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NARRATIVE REVIEW

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Antioxidative effects of N-acetylcysteine in patients with β–thalassemia: A quick review on clinical trials

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Abstract

Background and Aims: Several studies have highlighted the potent antioxidant properties of N-acetyl cysteine (NAC). This review aimed to assess the impact of NAC on oxidative stress biomarkers in patients with β-thalassemia.

Methods: The review included articles published before 2024 that investigated the effects of NAC on oxidative stress in individuals with β-thalassemia. A comprehensive search was conducted across various databases, including Scopus, PubMed, Web of Science, Trip, and CENTRAL. Only English-language clinical trials were considered for inclusion in this review. Besides, the number needed to treat (NNT) was calculated based on the included studies.

Results: Ninety-nine articles were retrieved from electronic databases, and after a thorough review, eight articles were selected for comprehensive text analysis. The highest dose of NAC administered was 10 mg/kg/day (equivalent to 600 mg/day) over a period of 3–6 months. All the studies assessing the impact of NAC on oxidative stress indicators in β-thalassemia patients demonstrated positive effects during the 3-month follow-up period. Most estimated NNTs fell into 1–5, suggesting significant clinical therapeutic value in this context.

Conclusion: The current potency of NAC alone appears to be effective in ameliorating oxidative stress in patients with β-thalassemia major. While a 3-month duration seems adequate to demonstrate the antioxidant properties of NAC in this population, larger and well-designed clinical trials are warranted. Current clinical evidence possesses a high risk of bias.

KEYWORDS

antioxidants, N-acetylcysteine, oxidative stress, β–thalassemia

1 | INTRODUCTION

Hemoglobinopathies that result in persistent hemolysis are now recognized as significant contributors to heightened oxidative stress. Over time, the accumulation of iron can lead to iron toxicity, causing various complications such as heart failure, malocclusion of teeth, infections, and endocrine abnormalities.[1] Hemolysis and frequent red cell transfusions can cause iron overload in several organs. For instance, elevated iron levels in the brain can result in oxidative stress and potentially irreversible damage to brain tissues, leading to cognitive impairment.[2] Besides, iron overload in β-thalassemia patients corresponds to other serious health sequela, such as cardiomyopathy and arrhythmias induced by myocardial siderosis. These conditions significantly contribute to both mortality and morbidity, accounting for 71% of global deaths associated with β-thalassemia major.[3] The clinical manifestation of cardiac failure can range from ventricular pathology to pulmonary hypertension, as well as symptomatic supraventricular arrhythmias, for example, atrial fibrillation, that may result in sudden fatality.[4,5] Endocrine dysfunction is also a common and significant repercussion observed in patients with transfusion-dependent thalassemia (TDT). A striking 88.4% of patients diagnosed with β-thalassemia major are expected to experience at least one endocrine complication during the second decade of life.[6,7]

Oxidative stress plays a big role in the progression of disease in TDT cases. The excessive iron present in these patients exacerbates oxidative stress as it acts as a catalyst in the formation of reactive oxygen species (ROS).[8] Furthermore, secondary iron overload resulting from regular red cell transfusions in TDT patients, shorter red blood cell (RBC) lifespan, and increased absorption of dietary iron, particularly in non-transfusion-dependent patients, perpetuates oxidative stress.[9] When transferrin becomes fully saturated, the surplus iron binds to low-molecular-weight ligands, forming non-transferrin-bound iron (NTBI).[10] NTBI can be highly toxic due to its unregulated movement across cell membranes and its ability to stimulate the synthesis of ROS.[8] Elevated levels of ROS can cause oxidative damage to biomolecules, thereby resulting in cellular toxicity and impaired organ function on account of the accumulation of NTBI.[11,12] The accumulation of NTBI in β-thalassemia patients sparks oxidative stress by ramping the presence of ROS up across the Haber–Weiss and Fenton reactions. This process subsequently brings about cellular damage, including lipid peroxidation, the impairment of cellular proteins and nucleic acids, mitochondrial malfunction, and ultimately, apoptosis.[13,14] Additionally, the accumulation of excess α-chains triggers the generation of free radicals and reactive iron, compromising the antioxidant defense system and contributing to hemolysis via the oxidation of various RBC components.[15,16] Moreover, the combination of oxidative stress and hepatic hemosiderosis reduces glutathione levels among individuals with β-thalassemia.[17]

Antioxidants make a significant contribution to protecting the body by neutralizing deleterious oxygen radicals.[18] The ability to counteract oxidative stress stems from the combined effects of water-soluble antioxidants, lipid-soluble antioxidants, and antioxidant enzymes. Studies have shown increased levels of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase (GPx), in individuals with β-thalassemia.[19,20] However, these heightened enzymatic activities were found to be insufficient in preventing oxidative damage, driving the patients to an imbalance in the body's antioxidant defense system.[17,21,22]

N-acetyl cysteine is a modified version of the amino acid L-cysteine, possessing the unique ability to easily enter cells and convert to L-cysteine, which is an essential precursor to GSH.[23] This antioxidant largely participates in various intracellular processes.[24] NAC carries antioxidant properties, both direct and indirect, exerts anticarcinogenic effects, and offers protection against DNA damage.[25] Research has shown that the administration of combinations of antioxidants, as with NAC, along with iron chelators, can effectively offset the damaging effects of ROS.[24] NAC's unbound thiol moiety can interact with ROS, resulting in the formation of NAC thiol as an intermediate, with NAC disulfide being the primary end product. In addition, NAC serves as a precursor to GSH,[25] thereby demonstrating an indirect antioxidant effect. To mitigate the deleterious influences of harmful substances, maintaining optimal intracellular GSH levels is indispensable. The synthesis of GSH takes place in the cytoplasm of cells through two distinct enzymatic phases. In the initial step, the enzyme γ-glutamylcysteine synthetase mediates the combination of glutamic acid and cysteine (Cys). Subsequently, GSH synthetase catalyzes the addition of glycine to the dipeptide γ-glutamylcysteine to produce GSH. In laboratory settings, NAC serves as a precursor for GSH due to its efficient cell penetration and deacylation to yield Cys.[26] The widespread use of NAC is attributed to its well-documented antioxidant and radical scavenging properties, as well as its stability as a thiol compound and cost-effective availability. With proven decent bioavailability and a favorable safety profile, NAC has been utilized as an antioxidant in numerous in-vivo investigations.[27]

