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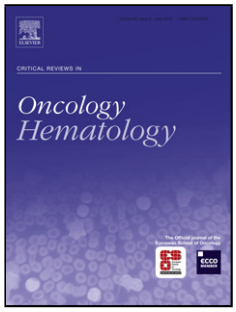

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Total Antioxidant Capacity in beta-thalassemia: a systematic review and meta-analysis of case-control studies

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Highlights

- Total Antioxidant Capacity (TAC) is decreased in thalassemics versus healthy subjects.
- Similar decreased TAC levels were found in beta-thalassemia major and trait.
- No relationship between TAC and disease severity could be established.
- The possible interference of uric acid and bilirubin must be taken into account.
- Uric acid-independent TAC might be the better approach to evaluate antioxidant status.

Abstract

Total Antioxidant Capacity (TAC), a biomarker measuring the antioxidant potential of body fluids, including redox synergistic interactions, is influenced by the presence of products of catabolism such as bilirubin (BR) and uric acid (UA). Hyperuricaemia and increased BR levels were observed in thalassemia. In order to evaluate the differences in TAC values between thalassemic patients and healthy subjects, we performed a systematic review and meta-analysis of case-control studies. After the exclusion of data deemed unsuitable for meta-analysis inclusion and a study imputed of bias by Trim-and-fill analysis, mean difference (MD) and confidence intervals 95% (CI 95%) were calculated by the random effect model for beta-thalassemia major (BTM) (1351 subjects: 770 thalassemic and 581 controls, from 15 studies) and Trait (BTT) or Hemoglobin E (BTE) (475 subjects: 165 thalassemic and 310 controls, from 5 studies). Despite the differences in clinical symptoms and severity, similar decreased levels of TAC were found in BTM [MD -0.22 (-0.35 -0.09) $p<0.001$] and BTT or BTE [MD -0.22 (-0.44 -0.01) $p<0.05$]. In conclusion, UA and BR interference on TAC suggests that corrected TAC and in particular the UA-independent TAC, considering the prominent influence of UA, might be the better approach to evaluate body antioxidant status.

Keywords: Beta-thalassemia, bilirubin, meta-analysis, Total Antioxidant Capacity, uric acid.

1. Introduction

Beta-thalassemia major (BTM) is the most prevalent type of beta-thalassemia (BT), comprising also thalassemia Trait (BTT or minor), thalassemia Intermedia (BTI) and Hemoglobin E thalassemia (BTE) [1]. BTM requires regular transfusion therapy to maintain hemoglobin levels of at least 9 to 10 g per deciliter and to reduce hepatosplenomegaly due to extramedullary hematopoiesis [1]. Both haemolysis and transfusional iron overload cause excessive generation of free radicals (through the Fenton reaction), and, consequently, iron-chelation therapy is largely responsible for doubling the life expectancy of patients with BTM [1]. Excessive generation of free radicals can cause oxidative damage to biological macromolecules such as DNA, lipids, carbohydrates and proteins [2]. In beta-thalassemia, malonyldialdehyde (MDA; a by-product of lipid peroxidation) levels correlate positively with serum iron and oxidative stress levels were shown to largely normalize in response to oral therapy with antioxidants [1]. Oxidative stress is the imbalance between reactive oxygen species (ROS) and antioxidant defense [3]. ROS is a collective term used by biologists to include not only oxygen-derived radicals, such as the superoxide anion, hydroxyl radical, peroxyl, alkoxyl and oxides of nitrogen, but also some derivatives of oxygen that do not contain unpaired electrons, such as the hydrogen peroxide and the hypochlorous acid produced by inflammatory cells [3]. The human body has a complex strategy for countermanding the deleterious effects of ROS, which include both antioxidative and repair mechanisms [4]. Antioxidant defenses of the body are composed of enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) and low molecular weight antioxidants, including glutathione (GSH), uric acid (UA), bilirubin (BR), thiols (SH), vitamin E (Vit. E), ascorbic acid (Vit. C), carotenoids and other nutritional antioxidants [4].

Synergistic interactions between antioxidants, in part involving antioxidant regeneration, need to be taken into account in order to properly assess antioxidant status in vivo. Total Antioxidant Capacity (TAC), defined as the moles of oxidants neutralized by one litre of plasma [5], is a biomarker measuring the antioxidant potential of body fluids, including redox synergistic interactions [5].

Over the past decade, a large number of assays and kits for the measurement of TAC in biological matrices have been developed and the most commonly used assays, their basic features, and their points of strength and weakness, have been extensively discussed in several comprehensive reviews [6-9].

