,25]. On meta-analysis of the studies by Siriwerdena et al. and Bhardwaj et al., these were found to be significantly higher in the antioxidant group compared to placebo (19.2% v/s 4.3%; $Z = -2.79$; $p = 0.005$) (Fig. 3b). We did not put the study by Kirk et al. into the meta-analysis in view of the high risk of bias. Though significantly higher in the antioxidant group, the adverse events were mild, and included headache, constipation, nausea, bad taste and heartburn. There were only two moderately severe adverse events in the form of increased frequency of stool with occasional diarrhea and convulsion in the antioxidant group. No mortality was observed in any of the study.

Analgesic requirement

Four studies (three RCTs [21,22,24] and one non-RCT [23]) reported the requirement of analgesics during the study period. Among the three RCTs, one study [21] reported the number of patients requiring analgesics, one [22] reported the dose of opioids and the third [24] reported the number of oral and parenteral analgesics required. These studies could not be meta-analyzed in view of the variability in the reported parameters.

Blood levels of antioxidant micronutrients

Changes in the levels of alpha-tocoferol and beta-carotene were reported by three studies [22,24,25,27], while three each reported that of ascorbate [22,24,25] and organic selenium [22,24,27]. As shown in Fig. 4a–d, there was a significant improvement in the blood levels of these antioxidant micronutrients in favor of methionine containing antioxidant combinations.

Antioxidant capacity

Antioxidant capacity was evaluated using ferric reducing activity of plasma (FRAP) in two studies [21,24] and total antioxidant capacity (TAC) in another [25]. Meta-analysis of these 3 studies demonstrated a significant increase in antioxidant capacity after use of antioxidant combination with a statistically significant effect size (Z) of −2.702 ($p = 0.007$) (Fig. 4e).

Publication bias

No publication bias was found when the four studies reporting pain scores were evaluated (Egger's intercept: −6.295 [95%CI −23.8 to 11.2]; $p = 0.262$). Publication bias could not be evaluated for the

Table 2
Tools used for evaluating outcomes in the included studies.

Author; year	Pain scores	Number of patients who had reduction in pain	Number of patients who were pain free	Number of hospital visit/ hospitalization	Analgesics use	Quality of life	Antioxidant levels in blood	Antioxidant capacity	Oxidative stress markers in blood
Dhingra et al.; 2013 [21]	NR	Yes	NR	NR	Yes (Number of patients requiring analgesics)	NR	NR	Yes (FRAP)	Yes (TBARS)
Siriwerdena et al.; 2012 [22]	Yes (NRS; BPI)	NR	NR	Yes (Number of days visited/ hospitalized)	Yes (Opiate dose)	Yes (EORTC QLC30 & PAN28)	Yes (Vit C, E, beta carotene; selenium; GSH)	NR	Yes (Linoleic acid)
Shah et al.; 2010 [23]	Yes (VAS)	NR	NR	NR	Yes (Number of patients requiring analgesics)	Yes (EORTC QLC30 & PAN28)	NR	NR	NR
Bhardwaj et al.; 2009 [24]	NR	NR	Yes	Yes (Number of admissions)	Yes (Number of oral and parenteral analgesics)	NR	Yes (Vit A, C, D; TGSH)	Yes (FRAP)	Yes (TBARS/SOD)
Kirk et al.; 2006 [25]	Yes (VAS)	NR	NR	NR	NR	Yes (SF36)	Yes (Vit C, retinol, tocoferol, beta carotene; selenium; GPX)	Yes (TAC)	Yes (MDA)
Castano et al.; 2000 [26]	Yes (VAS)	Yes	Yes	Yes (Number of admissions)	NR	NR	NR	NR	NR
Uden et al.; 1992 and 1990 (same cohort of patients) [27,28]	NR Yes (Pain vocabulary score sheet)	NR NR	NR Yes	NR NR	NR NR	NR NR	Yes (Vit E; beta carotene; selenium; SAMe) NR	NR NR	Yes (Linoleic acid) NR

randomized studies that reported reduction in pain score and proportion of pain free patients since the number of studies was inadequate.