In 2023, a meta-analysis was conducted to evaluate the antioxidant effects of NAC and vitamin E on patients with TDT. The study revealed a noticeable rise in hemoglobin levels in pediatric patients with TDT, with a weighted mean difference (WMD) of 1.10, 95% CI, 0.47–1.73 (one study). However, no major changes in the WMD of total antioxidant capacity were observed in pediatric or adult individuals with TDT. Using NAC displayed a substantial reduction in total oxidative stress in children, with a WMD of −7.80, 95% CI, −9.30 to −6.30 (one study). Likewise, the study estimated a WMD of −1.26, 95% CI, −1.58 to −0.94 for oxidative stress index (OSI) in children (one study).[28] To fully grasp the beneficial effects of NAC, a thorough evaluation of the clinical trials can help in devising an appropriate approach for NAC intake to alleviate the consequences of excessive iron, particularly the oxidative stress experienced by individuals with β-thalassemia. This study, as such, aimed to explore the impact of NAC therapy on oxidative biomarkers in light of estimating the index of the NNT in this specific population.

2 | METHOD

2.1 | Systematic search

In our review study, we systematically searched the Scopus, PubMed, Web of Science, Trip, and CENTRAL databases to gather relevant literature. We specifically targeted articles published from the first of January 2000 until the first of January 2024 that examined the impact of NAC on oxidative stress in cases of β-thalassemia. We focused on keywords such as "beta-thalassemia," "oxidative stress," and "N-acetyl cysteine."

2.2 | Inclusion and exclusion criteria

The inclusion criteria for the study were as follows: (a) articles recruiting human subjects. (b) patients diagnosed with β–thalassemia (c) clinical trials designed to apprise the antioxidant function of NAC.

Excluded articles comprised: (a) case studies, reviews, animal studies, brief reports, conference abstracts, editorials, letters, and book chapters. (b) articles written in languages other than English.

2.3 | Included studies

Five studies had control groups,[29–33] whereas the rest had no control arm.[32,34,35] Five studies investigated the impact of NAC 10 mg/kg/day on β-thalassemia major patients over a 3-month follow-up. Three studies demonstrated significant improvements in oxidative stress biomarkers in β-thalassemia major patients after 3 months of consuming 10 mg/kg/day dose of NAC, as indicated by estimated effect sizes. Three studies were set with a follow-up time of 6 months, two of which focused on patients with β-thalassemia major and one on β-thalassemia/HbE patients. Two of the three studies used a combination of antioxidants. The dosage of NAC differed, with 600 mg/day for two studies and one set with 200 mg/day. The study findings are summarized in Table 1. Besides, the estimated Hedges's *g* and the NNT with 95% CI as per posttreatment data between NAC and comparison groups in patients with β-thalassemia are shown in Table 2.

2.4 | Quality assessment

We employed the Cochrane Risk of Bias Tool version 2.0 to assess the included clinical trials. Two independent reviewers evaluated the potential risk of bias across several categories, including random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective reporting bias, and other sources of bias. The results of the risk of bias assessment were defined as follows: "low risk of bias": no domains are rated as high risk of bias, and at least one domain is rated as low risk of bias; "some concerns": no domains are rated as high risk of bias, but at least one domain is rated as some concerns and "high risk of bias": at least one domain is rated as high risk of bias.[38] Any disagreements were resolved by the corresponding author.

2.5 | Data extraction and NNT calculation

First, the retrieved studies were reviewed based on whether their titles and abstracts were aligned with the study' question. Upon excluding studies that did not fit the study's objectives, the remaining studies moved on. To avoid missing the articles, the references of the included articles were scanned to spot articles relating to our study. The whole process of selecting the included articles was autonomously undertaken by two researchers. Data extracted from the included studies encompassed the publication year, type of study, the study population, sample size, the intervention properties, the dose of NAC, the study duration, and the key findings. Data were prepared in the form of mean ± SD for all biomarkers pertaining to oxidate stress and antioxidants. Web-Plot-Digitizer 4.7 was also used to extract data depicted as plots. To compute Hedges's *g* with 95% confidence intervals (CI), data belonging to pre and post-in NAC groups were utilized using STATA 14.0 (StataCorp). Besides, the values of Hedges's *g* and NNT with 95% CI according to posttreatment data between NAC and comparison groups were estimated. Computed effect sizes were eventually turned into NNT (https://www.psychometrica.de/effect_size.html). The NNT is the number from which one case takes advantage of the treatment compared to an alternative intervention or control.[39]

2.6 | Ethical review

Ethical clearance was deemed unnecessary for this review since it did not involve any animal or human participants.

3 | RESULTS

3.1 | Study findings

In the electronic database search, 99 articles were identified, with 52 duplicates. The titles and abstracts of the remaining 47 papers were carefully analyzed. Having applied the inclusion and exclusion criteria, eight articles were selected for full-text analysis, ultimately resulting in eight articles eligible for NNT calculation. The evaluation of oxidative stress biomarkers' effect sizes depicted a significant improvement, representing a decent adjuvant therapy for NAC. The calculated NNTs are mentioned in Tables 1 and 2.

A quasi-experimental study including 35 individuals with β-thalassemia major, mean age 18.97 ± 6.24, revealed that NAC monotherapy 10 mg/kg/day was not able to make a significant improvement in hemoglobin and ferritin levels after 3 months. Four

TABLE 1 The summary of primary and secondary outcomes in clinical trials carried out concerning using N-acetylcysteine in β-thalassemia patients.