The more commonly used methods are the Ferric Reducing Antioxidant Potential (FRAP) and Trolox Equivalent Antioxidant Capacity (TEAC) within the single electron transfer (SET)-based assays and the Total-radical Trapping Antioxidant Parameter (TRAP) and the Oxygen Radical Antioxidant Capacity (ORAC) within the hydrogen atom transfer (HAT)-based methods [4, 6-9]. Although the discussion on the method used for determining TAC is not a central issue in the current study, it must be stated that the results obtained from FRAP and ORAC measurements correlate well, but not between the aforementioned methods and TEAC [10]. On the other hand, the copper(II) reduction assay (CUPRAC) does significantly correlate with FRAP and TEAC, but not with the 1,1-diphenyl-2-picrylhydrazyl assay (DPPH) for plasma samples [11]. Despite the different features of the TAC methods, their common major bias is that they are influenced by the presence of products of catabolism, such as bilirubin (BR) and uric acid (UA) [10]. Both hyperuricaemia [12] and increased BR levels [13] can be observed in thalassemia.

The objective of this meta-analysis was to evaluate the differences in TAC values between thalassemic patients and healthy subjects. To this aim, a systematic review of the available literature and meta-analysis of included case-control studies was conducted in this work.

2. Methods

2.1 Literature Screening

We performed a systematic search in the MEDLINE, EMBASE, ProQuest and Google scholar databases for relevant literature up to September 2015 with the search string: [thalassem* AND (antioxidant* OR ox*)]. The flowchart outlining the process of search criteria and study selection is shown in Figure 1.

2.2 Study Selection

Studies that met the following criteria were included for meta-analysis: (1) The outcome had to be thalassemia; (2) at least two comparison groups (case vs. control group); (3) studies in which the plasma TAC levels were expressed as millimolar (mM). Exclusion criteria included: (1) Review articles; (2) in vitro studies; (3) animal models; (4) patients with other diseases. Trials were initially identified through title or abstract. Study selection was performed independently by two reviewers (H.M. and M.P.) to ensure uniformity. Discrepancies were resolved by discussion with a third reviewer (I.P.).

2.3 Data Extraction

The data extracted from each study included the first-author's name, year of publication, country of the study performed, ethnicity, subject characteristics, type of BT, comorbidities, method of evaluation of TAC and other markers of plasma redox status. For each study with more than one beta-thalassemic group, we divided the control group evenly according to the number of disease groups [14]. When the data were presented as standard error of means (SEM), standard deviation (SD) was obtained by multiplying SEM by the square root of the sample size [14]. Data extraction was performed independently by two reviewers (H.M. and G.D.) to ensure uniformity. Discrepancies were resolved by discussion with a third reviewer (I.P.).

2.4 Statistics and Analysis

Meta analysis was performed with MIX software (BiostatXL) [15]. Mean difference (MD) and confidence intervals 95% (CI 95%) were calculated by the random effect model (DerSimonian & Laird) for continuous data. Statistical heterogeneity was assessed by using the τ^2 statistic and Egger's weighted regression statistics [14]. Symmetry or asymmetry of funnel plots and trim-and-fill sensitivity analysis were used to determine the presence of publication bias [14].

3. Results

3.1 Data Extraction

Figure 1 depicts the flow of studies in this review and the four-phase diagram of meta-analysis according to the PRISMA Statement [16]. After exclusion of irrelevant references, a total of 27 studies [17-43] were identified as suitable and were retrieved for complete review. In general, normally distributed data are given as mean ± SD and not normally distributed variables as medians (25–75 percentiles). Therefore, we excluded studies with data presented as medians [17-19], as well as one study in which results were presented exclusively in figures [20]. In addition, considering that TAC ranges between 10^{-3} M and 10^{-4} M, we also excluded studies that reported TAC values of 10^{-2} M [21], 10^{-6} M [22] and 10^{-9} M [23]. The remaining 19 studies met our inclusion criteria and were suitable to provide data for the analysis (Figure 1). With regard to the study by Ozdemir et al. [38], we only included the non supplemeted group in the analysis, but retained the *N*-acetylcysteine (NAC) and Vit. E-supplemented groups for the discussion. Due to the differences in clinical symptoms and severity of the disease [1], we performed the analysis separately for BTM and BTT, BTI or BTE. Since there were 8 studies with different disease groups, 28 datasets from 19 studies were analyzed, of which 21 on BTM and 7 on BTT, BTI or BTE.

Concerning BTM, the number of subjects was 1379, of which 778 thalassemic and 591 controls.

Conversely, 493 subjects (173 thalassemic and 320 controls) were analyzed for BTT, BTI or BTE.