Discussion

In this systematic review and meta-analysis, we evaluated the efficacy of methionine containing antioxidant combination in the management of pain in patients with CP. We meta-analyzed eight studies [21–28], of which six [21,22,24,25,27,28] were RCTs. Even though there was heterogeneity among the studies, meta-analyses of the RCTs showed a significantly higher proportion of patients taking methionine containing antioxidants were pain-free compared to placebo (47.9% vs 27.8%; $p = 0.001$). There was also a trend towards lesser requirement of hospital visits/hospitalization in favor of methionine containing antioxidants ($p = 0.063$). Even though higher proportion of patients receiving antioxidants experienced adverse events compared to those who received placebo (19.2% vs 4.3%; $p = 0.005$), all but two were mild. This testifies the relatively fair safety profile of methionine containing antioxidant combination even at a fairly high dose.

The role of antioxidants in the management of pain in CP has been evaluated in many studies including three recent systematic reviews and meta-analysis [29–31]. Several antioxidant preparations like methionine, ascorbic acid, selenium, dimethyl sulfoxide, alpha-tocoferol, beta-carotene, curcumin and S adenosyl methionine, have been studied so far as single agent or in combination, with variable results [15]. In the meta-analysis of nine RCTs by Cai et al. [29], it was shown that single agent antioxidant therapy did not have a significant impact on pain relief (SMD −0.12 [95% CI −1.23 to 0.99]; $p = 0.83$); whereas use of a combination of methionine, ascorbic acid, selenium, alpha-tocoferol and beta-carotene resulted in a significant improvement in pain (SMD −0.93 [95%CI −1.72 to −0.14]; $p = 0.02$). Furthermore, even though antioxidants were associated with adverse events most of them were mild. A subsequent meta-analysis of eight studies by Zhou et al. [30] concluded that there was a significant reduction in the proportion of patients with pain (RR 2.15 [95%CI 1.72–2.69]; $p < 0.00001$) along with a significant reduction in analgesic requirement (RR 0.56 [95% CI 0.40–0.78]; $p = 0.0006$). On the other hand, the most recent Cochrane Systematic Review [31] of 18 studies concluded that antioxidants could result in a slight reduction in pain in patients with CP (SMD −0.33 95% CI −0.64 to −0.02];

Table 3
Risk of bias of the included studies.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data addressed (attrition bias)	Selective reporting (reporting bias)	Other bias
Dhingra et al.; 2013 [21]	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Siriwerdena et al.; 2012 [22]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Shah et al.; 2010 [23]	High risk	High risk	High risk	High risk	Low risk	Low risk	High risk
Bhardwaj et al.; 2009 [24]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Kirk et al.; 2006 [25]	Low risk	Low risk	Low risk	Unclear risk	High risk	High risk	High risk
Castano et al.; 2000 [26]	High risk	High risk	High risk	High risk	Low risk	Low risk	High risk
Uden et al.; 1992 [27]/1990 [28]	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Unclear risk

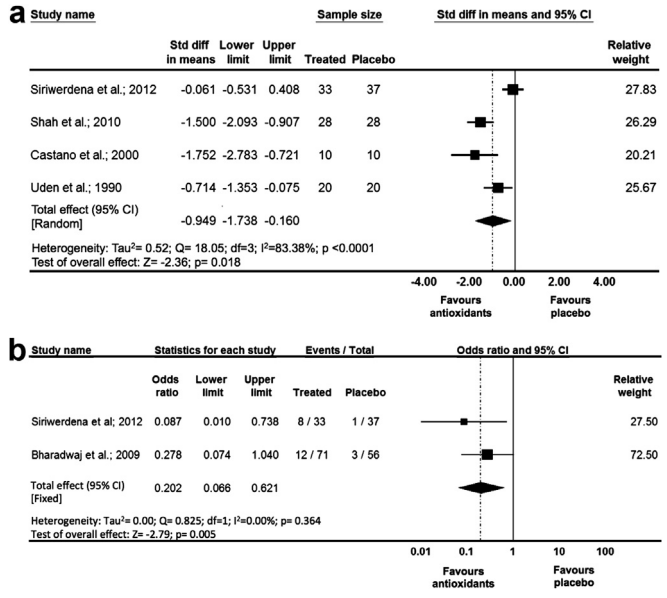

Fig. 3. a) Forrest plot of all studies that reported pain score. b) Forrest plot of studies that reported adverse events during the study.