References	Sample size, NAC, and control	NAC dose	NAC duration	Index	Outcome							
					NAC					Control		
					Pre	Post	MD	Hedges's *g*, 95% CI	NNT, 95% CI	Pre	Post	MD
Bahoush et al.[32]	35[a]	10 mg/kg/day	3 months	Hb, mg/dL Ferritin	8.35 ± 0.90 1531 ± 946	8.33 ± 0.64 1306 ± 997	−0.02 −225	−0.02, −0.48 to 0.43 −0.22, −0.69 to 0.23	89 9	-	-	-
Haghpanah et al.[33]	78, 25, and 25[b]	10 mg/kg/day	3 months	TAC, mmol Eqv./L TOS, μmol H_2O_2 Eqv./L OSI Hb, g/dL Ferritin, ng/mL	0.80 ± 0.22 3.24 ± 0.89 0.42 ± 0.14 9.78 ± 0.79 2932 ± 2504	0.70 ± 0.16 3.13 ± 2.08 0.46 ± 0.10 9.89 ± 0.82 3843 ± 3491	−0.10 −0.11 +0.04 +0.11 +911	−0.51, −1.06 to 0.04 −0.07, −0.61 to 0.48 0.32, −0.23 to 0.87 0.13, −0.41 to 0.68 0.29, −0.25 to 0.84	4, 2–45 26 6, 3–8 14 7, 3–8	0.80 ± 0.25[c] | 0.83 ± 0.20[d] 2.97 ± 1.04[c] | 3.25 ± 0.68[d] 0.42 ± 0.26[c] | 0.41 ± 0.12[d] 9.96 ± 0.66[c] | 9.96 ± 0.66[d] 3782 ± 3030[c] | 3782 ± 3030[d]	0.78 ± 0.16[c] | 0.74 ± 0.16[d] 2.81 ± 2.12[c] | 2.99 ± 1.12[d] 0.38 ± 0.15[c] | 0.42 ± 0.10[d] 9.93 ± 0.92[c] | 9.92 ± 0.73[d] 3364 ± 3071[c] | 2661 ± 5066[d]	−0.02[c] | −0.09[d] −0.16[c] | −0.26[d] −0.04[c] | −0.01[d] −0.03[c] | −0.04[d] −418[c] | −1121[d]
Khail et al.[29]	44, 22, and 22	10 mg/kg/day	3 months	TOS, pg/mL Ferritin, ng/dL	8.88 ± 2.21 3464 ± 1378	6.84 ± 2.18 3293 ± 1434	−2.04 −171	**−0.91, −1.52 to −0.30** −0.12, −0.70 to 0.46	3, 2–6 15	7.93 ± 2.05 1603 ± 938	8.66 ± 2.02 1886 ± 1352	+0.73 +283
Mohamed et al.[30]	100, 50, and 50	10 mg/kg/day	3 months	Hb, g/dL TOS, pg/mL TAC, mmol/L OSI Ferritin, ng/L	7.00 ± 1.01 9.00 ± 2.12 0.72 ± 0.26 14.54 ± 8.07 1639 ± 928	8.20 ± 0.72 6.84 ± 2.06 0.90 ± 0.18 7.80 ± 2.78 1053 ± 552	+1.2 −2.16 +0.18 −6.74 −586	1.36, **0.92–1.79** **−1.03, −1.44 to −0.61** 0.80, **0.39–1.20** **−1.11, −1.52 to −0.69** **−0.76, −1.16 to −0.36**	2 2 3, 2–5 2 3, 2–5	6.81 ± 0.73 8.10 ± 2.03 0.77 ± 0.22 11.36 ± 4.58 1666 ± 796	7.11 ± 0.61 8.53 ± 1.93 0.78 ± 0.19 11.52 ± 3.71 1705 ± 850	+0.3 +0.43 +0.01 +0.16 +39
Pattanakuhar et al.[36]	59, 30, and 29	600 mg daily	6 months	Hb, g/dL NTBI, μM TNF-α, ng/dL IL-10, ng/dL	7.14 ± 1.21 6.59 ± 2 17.14 ± 7.32 1.51 ± 0.51	7.44 ± 1.60 2.56 ± 0.8 12.11 ± 4.61 1.06 ± 0.52	+0.3 −4.03 −5.03 −0.45	0.21, −0.29 to 0.71 **−2.61, −3.29 to −1.92**	9 2 3, 2–7 3, 2– 6	7.16 6.98 16.16 1.00	7.39 ± 1.20 2.21 ± 1.66 12.54 ± 4.35 1.09 ± 1.28	+0.23 −4.77 −3.62 +0.09

TABLE 1 (Continued)

References	Sample size, NAC, and control	NAC dose	NAC duration	Index	Outcome							
					NAC					Control		
					Pre	Post	MD	Hedges's *g*, 95% CI	NNT, 95% CI	Pre	Post	MD
				8-isoprostane, ng/dL	18.02 ± 2.11	20.34 ± 5.21	+2.32	−0.81, **−1.33 to −0.29**	4, 2–30	17.95	21.74 ± 4.28	+3.79
				Ferritin, ng/dL	1561 ± 511	1603 ± 607	+42	−0.86, **−1.38 to −0.34**	26	1546	1785 ± 1142	+239
								0.58, **0.06–1.08**				
								0.07, −0.43 to 0.57				
Elsedfy et al.[34]	20	600 mg/day + L-carnitine 2 g/day	6 months	SDI	1.90 ± 0.33	2.46 ± 0.61	+0.56	1.09, **0.17–1.99**	2	-	-	-
				TZI	1.59 ± 0.22	1.86 ± 0.28	+0.27	1.03, **0.11–1.91**	2			
				AI	61.60 ± 12.18	46.60 ± 12.86	−15	−1.15, **−2.05 to −0.22**	2			
				DNA FI	8.5 ± 2.14	9.5 ± 1.33	+1	0.54, −0.33 to 1.39	4, 2– 6			
				GPX, U/L	3083 ± 790.99	3630 ± 1013.3	+547	0.58, −0.29 to 1.43	4, 2–7			
				GR, U/L	29.6 ± 12.18	36.2 ± 15.84	+6.6	0.45, −0.41 to 1.29	5			
				SOD, U/L	120.8 ± 25.5	146.7 ± 35.53	+25.9	0.80, −0.09 to 1.67	3, 2–20			
				Ferritin, ng/mL	1676 ± 775	1359 ± 424	−317	−0.49, −1.33 to 0.38	4, 2–5			
Yanpanitch et al.[35]	35, 16, and 19[e]	200 mg/day +- 50 mg/kg/day deferiprone + curcuminoids 500 mg/day	15 months	NTBI, μmol/L	5.3 ± 0.6	6 months	2.1 ± 0.2	−3.2	−6.97, **−8.85 to −5.08**	1	-	-	-
				RBC SOD, U/g Hb	5395 ± 278		4318 ± 179	−1077	−4.49, **−5.80 to −3.16**	1			
				RBC GPX, U/g Hb	63.7 ± 3.2		48.9 ± 1.9	−14.8	−5.48, **−7.01 to −3.93**	1			
				RBC GSH, mmol/L	1.74 ± 0.05		2.12 ± 0.06	+0.38	6.71, **4.88–8.52**	2			
				ROS, %MCF	51.1 ± 8.8		29.7 ± 3.3	−21.4	−3.14, **−4.17 to −2.09**	2			
				RBC MDA, nmol/g Hb	1542 ± 165		1150 ± 107	−392	−2.75, **−3.70 to −1.77**	2			
				Ferritin, pmol/L	3651 ± 855		1921 ± 426	−1730	−2.50, **−3.41 to −1.56**	2			