3.2 Publication Bias

Trim-and-fill analysis and Egger's test were performed to estimate the publication bias of the studies. Funnel plots showed asymmetric distribution of results (Figure 2). The drawback in using the funnel plot is that it is purely subjective. Therefore, trim-and-fill analysis was used to estimate how many studies are in the asymmetric part. In particular, Trim-and-Fill analysis creates a funnel plot that includes both the observed studies and the imputed studies. Trim-and-fill analysis showed no publication bias for BTM (Figure 2A) and a study imputed of bias for BTT, BTI or BTE (Figure 2B). The imputed study resulted from work by El Gendy et al. [28]. This dataset did not involve BTI patients, but BTT subjects [28]. Furthermore, this study reported TAC levels that were approximately 2-fold higher for healthy subjects compared to the majority of the other reviewed studies. Therefore, after the exclusion of this study involving BTI, BTT and BTM patients, a second analysis was performed for both BTM (1351 subjects: 770 thalassemic and 581 controls from 20 datasets) and BTT or BTE (475 subjects: 165 thalassemic and 310 controls from 5 datasets), as shown in Figure 1. Low statistical heterogeneity was found for BTM ($\tau^2 = 0.08$, Egger intercept -1.59, $p > 0.05$) and BTT or BTE ($\tau^2 = 0.07$, Egger intercept -9.15, $p > 0.05$). Funnel plots showed symmetric distribution of results and Trim-and-fill analysis showed no publication bias (Figure 2C and 2D).

3.3 Meta-Analysis

Decreased levels of TAC were found in both BTM [MD -0.22 (-0.35 -0.09) $p<0.001$] and BTT or BTE [MD -0.22 (-0.44 -0.01) $p<0.05$] (Table 1).

Table 1 shows the results of each study separately and the contribution of each study to the overall results of meta-analysis for BTM and BTT. Furthermore, we presented the effect of accumulating the results when each study is added (Table 1).

Despite the differences in clinical symptoms, disease severity and the reported lower TAC values in patients infected by transfusion (HIV+ and/or HCV+) [17], our study found that the MD between thalassemic and control subjects was similar for BTM and BTT or BTE (Table 1).

3.4 Characteristics of Selected Studies

Characteristics of the included studies are shown in Table 2.

TAC was measured by TEAC in the majority of the studies (n=13), but also TRAP (n=2), FRAP (n=1) and ELISA kit (n=1) were used. Furthermore, Labib et al. [39] referred to a method that used UA as a standard [44] instead of the more commonly used Trolox [6-9]. Despite these methodological differences, TAC was lower in patients versus controls in the majority of the studies, regardless of the assay used and of the ethnicity of the subjects (Table 2). However, unchanged or increased levels of TAC were also reported (Table 2).

Mean age of patients with BTM ranged between 7.5±4.3 [27] and 21.6±10.5 [25], whereas mean age of subjects with BTT or BTE ranged between 21.2±0.6 [42] and 36.6±7.9 [39] (Table 2). The difference in mean age was probably due to the fact that BTM is characterized by severe anemia (requiring regular transfusions beginning in infancy), severe iron overload (requiring chelation therapy), splenomegaly and bone disease (depending on the efficacy of the transfusion therapy), whereas other types of BT could range from asymptomatic, with mild or no anemia and variable microcytosis, to severe [1]. Some of the reviewed studies conducted on BTM reported that a number of patients exhibited clinical complications or comorbidities, such as cardiovascular diseases (CVD), insulin dependent diabetes mellitus (IDDM), or were hepatitis C virus positive (HCV+) and/or human immunodeficiency virus positive (HIV+) (Table 2).

Sixteen out of nineteen studies also measured other markers of redox status (Table 2).

Increases of UA and BR were measured in BTM, but also unchanged levels of UA were found (Table 2). Despite the increased TAC and the direct correlation between TAC and BR or UA found by Bazvad et al. [9], other studies reported decreased TAC irrespective of UA and/or BR increases (Table 2). On the other hand, when TAC, Vit. C, Vit. E and carotenoids were measured in the same study, it turned out that there was a clear accordance between the antioxidant concentration and TAC (Table 2). Furthermore, in the majority of the studies on BTM and in all studies on BTT, the

markers of oxidation [Total Oxidant Status (TOS), carbonyls, peroxides (ROOH), MDA or thiobarbituric acid reactive substances (TBARS)] were associated with decreased TAC (Table 2).

Conversely, contrasting results were obtained from antioxidant enzymes evaluation, probably due to the great variability of the various samples analyzed, such as erythrocytes (red blood cells, RBC) [30, 33, 34, 42], peripheral blood mononuclear cells (PBMC) [34] or serum [24]. For example, Kuppusamy et al. [34] reported that CAT activity was increased in PBMC, but not in RBC.

4. Discussion

Overall, our meta-analysis indicates that TAC is decreased in thalassemic patients versus healthy subjects (Table 1). However, despite the differences in clinical symptoms, similar decreased levels of TAC were found in BTM and BTT or BTE groups (Table 1), indicating that TAC is not related to disease severity. To understand this result several considerations should be made.