p = 0.04), but there are no conclusive data on analgesic requirement and quality of life measures. In actual clinical experience too, opinion regarding the efficacy of antioxidants in pain relief in patients with CP appears disparate. Few of the possible reasons for variability in results of antioxidant trials and non-efficacy of antioxidants in clinical practice could be the use of inappropriate antioxidant agents or use at a suboptimal dose.

In the current systematic review and meta-analysis, we evaluated specifically methionine containing antioxidant combination on pain relief, analgesic requirement, improvement in antioxidant capacity, quality of life and adverse events in patients with CP. Fig. 1 depicts the rationale for using methionine containing antioxidant cocktails in our meta-analyses. Irrespective of the etiology, it has been shown in several clinical and experimental studies that there is generation of significant oxidative stress within the intra- and extra-acinar components (including PSCs) of the pancreas in CP [2–9]. Generation of oxidative stress depletes intra-acinar thiol and methyl groups that are required to maintain the transsulfuration pathway, which is essential for exocytosis of zymogens from the acinar apical region to the pancreatic ductules. Disruption of the transsulfuration pathway eventually contributes, among other factors, to recurrent intrapancreatic inflammation, pain, further oxidative stress and progression of fibrosis [10–12]. Therefore, replenishing the thiol- and methyl stores by methionine supplementation along with other antioxidant compounds could ameliorate the severity of inflammation and resulting pain. Dose