(Continues)

TABLE 1 (Continued)

References	Sample size, NAC, and control	NAC dose	NAC duration	Index	Outcome								
					NAC						Control		
					Pre	Post		MD	Hedges's *g*, 95% CI	NNT, 95% CI	Pre	Post	MD
						12 months	2.0 ± 0.6	−0.3	−0.82, −7.43 to 0.20	4, 2–18			
							4024 ± 359	−568		3, 2–8			
							36.6 ± 1.8	−22.1	−0.92, **−3.82 to −0.30**	2			
							1.79 ± 0.04	−0.03		6, 2–9			
							31.4 ± 4.8	−19.3	−3.02, **−5.15 to −2.71**	2			
							9369 ± 8151	−698		3, 2–8			
							2018 ± 498	−1636	0.46, −0.23 to 1.04	2			
									−2.42, −3.31 to −1.50				
									−4.56, **−5.88 to −3.22**				
									−2.85, −2.21 to −0.88				
		200 mg/day +- 50 mg/kg/day deferiprone + vitamin E 400 IU/day			4.9 ± 0.6	6 months	1.8 ± 0.3	−3.1	−6.37, **−8.10 to −4.62**	1			
					5051 ± 188		4245 ± 196	−806		2			
					62.6 ± 2.6		48.9 ± 2.5	−13.7	−4.09, **−5.31 to −2.85**	1			
					1.81 ± 0.04		2.10 ± 0.05	+0.29		1			
					53.0 ± 7.0		28.6 ± 3.6	−24.4	−5.23, **−6.70 to −3.74**	2			
					1487 ± 138		815 ± 33	−672		1			
					4767 ± 773		2339 ± 532	−2428	6.24, **4.52–7.94**	2			
									−4.27, **−5.53 to −2.99**				
									−6.53, **−8.30 to −4.74**				
									−3.57, **−4.68 to −2.43**				
						12 months	1.8 ± 0.3	−3.1	−6.37, **−8.10 to −4.62**	1			
							4075 ± 219	−976		2			
							36.6 ± 1.7	−26	−4.66, **−6.00 to −3.30**	1			
							2.04 ± 0.05	+0.23		1			
							33.5 ± 7.4	−19.5	−11.5, **−14.5 to −8.55**	2			
							698 ± 24	−789		1			
							2065 ± 655	−2702	4.95, **3.52–6.36**	2			
									−2.64, **−3.57 to −1.68**				

TABLE 1 (Continued)

References	Sample size, NAC, and control	NAC dose	NAC duration	Index	Outcome							
					NAC					Control		
					Pre	Post	MD	Hedges's *g*, 95% CI	NNT, 95% CI	Pre	Post	MD
								−7.76, **−9.82 to −5.69**				
								−3.68, **−4.81 to −2.52**				
						15 months	5.2 ± 0.7	0.45, −0.24 to 1.13	5, 2–8			
							+0.3					
							5097 ± 293		10			
							+46					
							47.3 ± 2.1	0.18, −0.50 to 0.86	1			
							−15.3					
							1.82 ± 0.05		9			
							+0.01					
							48.8 ± 15.1	**−6.31, −8.03 to −4.57**	6			
							−4.2					
							1175 ± 79		2			
							−312					
							2765 ± 622	0.21, −0.46 to 0.89	2			
							−2002					
								−0.35, −1.03 to 0.34				
								−2.70, **−3.65 to −1.73**				
								−2.78, **−3.74 to −1.80**				
Ozdemir et al.[31]	98, 25, and 25[f]	10 mg/kg/day	3 months	Hb, g/dL	8.4 ± 1.3	9.6 ± 0.8	+1.2	1.09, **0.50–1.68**	2	8.0 ± 1.1[c] 8.7 ± 1.1[d]	8.1 ± 0.9[c] | 9.7 ± 1.2[d]	+0.1
				TOS, mmol Eqv/L	16.3 ± 4.1	7.8 ± 2.3	−8.5		2	8.9 ± 2.1[c] | 11.9 ± 2.8[d]	8.2 ± 2.1[c] | 7.8 ± 2.1[d]	−0.7
				TAC, mmol Eqv/L	0.72 ± 0.09	0.84 ± 0.13	+0.12	−2.52, **−3.25 to −1.77**	2	0.76 ± 0.11[c] | 0.74 ± 0.12[d]	0.81 ± 0.13[c] | 0.84 ± 0.21[d]	+0.05
				OSI, AU	2.29 ± 0.75	0.94 ± 0.23	−1.13		2	1.19 ± 0.36[c] | 1.65 ± 0.05[d]	1.10 ± 0.27[c] | 0.92 ± 0.30[d]	−0.09
				DNA damage, AU	8.9 ± 6.0	2.5 ± 2.0	−6.4	1.06, **0.47–1.64**	2	3.2 ± 3.9[c] | 4.2 ± 5[d]	4.0 ± 3.4[c] | 4.4 ± 3.6[d]	+0.8
								−2.39, **−3.11 to −1.66**				
								−1.41, **−2.02 to −0.79**				

Note: The confidence interval in bold means statistical significance. The values of Hedges's *g* (95% CI) were estimated based on pre-and-postdata belonging to the intervention.

[a]A quasi-experimental study on 35 individuals with β-thalassemia major.

[b]There were three groups, including NAC (*N* = 25), vitamin E (*N* = 26), and nonsupplemented group (*N* = 25).

[c]Data belong to the no-treatment group.

[d]Data belong to the vitamin E group.

[e]Sample sizes for curcuminoids and vitamin E cocktails were 16 and 19, respectively.

[f]There were three separate groups, namely NAC, vitamin E, and nonsupplemented group, with an equal sample size of 25.

TABLE 2 The estimated Hedges's *g* and the number needed to treat (NNT) with 95% CI based on posttreatment data between N-acetylcysteine and control groups in patients with β-thalassemia.