Since the first study that evaluated TAC in BTM [35], it has been argumented that blood antioxidants such as UA and BR, known to contribute significantly to the plasma TAC value, may be expected to increase in thalassemia patients because of haemolysis and liver damage.

Among the reviewed studies measuring TAC, UA and/or BR (Table 2) 40% (2/5) reported increases of both TAC and UA, whereas increased TAC and BR levels were observed in one out of six studies, concurrently with high UA levels [26].

UA provides 60-80% of TAC in plasma [45, 46] and it has been reported that TAC is mainly related to the UA concentration of plasma, urine and saliva [47-49]. Therefore, due to the dominating influence of UA in biological fluids, it has been suggested [49] that TAC loses its sensitivity considerably, since UA obscures the influence of those compounds with antioxidant capacity that are present at lower concentrations, and that the determination of the UA-independent TAC appears more meaningful. Therefore, methods for UA-independent TAC have been proposed that utilize the uricase-reaction [50] or by using a corrected TAC (TAC_{corr}); the calculated parameter that represents the fraction of circulating antioxidants after the elimination of the interference by

endogenous antioxidants [49, 51]. However, uricase methods might be inherently biased because of the mechanism of the action of uricase, which generates one molecule of H_2O_2 for every molecule of UA [52]. Therefore, TAC_{corr} might represent the better approach. The plasma level of UA is regulated by renal function [53]. In this context, plasma TAC values of hemodialyzed patients were high [51] compared to those of control individuals before dialysis and during dialysis these elevated values decreased due to UA and BR decreases. In contrast, while initial TAC_{corr} was significantly lower than that of controls, it increased during the dialysis procedure and reached normal values at the end of dialysis.

Hamed et al. [32] reported glomerular and tubular dysfunctions in BTM patients. However, none of the reviewed studies reported data on TAC_{corr} and only two studies reported data on calculated TAC (TAC_{cal}). In these studies [26, 36], TAC_{cal} was determined according to the relative activity of major endogenous plasma antioxidants of the different methods used [i.e. 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and TRAP]. However, in the study by Mastroiacovo and co-workers [36], TAC_{cal} was biased by the inclusion of vit. E (an exogenous antioxidant) in the calculation.

Vit. C, vit. E and beta-carotene, when measured, were decreased in 75% of the case-control studies on BTM (Table 1). It has been suggested that the decreased TAC could be the result of the marked decrease in vitamin levels [35]. In this context, in the study by Ozdemir et al. [38], children with BTM and supplemented with NAC (10 mg/kg/day) or Vit. E (10 IU/kg/day) had higher TAC and lower TOS after 3 months of treatment compared to before the start of treatment. Therefore, despite the fact that TAC was not related to disease severity, it might still be a valid marker of antioxidant status during antioxidant treatment in BT.

The major limitation of this meta-analysis is that the number of studies that contributed substantial data to the meta-analysis was limited. Therefore, we did not perform a subgroup analysis for treatment and we could not adequately investigate sex and age differences.

As a consequence, a number limitations should be allowed for.

First of all, it has been reported that ferritin was higher in older patients [35] and negatively correlated with TAC [29], whereas Bazvand et al. [26] found no correlation between age and TAC.

Secondly, the current "normal" range set for hyperuricaemia is gender-specific [54-57] and increase in serum UA levels may also be due to dietary factors, such as increased intake of purine [58], alcohol [59] and fructose [60]. None of the reviewed study included data regarding the food frequency questionnaire and only one study reported data of men and women separately [26]. In the latter study [26], TAC and BR levels in male patients were significantly higher than in females.

Again, some of the reviewed studies conducted on BTM reported cases of splenectomy [27, 29, 32, 35, 38]. Both decreased [29] and unchanged [34] TAC levels were reported in splenectomized compared to nonsplenectomized patients with BT.

Finally, the effect of chelation therapy should be taken into account. Thalassemic patients not receiving or on irregular chelation therapy had significantly lower TAC, irrespective of serum UA [32] and BR [34]. On the contrary, although Hamed et al. [32] reported glomerular and tubular dysfunctions in patient with and without chelation, renal damage was higher in patients receiving chelation therapy (deferoxamine).

In summary, many factors affect TAC values and consequently these must be taken into account when interpreting TAC values.

5. Conclusion

Our meta-analysis suggests that, although thalassemic patients had lower TAC compared to healthy subjects, no relationship to disease severity could be established, probably due to UA and BR interference. In particular the prominent influence of UA suggests that UA-independent TAC would represent a significantly better approach when evaluating the antioxidant status of the body.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Figure legends

Figure 1: Four-phase flow diagram of systematic review and meta-analysis.