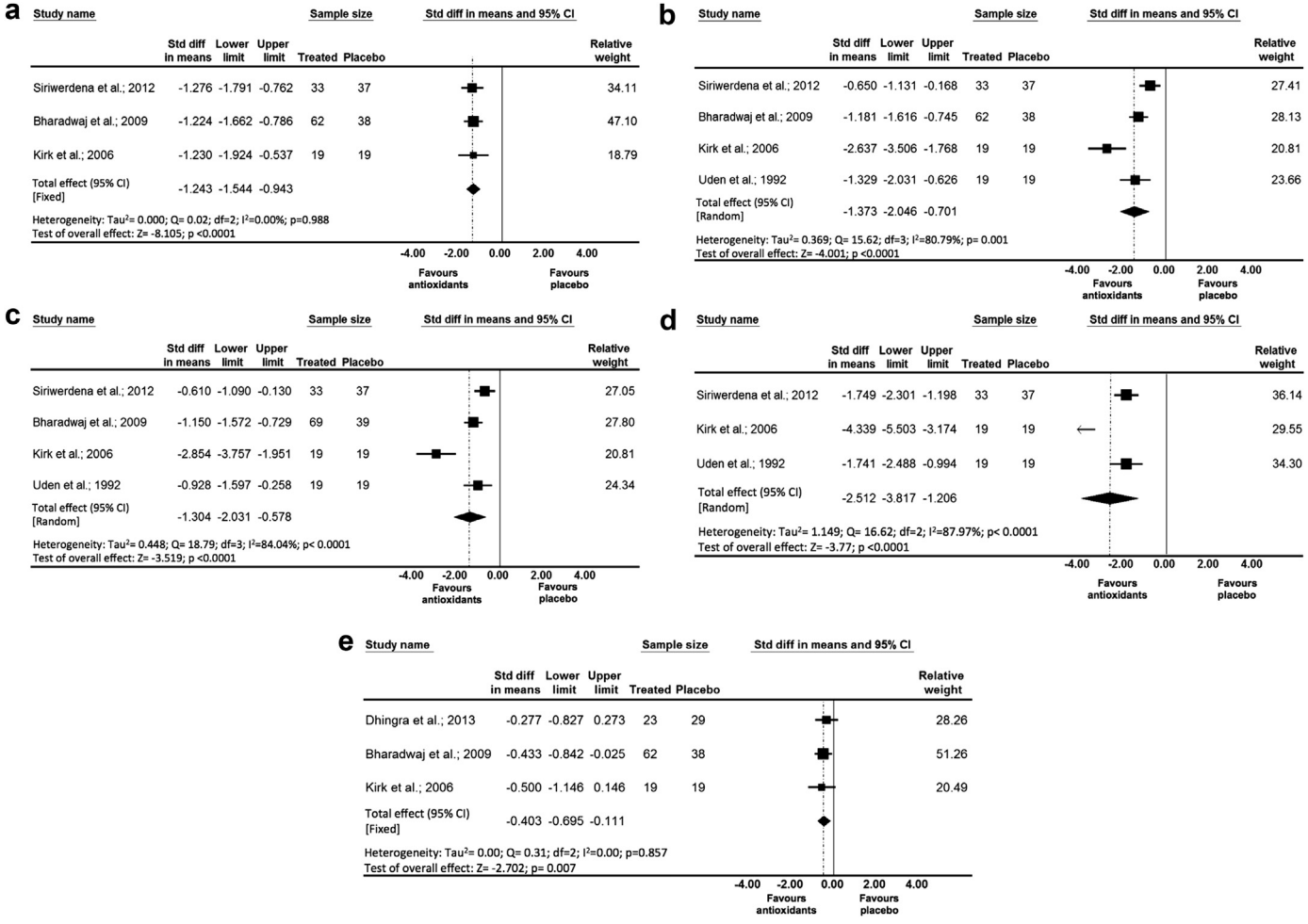

Fig. 4. Forrest plot of studies that reported blood levels of: a) Ascorbic acid. b) Alpha-tocoferol. c) Beta-carotene. d) Organic selenium. e) Antioxidant capacity.

finding studies have demonstrated that at least 2–4 gms of methionine is required daily for this action [28]. It is currently not entirely clear if risk factors (eg. alcohol, smoking and genetic polymorphisms) have an impact on the response to antioxidants. Based on population-based studies, it is currently accepted that smoking is an independent risk factor for CP [32]; and cigarette smoke contains free radicals sufficient to produce substantial intrapancreatic oxidative stress. In the study by Uden et al. [28], six out of seven patients who did not experience a significant reduction in pain scores were smokers. Therefore, it would be important that factors such as continuation and cessation of alcohol consumption and smoking, along with presence or absence of genetic polymorphisms be considered in the analyses of response to antioxidants in future RCTs.

The eight methionine containing studies that we identified for the meta-analyses also contained ascorbic acid, selenium, alpha-tocoferol and beta-carotene. Another source of thiol/methyl donor, S adenosyl methionine was used in the study by Uomo et al. [33]. However, this was a single agent pilot study performed on three patients with hereditary pancreatitis and did not meet inclusion criteria of the current meta-analysis. Overall, seven [21,23–28] out of the eight studies demonstrated efficacy of methionine containing antioxidant combination in the management of pain in CP. The RCT by Siriwerdena et al. [22], which did not show any benefit of using antioxidants included patients who were on high doses of opiates. It appears likely that these patients had opiate dependence, which could have overridden the effect of methionine and other antioxidants. Furthermore, opiates can also cause mast cell degranulation [34]. It is now known that there is mast cell infiltration into the pancreas in CP and mast cell tryptase can contribute to the inflammation thereby resulting in pain [35]. This could be another factor for non-response in patients on high dose of opiates.