References		Hedges's *g*, 95% CI		NNT, 95% CI	
Haghpanah et al.[33]	TAC, mmol Eqv./L	−0.49, −1.04 to 0.06[a]	−0.25, −0.79 to 0.30[b]	4, 2 to 30[a]	8[b]
	TOS, μmol H_2O_2 Eqv./L	0.14, −0.40 to 0.69[a]	0.08, −0.46 to 0.62[b]	13[a]	23[b]
	OSI	0.62, **0.05–1.17**[a]	0.39, −0.15 to 0.94[b]	3, 2–36[a]	5, 3–12[b]
	Hb, g/dL	−0.04, −0.59 to 0.50[a]	−0.04, −0.58 to 0.50[b]	45[a]	45[b]
	Ferritin, ng/mL	0.14, −0.40 to 0.69[a]	0.27, −0.28 to 0.81[b]	13[a]	7[b]
Khail et al.[29]	TOS, pg/mL	−0.85, **−1.45 to −0.24**		3, 2–8	
	Ferritin, ng/dL	0.99, **0.37–1.60**		2	
Mohamed et al.[30]	Hb, g/dL	1.62, **1.17–2.07**		2	
	TOS, pg/mL	−0.84, **−1.24 to −0.43**		3, 2–5	
	TAC, mmol/L	0.64, **0.24–1.04**		3, 2–8	
	OSI	−1.13, **−1.54 to −0.70**		2	
	Ferritin, ng/L	−0.90, **−1.31 to −0.49**		3, 2–4	
Pattanakuhar et al.[36]	Hb, g/dL	0.03, −0.47 to 0.54		60	
	NTBI, μM	0.27, −0.24 to 0.77		7, 3–8	
	TNF-α, ng/dL	−0.09, −0.60 to 0.41		20	
	IL-10, ng/dL	−0.03, −0.53 to 0.47		60	
	8-isoprostane, ng/dL	−0.29, −0.79 to 0.22		7, 3–9	
	Ferritin, ng/dL	−0.20, −0.70 to 0.31		9	
Ozdemir et al.[31]	Hb, g/dL	1.73, 1.08 to 2.37[a]	−0.10, −0.64 to 0.45[b]	2[a]	18[b]
	TOS, H_2O_2 Eqv/L	−0.18, −0.72 to 0.37[a]	0[b]	10[a]	-[b]
	TAC, Trolox Eqv/L	0.23, −0.32 to 0.77[a]	0[b]	8[a]	-[b]
	OSI, AU	−0.63, −1.18 to −0.06[a]	0.07, −0.47 to 0.62[b]	3, 2–30[a]	26[b]
	DNA damage, AU	−0.53, −1.08 to 0.03[a]	−0.64, −1.2 to −0.1[b]	4, 2–60[a]	3, 2–18[b]

Note: The effect sizes show the effectiveness of the N-acetylcysteine than control. Bold values indicate significant finding.

Abbreviations: AU, arbitrary unit; CI, confidence interval; Hb, hemoglobin[37]; IL-10, interleukin-10 (ng/dL); NNT, number needed to treat; NTBI, non-transferrin-bound iron (μM/L); OSI, oxidative stress index; TAC, total antioxidant capacity (mmol; Trolox Eqv/L); TNF-α, tumor necrotic factor alpha (ng/dL); TOS, total oxidant status (mmol; H_2O_2 Eqv/L).

[a]Compared to the no-treatment group.

[b]Compared to the vitamin E group.

participants in the NAC group were unable to finish the course of treatment due to mild complications. The study demonstrated a nonsignificant decrease in the hemoglobin levels following the NAC treatment, mean difference −0.02 mg/dL, Hedges's *g* −0.02 (95% CI, −0.48 to 0.43), NNT 89. A nonsignificant improvement in reducing ferritin levels post-NAC therapy was also found by that study, with a mean difference of −225, Hedges's *g* −0.22 (95% CI, −0.69 to 0.23), NNT 9[32] (Table 1).

In a 3-month open-label randomized controlled trial (RCT), 78 eligible patients diagnosed with TDT were recruited, with an average age of 28.5 ± 5.1 years. The patients were randomly assigned to three groups: the NAC group (*n* = 25, receiving 10 mg/kg/day orally), the vitamin E group (*n* = 26, receiving 10 U/kg/day orally), and the control group (*n* = 25). Upon NAC treatment, a nonsignificant change observed in TAC values, with a mean difference of −0.10, Hedges's *g* −0.51 (95% CI, −1.06 to 0.04), NNT 4 (95% CI, 2–45). Nevertheless, no notable alteration was detected in the total oxidant status (TOS), with a mean difference of −0.11, Hedges's *g* −0.07 (95% CI, −0.61 to 0.48), NNT 26 and OSI, with a mean difference 0.04, Hedges's *g* 0.32 (95% CI, −0.23 to 0.87), NNT 6 (95% CI, 3–8) post-NAC treatment after the completion of the trial. The study exhibited a nonsignificant improvement in Hb with a mean difference 0.11, Hedges's *g* 0.13 (95% CI, −0.41 to 0.68), NNT 14 and ferritin levels with a mean difference 911, Hedges's *g* 0.29 (95% CI, −0.25 to 0.84), NNT 7 (95% CI, 3–8) after NAC therapy. Adverse events were also reported, with four patients experiencing mild adverse effects attributed to NAC, including gastrointestinal symptoms, for example, abdominal pain, nausea, constipation, diarrhea, as well as skin rash[33] (Table 1).

In a randomized controlled trial, the TOS of 44 children with β-thalassemia major, mean aged at 8.05 ± 3.35 years, was assessed for a 3-month treatment with 10 mg/kg/day of NAC. The study exhibited a significant decrease in TOS after NAC therapy, mean difference −2.04 pg/mL, Hedges's *g* −0.91 (95% CI, −1.52 to −0.30), NNT 3 (95% CI, 2–6). As per the comparison between pre-and posttreatment ferritin levels, the administration of NAC did not demonstrate a significant impact on ferritin levels, with a mean difference of −171 ng/dL, Hedges's *g* −0.12 (95% CI, −0.70 to 0.46), NNT 15[29] (Table 1).

In a randomized controlled trial involving 100 children aged 2–13 with β-thalassemia major, the effectiveness of a 10 mg/kg dose of NAC was demonstrated after a 3-month period. The analysis of oxidative biomarkers indicated a significant improvement following NAC therapy, with notable enhancements in hemoglobin levels posttreatment, with a mean difference of +1.2 g/dL, Hedges's *g* 1.36 (95% CI, 0.92–1.79), NNT 2. The TOS rose after NAC treatment, mean difference −2.16 pg/mL, Hedges's *g* −1.03 (95% CI, −1.44 to −0.61), NNT 2. The impact of NAC on TAC levels was also significant, mean difference +0.18 mmol/L, Hedges's *g* 0.80 (95% CI, 0.39–1.20), NNT 3 (95% CI, 2–5). A remarkable reduction was also reported in OSI after using NAC, with a mean difference of −6.74, Hedges's *g* −1.11 (95% CI, −1.52 to −0.69), NNT 2. NAC therapy caused the ferritin levels to subside significantly, mean difference −586 ng/L, Hedges's *g* −0.76 (95% CI, −1.16 to −0.36), NNT 3 (95% CI, 2–5)[30] (Table 1).