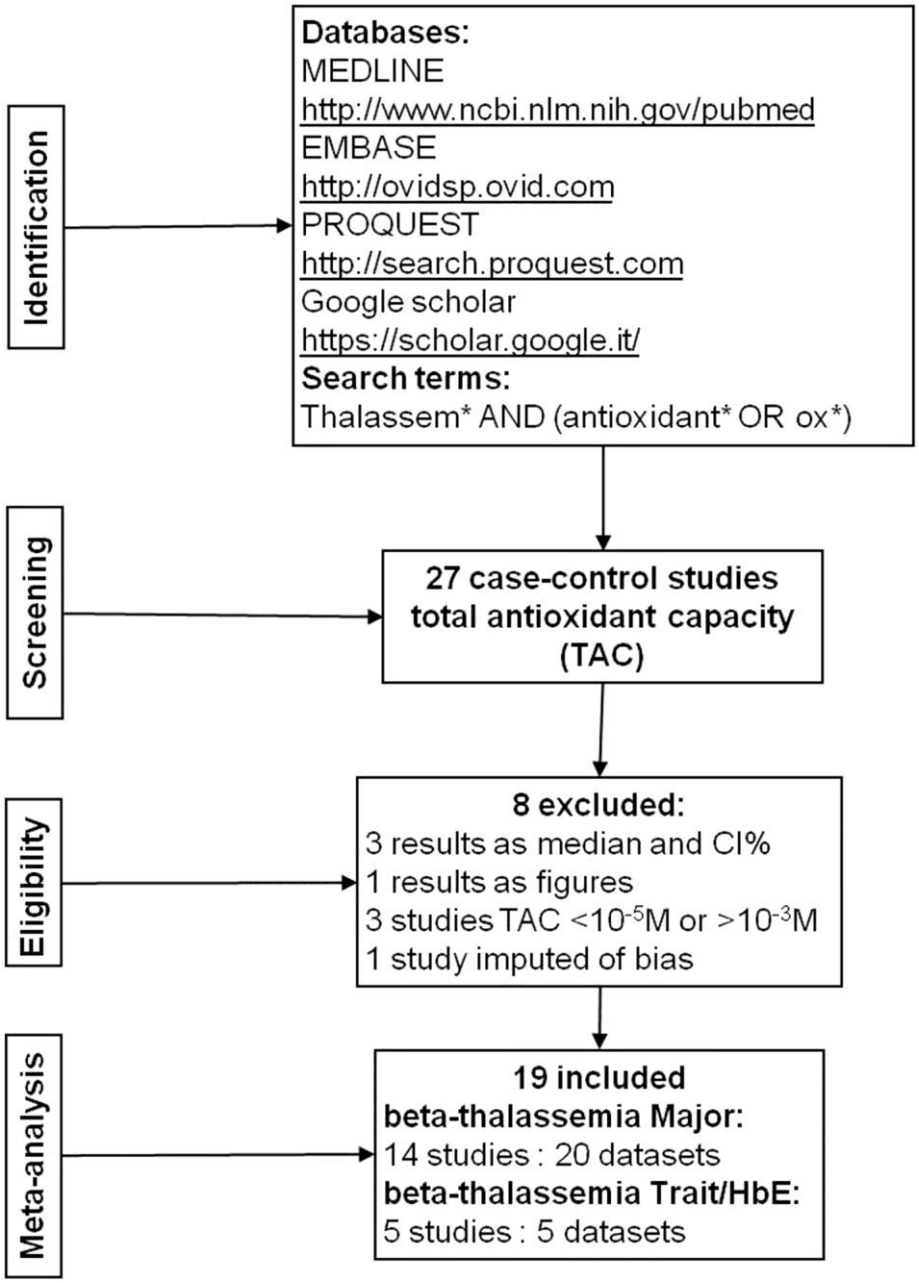

Figure 2. Trim-and-fill funnel plot of BTM (A) and BTT or BTE (B). The solid circles represent actual identified studies, whereas the open circle in (B) represents the imputed study from a trim-and-fill analysis. The imputed study causes a shift in the mean difference (MD). Trim-and-fill funnel plot of BTM (C) and BTT or BTE (D) after the exclusion of the study imputed of bias [28].