Even though we demonstrated that methionine containing antioxidant combination was associated with a significant reduction in the number of pain free patients and a trend towards lesser requirement of hospital visit/admissions, there was heterogeneity among the studies for these outcomes. A limitation of this meta-analysis was the non-uniformity in the manner in which the clinical outcomes were reported in the individual studies. On the other hand, there was significant improvement in the oxidative stress markers and antioxidant capacity after treatment with methionine containing antioxidant combination; and there was no heterogeneity in the reporting of these outcomes. This improvement in antioxidant capacity has likely translated in the clinical improvement in pain in a significantly higher proportion of patients via restoration of the transsulfuration pathway and reduction of intrapancreatic inflammation. Therefore, it appears that methionine containing antioxidant combination have the potential to ameliorate pain in patients with CP when used in the appropriate dose. Other limitations of the study were low sample size, short study duration (maximum 6 months in the RCTs) and moderate to low quality evidence from the individual studies. However, the latter opens up room for more RCTs designed for high quality evidence. Furthermore, a response was also observed in the placebo groups in few of the studies, which could have been due to concomitant use of pancreatic enzyme supplements, abstinence from alcohol or smoking, or supplementation of adequate micro- and macronutrients.

In conclusion, results of the current systematic review and meta-analysis suggests that it would be currently too early to refute the role of methionine containing antioxidant combination in treating pain in CP. In view of the findings from recent meta-analyses including the current one, and relatively fair safety profile, methionine containing antioxidant combination should be considered as part of medical therapy for patients with chronic pancreatitis. However, multicenter RCTs with methionine as a mandatory component, involving an adequate sample size using standardized tools for evaluating outcomes need to be conducted to obtain high quality evidence in this regard.

Specific authors contributions

- Rupjyoti Talukdar reviewed the literature, abstracted data, performed statistical analyses, and drafted the manuscript.
- H.V.V. Murthy abstracted data, performed statistical analyses, and provided intellectual inputs.
- D. Nageshwar Reddy drafted the manuscript and provided intellectual inputs.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.pan.2015.01.003.

References

[1] Talukdar R, Reddy DN. Pain in chronic pancreatitis: managing beyond the pancreatic duct. World J Gastroenterol 2013;19:6319–28.
[2] Schoenberg MH, Büchler M, Pietrzyk C, Uhl W, Birk D, Eisele S, et al. Lipid peroxidation and glutathione metabolism in chronic pancreatitis. Pancreas 1995;10:36–43.
[3] Morris-Stiff GJ, Bowrey DJ, Oleesky D, Davies, Clark GW, Puntis MC. The antioxidant profiles of patients with recurrent acute and chronic pancreatitis. Am J Gastroenterol 1999;94:2135–40.
[4] Zeki S, Miura S, Suzuki H, Watanabe N, Adachi M, Yokoyama H, et al. Xanthine oxidase-derived oxygen radicals play significant roles in the development of chronic pancreatitis in WBN/Kob rats. J Gastroenterol Hepatol 2002;17:606–16.
[5] Santini SA, Spada C, Bononi F, Foschia F, Mutignani M, Perri V, et al. Liver, pancreas and biliary tract enhanced lipid peroxidation products in pure pancreatic juice: evidence for organ-specific oxidative stress in chronic pancreatitis. Dig Liver Dis 2003;35:888–92.
[6] Quillot D, Walters E, Bonte JP, Fruchart JC, Duriez P, Ziegler O. Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis. Am J Clin Nutr 2005;81:1117–25.
[7] Verlaan M, Roelofs HM, van-Schaik A, Wanten GJ, Jansen JB, Peters WH, et al. Assesment of oxidative stress in chronic pancreatitis patients. World J Gastroenterol 2006;12:5705–10.
[8] Tasci I, Deveci S, Isik AT, Comert B, Akay C, Mas N, et al. Allopurinol in rat chronic pancreatitis: effects of pancreatic stellate cell activation. Pancreas 2007;35:366–71.
[9] Girish BN, Rajesh G, Vaidyanathan K, Balakrishnan V. Assessment of oxidative status in chronic pancreatitis and its relation to zinc status. Indian J Gastroenterol 2011;30:84–8.
[10] Braganza JM. A framework for the pathogenesis of chronic pancreatitis. Digestion 1998;59(Suppl. 4):1–12.
[11] Braganza JM. Towards a novel treatment strategy for acute pancreatitis. 1. Reappraisal of the evidence on aetiogenesis. Digestion 2001;63:69–91.
[12] Braganza JM, Lee SH, McCloy RF. Chronic pancreatitis. Lancet 2011;377:1184–97.
[13] Tandon RK, Garg PK. Oxidative stress in chronic pancreatitis: pathophysiological relevance and management. Antioxid Redox Signal 2011;15:2757–66.
[14] Bhardwaj P, Yadav RK. Chronic pancreatitis: role of oxidative stress and antioxidants. Free Radic Res 2013;47:941–9.
[15] Pezzilli R. Antioxidants are not useful in reducing both pain and inflammation in chronic pancreatitis. Recent Pat Inflamm Allergy Drug Discov 2014;8:19–23.
[16] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009;339:b2700.
[17] Higgins JPT, Green S, editors. The cochrane collaboration handbook for systematic reviews of interventions; 2011. Version 5.1.0 [updated March 2011] Available from: www.cochrane-handbook.org.
[18] Atkins D, Eccles M, Flottorp S, Guyatt GH, Henry D, Hill S, et al. GRADE working group. Grading quality of evidence and strength of recommendations. BMJ 2004;328:1490–4.
[19] Higgins JPT, Green S. The Cochrane Library, Issue 3. In: Cochrane handbook for systematic reviews of interventions 4.2.6. Chichester, UK: Wiley; 2006.
[20] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959;22:719–48.