In a 6-month RCT on 59 TDT patients with a mean age of 27 ± 8 years, the therapeutic effectiveness of NAC 600 mg/daily on iron overload-related biomarkers was studied. The levels of NTBI plummeted by −4.03 µM following NAC therapy, indicating an advantageous trait on iron surplus in these cases, Hedges's *g* −2.61 (95% CI, −3.29 to −1.92), NNT 2. However, The ferritin levels showed no significant improvement subsequent to NAC treatment, mean difference +42 ng/dL, Hedges's *g* 0.07 (95% CI, −0.43 to 0.57), NNT 26. Similarly, the effect of NAC on hemoglobin levels was not significant, mean difference +0.3 g/dL, Hedges's *g* 0.21 (95% CI, −0.29 to 0.71), NNT 9. The serum levels of tumor necrosis factor-alpha (TNF-α) dwindled by −5.03 ng/dL after NAC therapy, which was a noticeable effect on TNF-α concentration, Hedges's *g* −0.81 (95% CI, −1.33 to −0.29), NNT 3 (95% CI, 2–7). A significant impact on interleukin-10 levels was also found following treatment with NAC, mean difference −0.45 ng/dL, Hedges's *g* −0.86 (95% CI, −1.38 to −0.34), NNT 3 (95% CI, 2–6). The efficacy of NAC on serum levels of 8-isoprostane was also significant, mean difference +2.32 ng/dL, Hedges's g 0.58 (95% CI, 0.06–1.08), NNT 4 (95% CI, 2–30)[36] (Table 1).

In a pilot study, the impact of antioxidants, including NAC 600 mg/day and L-carnitine 2 g/day, on 20 patients with β-thalassemia major was examined. The results indicated a significant effect on sperm deformity index (SDI), mean difference +0.56, Hedges's *g* 1.09 (95% CI, 0.17–1.99), NNT 2. The teratozospermia index[40] improved upon NAC therapy, as the value significantly rose by +0.27 at the end of the study, Hedges's *g* 1.03 (95% CI, 0.11–1.91), NNT 2. The treatment with NAC caused the acrosomal index (AI) to descend remarkedly by −15, which provided a Hedges's *g* of −15 (95% CI, −2.05 to −0.22) and a NNT of 2. Likewise, the effect of NAC on DNA fragmentation index (DNA FI) was not significant, mean difference +1, Hedges's *g* 0.54 (95% CI, −0.33 to 1.39), NNT 4 (95% CI, 2–6). Although the GPx levels improved by +547 U/L amongst the cases co-treated by NAC, the change was not statistically significant, Hedges's *g* 0.58 (95% CI, −0.29 to 1.43), NNT 4 (95% CI, 2–7). Similarly, the serum levels of glutathione reductase (GR) nonsignificantly rose by +6.6 U/L, Hedges's *g* 0.45 (95% CI, −0.41 to 1.29), NNT 5. The levels of SOD increased by +25.9 U/L at the end of the study, providing a Hedges's *g* of 0.80 (95% CI, −0.09 to 1.67) and a NNT 3 (95% CI, 2–20). The mean difference of ferritin levels between before and after the treatment was −317 ng/mL, Hedges's *g* −0.49 (95% CI, −1.33 to 0.38), NNT 4 (95% CI, 2–5)[34] (Table 1).

In a 15-month clinical study, 50 cases with β-thalassemia/hemoglobin E, mean age of 32.5 ± 1.7 years, were randomly placed on two regimes, either curcuminoids cocktail (*n* = 16, receiving curcuminoids 500 mg/day, NAC 200 mg/day, and deferiprone 50 mg/kg/day) or vitamin E cocktail (*n* = 19, receiving vitamin E 400 IU/day, NAC 200 mg/day, and deferiprone 50 mg/kg/day). Notably, the NTBI levels started scaling down following 15 months of treatment with the curcuminoids cocktail, with a mean difference of −0.5 µmol/L, Hedges's g −0.59 (95% CI, −1.28 to 0.10), NNT 4 (95% CI, 2–18), while the values in vitamin E arm were 0.3 µmol/L, Hedges's *g* 0.45, (95% CI, −0.24 to 1.13), NNT 5, (95% CI, 2–8), indicating the better performance of vitamin E and NAC in mitigating the amount of unbound iron in the circulation. The findings were the same when the iron-burden-lowering effects were compared between the groups based on pre-and-postserum ferritin levels, as mean differences for the arms were −1236 pmol/L (Hedges's *g* −1.63, 95% CI, −2.41 to −0.83, NNT 2 and −2002 pmol/L (Hedges's *g* −2.78, 95% CI, −3.74 to −1.80, NNT 2, correspondingly. The results were paradoxical when The RBC SOD levels were measured in the groups, underscoring a better function of vitamin E and NAC than curcuminoids and NAC in enforcing the enzyme to break down superoxide radicals in these cases (mean difference +46 U/g Hb, Hedges's *g* 0.18, 95% CI, −0.50 to 0.86, NNT 10. The mean difference stood at −301 U/g hemoglobin (Hedges's *g* −0.95, 95% CI, −1.66 to −0.23, NNT 3, (95% CI, 2–8) after co-administration of curcuminoids and NAC. Moreover, RBC GPx levels within two arms decreased after 15 months. The mean difference for the curcuminoids group was −12.1 U/g hemoglobin (Hedges's *g* −3.92, 95% CI, −5.11 to −2.71, NNT 2, whereas that was −15.3 U/g hemoglobin (Hedges's *g* −6.31, 95% CI, −8.03 to −4.57, NNT 1 for the vitamin E group. The changes in RBC GSH levels were almost the same between the arms, suggesting that curcuminoids, vitamin E, and NAC may have the least impact on RBC GSH. Based on the alternations between pre-and posttreatment ROS levels, the curcuminoids conjugated with NAC exhibited a notable impact on reducing ROS levels (mean difference −17.7%MCF, Hedges's *g* −2.43, 95% CI, −3.33 to −1.51, NNT 2, which excellences in combination antioxidant therapy with vitamin E and NAC (mean difference −4.2%MCF, Hedges's *g* −0.35, 95% CI,

−1.03 to 0.34, NNT 6. Co-therapy with vitamin E and NAC provided further decline in RBC malondialdehyde as much occurred after curcuminoids and NAC, mean difference −312 vs. −73 nmol/g Hb, respectively. The Hedges's g for RBC malondialdehyde after co-treatment with vitamin E and NAC was −2.70 (95% CI, −3.65 to −1.73, NNT 2, while that was estimated at −0.45, (95% CI, −1.13 to 0.24, NNT 5, (95% CI, 2–8) after curcuminoids and NAC treatment[35] (Table 1).