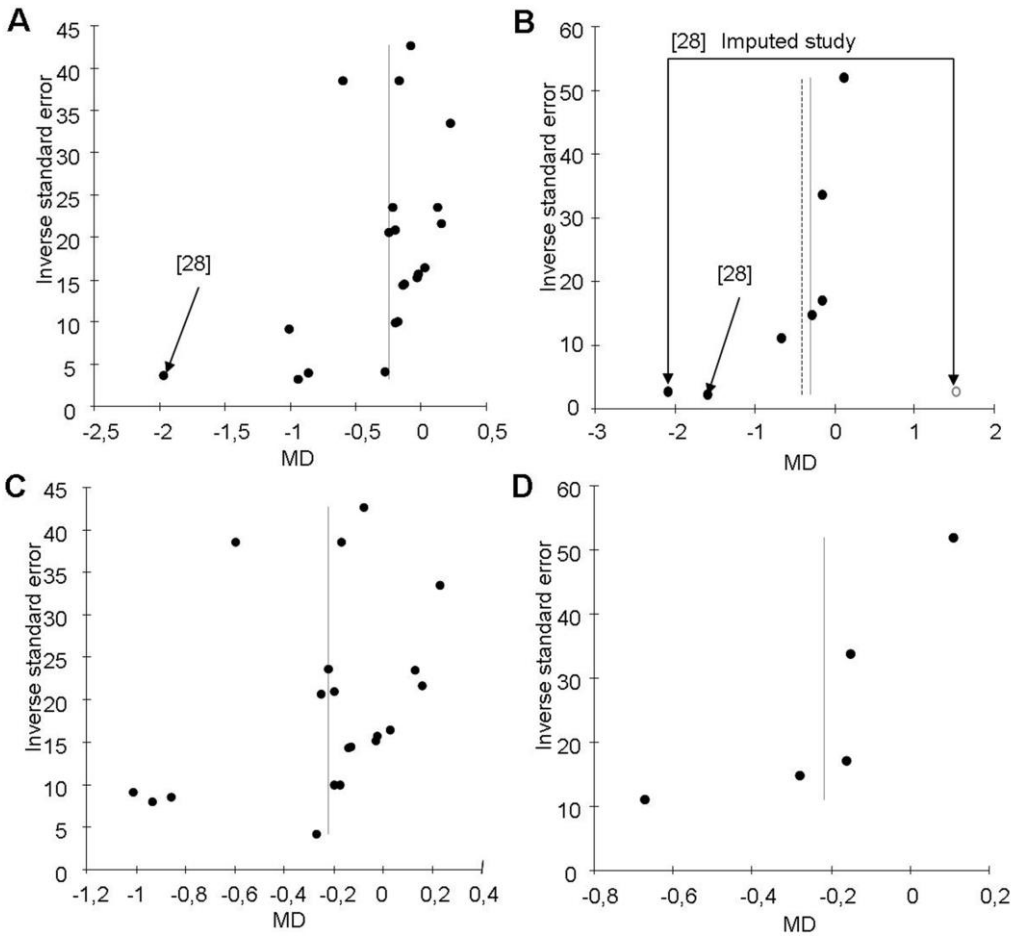

Table 1. Meta-analysis

First author Year [reference]	Dataset, BT type (comorbidity)	Method	Input summary MD (95% CI)	p	Weight (%)	N° of studies (Datasets)	Cumulative Meta-analysis MD (95% CI)
Asif 2015 [24]	1, BTM	TEAC	+0.23 (+0.17 +0.29)	<0.001	5.35%		
Awadallah 2013 [25]	2, BTM	TEAC	-0.08 (-0.12 -0.03)	0.001	5.37%	2 (1, 2)	+0.07 (-0.22 +0.38)
Bazvand 2011 [26]	3, BTM M(a)	TEAC	+0,16 (+0.06 + 0.25)	<0.001	5.25%	3 (1-3)	+0.10 (-0.11 +0.32)
Bazvand 2011 [26]	4, BTM F(b)	TEAC	+0.13 (+ 0.05 +0.21)	<0.01	5.29%	4 (1-4)	+0.11 (-0.05 +0.27)
Cakmak 2010 [27]	5, BTM	TEAC	-0.03 (-0.16 +0.09)	0.65	5.13%	5 (1-5)	+0.08 (-0.06 +0.22)
Elsayh 2013 [29]	6, BTM(a)	TEAC	-0.85 (-1.09 -0.63)	<0.001	4.61%	6 (1-6)	-0.04 (-0.22 +0.13)
Elsayh 2013 [29]	7, BTM+splenectomy(b)	TEAC	-0.94 (-1.18 -0.69)	<0.001	4.51%	7 (1-7)	-0.16 (-0.35 +0.03)
Ghone 2008 [30]	8, BTM	FRAP	-0.60 (-0.65 -0.55)	<0.001	5.36%	8 (1-8)	-0.23 (-0.49 +0.03)
Gunay 2015 [31]	9, BTM(a)	TEAC	-0.13 (-0.26 +0.01)	0.06	5.10%	9 (1-9)	-0.22 (-0.46 +0.02)
Gunay 2015 [31]	10, BTM+gingivitis(b)	TEAC	-0.14 (-0.27 -0.01)	<0.05	5.10%	10 (1-10)	-0.21 (-0.44 + 0.01)
Hamed 2010 [32]	11, BMT(a)	ELISA	-0.25 (-0.34 -0.15)	<0.001	5.25%	11 (1-11)	-0.21 (-0.42 -0.01)
Hamed 2010 [32]	12, BTM+chelation(b)	ELISA	-0.20 (-0.29 -0.10)	<0.001	5.26%	12 (1-12)	-0.21 (-0.40 -0.02)
Kassab-Chekir 2003 [33]	13, BTM	TRAP	-1.01 (-1.22 -0.79)	<0.001	4.70%	13 (1-13)	-0.27 (-0.46 -0.08)
Kuppusamy 2011 [34]	14, BMT(a)	FRAP	-0.17 (-0.37 +0.02)	0.07	4.81%	14 (1-14)	-0.26 (-0.44 -0.08)
Kuppusamy 2011 [34]	15, BTM+chelation(b)	FRAP	+0.03 (-0.09 +0.15)	0.63	5.17%	15 (1-15)	-0.24 (-0.41 -0.07)