[21] 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;42:589–95.
[22] 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;143:655–63.
[23] Shah NS, Makin AJ, Sheen AJ, Siriwardena AK. Quality of life assessment in patients with chronic pancreatitis receiving antioxidant therapy. World J Gastroenterol 2010;16:4066–71.
[24] 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;136:149–59.
[25] Kirk GR, White JS, McKie L, Stevenson M, Young I, Clements WD, et al. Combined antioxidant therapy reduces pain and improves quality of life in chronic pancreatitis. J Gastrointest Surg 2006;10:499–503.
[26] De las Heras Castaño G, García de la Paz A, Fernández MD, Fernández Forcelledo JL. Use of antioxidants to treat pain in chronic pancreatitis. Rev Esp Enferm Dig 2000;92:375–85.
[27] Uden S, Schofield D, Miller PF, Day JP, Bottiglier T, Braganza JM. Antioxidant therapy for recurrent pancreatitis: biochemical profiles in a placebo-controlled trial. Aliment Pharmacol Ther 1992;6:229–40.
[28] Uden S, Bilton D, Nathan L, Hunt LP, Main C, Braganza JM. Antioxidant therapy for recurrent pancreatitis: placebo-controlled trial. Aliment Pharmacol Ther 1990;4:357–71.
[29] Cai GH, Huang J, Zhao Y, Chen J, Wu HH, Dong YL, et al. Antioxidant therapy for pain relief in patients with chronic pancreatitis: systematic review and meta-analysis. Pain Physician 2013;16:521–32.
[30] 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 2014 Jul 5. http://dx.doi.org/10.1016/j.clnu.2014.07.003. pii: S0261-5614(14)00178-2 [Epub ahead of print].
[31] Ahmed Ali U, Jens S, Busch OR, Keus F, van Goor H, Gooszen HG, et al. Antioxidants for pain in chronic pancreatitis. Cochrane Database Syst Rev 2014 Aug 21;8. http://dx.doi.org/10.1002/14651858.CD008945.pub2. CD008945.
[32] Tolstrup JS, Kristiansen L, Becker U, Grønbaek M. Smoking and risk of acute and chronic pancreatitis among women and men: a population-based cohort study. Arch Intern Med 2009;23(169):603–9.
[33] Uomo G, Talamini G, Rabitti PG. Antioxidant treatment in hereditary pancreatitis. A pilot study on three young patients. Dig Liver Dis 2001;33:58–62.
[34] Sheen CH, Schleimer RP, Kulka M. Codeine induces human mast cell chemokine and cytokine production: involvement of G-protein activation. Allergy 2007;62:532–8.
[35] Zimnoch L, Szynaka B, Puchalski Z. Mast cells and pancreatic stellate cells in chronic pancreatitis with differently intensified fibrosis. Hepatogastroenterology 2002;49:1135–8.