A recent open-label, randomized trial involving 98 children with β-thalassemia, mean aged 10 years, found that monotherapy with NAC 10 mg/kg/day is likely efficacious in reducing oxidative stress after 3 months. The study observed a decrease in TOS following the treatment, mean difference −8.5 µmol; H_2O_2 Eqv/L, Hedges's g −2.52 (95% CI, −3.25 to −1.77), NNT 2. The levels of TAC were also escalated following NAC therapy (mean difference +0.12 mmol; Trolox Eqv/L), which provided a Hedges's g of 1.06 (95% CI, 0.47–1.64) and NNT of 2. The value of OSI experienced a significant decrease following NAC treatment in β-thalassemia cases, mean difference −1.13 AU (arbitrary unit), Hedges's *g* −2.39 (95% CI, −3.11 to −1.66), NNT 2. Posttreatment, DNA damage significantly dropped in the NAC group, mean difference of −6.4 AU, Hedges's *g* −1.41 (95% CI, −2.02 to −0.79), NNT 2. A rise in hemoglobin levels was observed before red cell transfusion after NAC therapy, mean difference +1.2 gr/dL, Hedges's *g* 1.09 (95% CI, 0.50–1.68), NNT 2[31] (Table 1).

Ultimately, the estimated values of Hedges's *g* and the NNT as per posttreatment data between NAC and control groups in patients with β-thalassemia pinpointed that the strongest relationship with using NAC therapy was geared by mitigating oxidative stress, particularly TOS and OSI, in these cases, although the preponderance of the evidence remained in high risk of bias (Table 2).

3.2 | Risk of bias assessment

Out of the eight studies assessed, only one was labeled as a low risk of bias, while the remaining were categorized as a high risk of bias. The results of the risk of bias assessment are shown in Table 3 and eTable S1.

4 | DISCUSSION

4.1 | Summary of evidence

The administration of NAC has been shown to effectively alleviate oxidative stress. Most estimated NNTs were below 5, indicating a clinically significant therapeutic value in this context. The highest dose of NAC used was 10 mg/kg/day (600 mg/day) over a period of 3–6 months. Across the six included studies, the impact of NAC monotherapy on oxidative stress in β-thalassemia major patients, three studies reported positive effects after 3 months, while one study showed favorable effects after 6 months of follow-up. Four out of the six studies were RCTs.

TABLE 3 The risk of bias assessment of the included studies.

References	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias	Risk of bias
Bahoush et al.[32]	High risk	High risk	High risk	Unclear	Low risk	Low risk	Low risk	High
Haghpanah et al.[33]	Low risk	High risk	High risk	Unclear	High risk	Low risk	Low risk	High
Khail et al.[29]	Low risk	Low risk	High risk	Unclear	Low risk	Low risk	Low risk	High
Mohamed et al.[30]	Low risk	Low risk	High risk	Unclear	Low risk	Low risk	Low risk	High
Pattanakuhar et al.[36]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low
Elsedfy et al.[34]	High risk	High risk	High risk	Unclear	Low risk	Low risk	Low risk	High
Yanpanitch et al.[35]	High risk	Low risk	High risk	Unclear	Low risk	Low risk	Low risk	High
Ozdemir et al.[31]	Low risk	High risk	High risk	Unclear	Low risk	Low risk	Low risk	High

The inclusion criteria for the five studies comprised children with TDT who received NAC monotherapy therapy as an additional treatment, and one study included adults with TDT.[36] In the conducted studies, NAC 10 mg/kg/day was orally administered for 3 months in five studies, and one study administered NAC 600 mg daily for 6 months.[36] The study population consisted of individuals undergoing chronic transfusion therapy and treatment with iron chelator agents. The type and number of chelators used varied across the studies. Notably, the comparison groups in these studies were heterogeneous; two studies lacked a comparison group, and two studies included nonsupplemented and vitamin E-supplemented groups as controls (Tables 1 and 2).

Despite the variations in the types of chelators used in the studies may influence the findings, the results of the three studies examining the impact of NAC on oxidative stress biomarkers in β-thalassemia patients over a 6-month period were acceptable. In these studies, NAC was administered orally to a heterogeneous population, including individuals with TDT and non-TDT. The studies also varied in terms of the type of chelators used. Two of the studies involved combination therapy with NAC,[34,41] while one focused on monotherapy with NAC.[36]

It is challenging to assess the effectiveness of combination therapy with NAC compared to NAC monotherapy due to the varied characteristics of the included studies. These differences constituted the study population, prescribed regimens, dosage, study design, and outcomes. Therefore, comparing studies on combination therapy with NAC to NAC monotherapy over a 6-month period may lead to misinterpretation due to the lack of consistency among the included studies. As a result, the exclusive effect of combination therapy with NAC on β-thalassemia patients remains unexplored.