Livrea 1996 [35]	16, BTM (6IDDM, 6CVD, 31HCV+)	TEAC	-0.17 (-0.22 -0.12)	<0.001	5.36%	16 (1-16)	-0.24 (-0.39 -0.09)
Mastroiacovo 1999 [36]	17, BTM(a)	TRAP	-0.02 (-0.15 +0.10)	0.70	5.15%	17 (1-17)	-0.22 (-0.37 -0.08)
Mastroiacovo 1999 [36]	18, BMT infected (b)	TRAP	-0.20 (-0.39 -0.01)	<0.05	4.80%	18 (1-18)	-0.22 (-0.36 -0.08)
Ozdem 2008 [37]	19, BTM	TEAC	-0.22 (-0.30 -0.13)	<0.001	5.29%	19 (1-19)	-0.22 (-0.35 -0.09)
Ozdemir 2014 [38]	20, BTM	TEAC	-0,27 (-0.74 +0.20)	0.26	3.14%	**20 (1-20)**	**-0.22 (-0.35 -0.09)**
Overall	**BTM**		**-0.22 (-0.35 -0.09)**	**<0.001**	**100%**		
Labib 2011 [39]	1, BTT	UAEAC	-0.67 (-0.85 -0.49)	<0.001	18.42%		
Ondei 2013 [40]	2, BTT	TEAC	+ 0,11 (+0.07 + 0.15)	<0.001	21.11%	2 (1, 2)	-0.27 (-1.04 +0.49)
Palasuwan 2005 [41]	3, BTE	TEAC	-0.16 (-0.28 -0.05)	<0.01	19.97%	3 (1-3)	-0.23 (-0.62 +0.15)
Palasuwan 2015 [42]	4, BTE	TEAC	-0.28 (-0.41 -0,15)	<0,001	19.59%	4 (1-4)	-0.24 (-0.55 +0.07)
Selek 2007 [43]	5, BTT	TEAC	-0.15 (-0.21 -0.09)	<0.001	20.91%	**5 (1-5)**	**-0.22 (-0.44 -0.01)**
Overall	**BTT/BTE**		**-0.22 (-0.44 -0.01)**	**<0.05**	**100%**		

Random Effects estimate in BTM and BTT or BTE. MD: mean difference. CI 95%: confidence intervals 95%.

BT: beta-thalassemia; BTE: Hemoglobin E talassemia; BTM: beta-thalassemia major; BTT: beta-thalassemia Trait (or minor); F: females; FRAP: Ferric Reducing Antioxidant Potential (µmol Fe^{+2} equiv./l); HCV+: hepatitis C virus positive; HIV+: human immunodeficiency virus positive; M: males; TEAC: Trolox Equivalent Antioxidant Capacity (mmolTrolox Equiv./l); TRAP: Total-radical Trapping Antioxidant Parameter; UA: uric acid; UAEAC: µmol UAequiv./l.

Table 2. Characteristics of the included case-control studies.

First author Year [reference]	Study country	Ethnicity	Healthy n (age years)	Disease n (age years)	BT type (comorbidity)	TAC (method)	Other redox Markers
Asif 2015 [24]	Pakistan	Asian	90 (1-10)	90 (1-10)	BTM	↑ (TEAC)	TOS, MDA, CAT↑ GPX↓
Awadallah 2013 [25]	United Arab Emirates	Middle-Eastern	43 (23.8±6.4)	55 (21.6±5.7)	BTM (5% HCV+)	↓ (TEAC)	BR↑
Bazvand 2011 [26]	Iran	Middle-Eastern	66 (34a/32b) (15.1±7.3)	66 (34a/32b) (14.7±6.9)	BTM M(a) BTM F(b)	↑ (TEAC)	BR, UA↑ TACcal↑
Cakmak 2010 [27]	Turkey	Middle-Eastern	33 (8.6±3.3)	87 (7.5±4.3)	BTM	↔ (TEAC)	TOS↑
Elsayh 2013 [29]	Egypt	Middle-Eastern	46 (7.9±0.6)	56 (32a/24b) (8.7±0.6)	BTM(a) BTM+splenectomy(b)	↓ (TEAC)	ROOH ↔ MDA↑
Ghone 2008 [30]	India	Asian	72 (7-12)	72 (7-12)	BTM	↓ (FRAP)	MDA↑ Vit. E, SOD ↔
Gunay 2015 [31]	Turkey	Middle-Eastern	25 (11-16)	48 (25a+23b) (11-16)	BTM(a) BTM+gingivitis(b)	↓ (TEAC)	
Hamed 2010 [32]	Egypt	Middle-Eastern	15 (8.4±4.1)	69 (34a+b35) (8.7±3.7)	BMT(a) BTM+chelation(b)	↓ (ELISA)	MDA↑ UA↑
Kassab-Chekir 2003 [33]	Tunesia	Middle-Eastern	51 (10.6±2.4)	56 (8.0±3.4)	BTM	↓ (TRAP)	TBARS, SOD, GPX, BR↑ Vit. E, UA ↓
Kuppusamy 2011 [34]	Malaysia	Asian[1]	20 (15.0±6.0)	39(6°+33b) (10.0±5.0, 8.0±2.0)	BMT(a) BTM+chelation(b)	↓ (a) ↔ (b) (FRAP)	BR↑ (a) CAT-RBC ↔ ROOH, CAT- PBMC ↑ GPX-RBC ↓ GPX-PBMC ↓ (b)
Livrea 1996 [35]	Italy	Caucasian	35 (29.0±5.0)	42 (21.0±10.0)	BTM (6IDDM, 6CVD, 31HCV+)	↓ (TEAC)	MDA, dienes, carbonyls, UA, BR↑ Vit. C, Vit, E, β-car., Licopene, SH ↓ GSH ↔
Mastroiacovo 1999 [36]	Italy	Caucasian	33 (17.5, 8-33)	33 (26a+7b) (17.6, 8-33)	BTM(a) BMT infected (b) (3HCV+, 3HIV+, 1HCV+/HIV+)	↓ (TRAP)	UA ↔ BR, SH ↑ Vit. C, VIT. E, β-car., TRAPcal↓
Ozdem 2008 [37]	Turkey	Middle-Eastern	27 (17.0±9.0)	32 (18.0±8.0)	BTM	↓ (TEAC)	
Ozdemir 2014 [38]	Turkey	Middle-Eastern	25 (8.0±3.8)	25 (8.6±4.1)	BTM	↔ (TEAC)	TOS↑
Labib 2011 [39]	Egypt	Middle-Eastern	20 (38.1±8.3)	60 (36.6±7.9)	BTT	↓ (UAEAC)	MDA↑
Ondei 2013 [40]	Brazil	Caucasian[2]	81 (18-62)	49 (18-79)	BTT	↑ (TEAC)	TBARS↑
Palasuwan 2005 [41]	Thailand	Asian	171 (38.2±17.0)	14 (25.8±13.9)	BTE	↓ (TEAC)	

Palasuwan 2015 [42]	Thailand	Asian	10 (21.4±0.5)	10 (21.2±0.6)	BTE	↓ (TEAC)	SOD, GPX, TBARS ↔
Selek 2007 [43]	Turkey	Middle-Eastern	28 (27.0±4.9)	32 (28.0±2.0)	BTT	↓ (TEAC)	TOS, ROOH ↑

[1] All Chinese; [2] Patients 100%, Controls 74%; β-Car.: β-Carotene; BR: bilirubin; BT: beta-thalassemia; BTE: Hemoglobin E talassemia; BTM: beta-thalassemia major; BTT: beta-thalassemia Trait (or minor); CAT: catalase; F: females; FRAP: Ferric Reducing Antioxidant Potential (μmol Fe^{+2} equiv./l); GPX: glutathione peroxidise; GSH: glutathione; HCV+: hepatitis C virus positive; HIV+: human immunodeficiency virus positive; M: males; MDA: malonyldialdehyde; n: number of subjects; PBMC: Peripheral Blood Mononuclear Cells; RBC: Red Blood Cells; ROOH: peroxides; SH: thiols; SOD: superoxide dismutase; TAC: Total Antioxidant Capacity; TACcal: calculated TAC; TBARS: thiobarbituric acid reactive substances; TEAC: Trolox Equivalent Antioxidant Capacity (mmolTrolox Equiv./l); TOS: μmol H2O2 equiv./l; TRAP: Total-radical Trapping Antioxidant Parameter; TRAPcal: calculated TRAP; UA: uric acid; UAEAC: μmol UAequiv./l; Vit.: vitamin.