4.2 | Iron induced oxidative stress

The production of oxidants during the redox cycling of Fe(II)/Fe is a well-established mechanism for the pathological effects observed in cases of iron overload. Iron acts as a catalyst in the generation of ROS, specifically the hydroxyl radical (•OH) functioning as a Fenton reagent.[42] Additionally, hemichrome, a group of denatured ferric proteins ranging from methemoglobin to the full separation of heme from globin, also serves as Fenton reagents.[43] The generation of such radicals as hydroxyl radicals by iron can be particularly harmful, as they are able to cause damage in close proximity to lipid and protein components of the cell membrane because they are somewhat isolated from the cellular antioxidant capacity. The harm inflicted on the membranes by hydroxyl radicals is directly associated with an iron-containing Fenton reagent located within the membrane.[44]

In cases of β-thalassemia major, excessive release of catabolic iron, known as a Fenton reagent, can overwhelm the iron-binding capacity of plasma transferrin. This process generates redox-active forms that have the potential to cause tissue iron accumulation, leading to damage to vital organs, for instance, the heart, liver, and endocrine glands. Individuals with β-thalassemia not only experience iron overload due to hemolysis and red cell transfusions but also surprisingly demonstrate heightened iron absorption from the gastrointestinal tract.[45]

Hepcidin is a key regulator of iron absorption, playing a critical role in inhibiting the uptake of iron in the duodenum, the release of iron from macrophages in the reticuloendothelial system, and the transport of iron across the placenta.[46] Hepcidin interacts with ferroprotein, also known as Iregl, which is an iron-exporting protein present on the surface of absorptive enterocytes, macrophages, hepatocytes, and placental cells. This interaction yields a reduction in the export of cellular iron from these cells, including liver cells.[47] The increased production of RBCs following hemolysis ends up with a greater demand for iron, necessitating an increase in iron absorption from the gastrointestinal tract. Dysregulation of hepcidin expression influenced by ineffective erythropoiesis contributes to the development of iron overload in β-thalassemia patients.[48] Research has also shown a correlation between hepcidin expression and the degree of transferrin saturation in these individuals.[49]

The iron forms found in the bloodstream, which are weakly bound to plasma transferrin, are known as NTBI.[50] NTBI is detected when there is a compromise in transferrin's ability to take in iron from the gastrointestinal tract or reticuloendothelial cells. In patients receiving frequent red cell transfusions, a rise in both NTBI and labile plasma iron pool is mainly observed as the capacity of transferrin to bind iron is exceeded.[10,51] The transport of NTBI through the cell membrane appears to be irregular and pathologically relevant in these circumstances. This specific fraction, known as LPI, refers to iron forms capable of penetrating cells, displaying redox activity, and being susceptible to chelation.[52] The destruction of cells associated with iron overload is thought to result from the excessive presence of LPI within cells, which promotes the generation of ROS that overwhelms the cellular defense mechanisms.[53] A link has been established between the presence of NTBI and the oxidative damage of lipid membranes, as indicated by the correlation between NTBI concentrations and increased levels of malondialdehyde.[54]

The oral consumption of vitamin E, a lipid-soluble antioxidant, may result in the normalization of elevated ROS levels and demonstrated improvements in the balance between oxidants and antioxidants in the circulation.[16] It is probable that vitamin E alone may not be adequate to significantly impact the intensity of RBC lysis to extend their lifespan and increase hemoglobin levels. One potential strategy to decrease iron absorption could involve pharmacologically regulating hepcidin expression, employing gene therapy, or through administration. A combination therapy incorporating antioxidants, such as NAC for proteins and vitamin E for lipids, along with iron chelators, may mitigate the detrimental effects of ROS. NAC, another antioxidant-targeting protein, has demonstrated improvements in specific parameters affected by oxidative damage.[15] Evaluation through the measurement of LPI could assess its effectiveness.[55]

Histomorphometry of bone in children and teenagers with thalassemia has shown a typical trabecular bone volume, but it also indicates iron-related focal osteomalacia, marked by higher osteoid thickness, reduced mineralization, and localized iron build-up.[56]

Studies in adults with thalassemia have found notable iron buildup at the mineral front through histomorphometric analysis.[57] An increasing body of research indicates the potential of pharmacological treatments targeting the root causes of bone complications linked to excess iron in the body. These treatments encompass iron-modifying agents, antioxidants, sex hormone therapy, and mammalian target of rapamycin (mTOR) inhibitors. It is worth noting that antioxidants such as NAC, resveratrol, and melatonin have shown positive effects on the function of osteoblasts. NAC is recognized for its robust antioxidant and reducing properties.[58] NAC treatment may enhance the expression of Forkhead box protein O1 (FOXO1), which in turn reduces apoptosis.[59] Furthermore, antioxidants like NAC have demonstrated significant benefits in bone remodeling, as evidenced by in vivo studies. In models of iron overload, NAC was found to decrease bone resorption while simultaneously promoting bone formation, resulting in improved trabecular and cortical bone parameters.[59–61] These beneficial effects are attributed to a reduction in oxidative stress, without affecting serum ferritin levels[61] or tissue iron deposition.[60] In previous studies, there have been inconsistent results, possibly on account of diverse measurement methods, such as different NAC treatment models.[59,62–65] These findings indicate that NAC may have the potential to halt bone loss from iron overload.

4.3 | The mechanism and rationale of NAC therapy

ROS can trigger lipid oxidation that navigates the release of a wide variety of reactive carbonyls. These carbonyls are capable of spreading throughout the cells and causing damage to various cellular components. The most noxious byproducts of lipid oxidation are α and β-unsaturated aldehydes, specifically acrolein, crotonaldehyde, and 4-hydroxy-2-nonenal (4HNE). The toxicity of these carbonyl compounds stems from their exceptional reactivity with protein-SH groups, achieved through Michael addition reactions at the β-carbon of the double bond (R-CH═CH—CHO). The carbonyl groups possess a strong electron-shifting capacity, increasing the electrophilicity of the β-carbon and making it highly susceptible to Michael addition. Enzymatic mechanisms for eliminating α,β-unsaturated aldehydes are notably less efficient compared to ROS.[8] This leads to higher cellular concentrations of these aldehydes than ROS. For example, 4HNE is found in the low micromolar range, while H_2O_2 is in the low nanomolar range, and $O_2^{\bullet-}$ is in the picomolar range.[66] The sulfhydryl (SH) group in NAC rapidly creates Michael adducts with α,β-unsaturated aldehydes, inhibiting their binding to proteins and preventing their harmful effects.[67,68]

The chemoprotective potential of NAC has been well-documented across various disease models, xenobiotics, and conditions that trigger cellular stress. NAC's effectiveness originates from multiple mechanisms, although its direct role in neutralizing primary ROS like H_2O_2 and $O_2^{\bullet-}$ is minimal, as these actions are predominantly governed by highly efficient enzymatic processes.[27,66] The reaction rates between NAC and the primary ROS are typically slow, particularly for the superoxide radical. The effectiveness of thiols in neutralizing oxidants is closely tied to the pKa values of the sulfhydryl (SH) groups. It is evident that the reaction rates with most ROS (H_2O_2, $^{\bullet}NO_2$, $O_2^{\bullet-}$, $CO_3^{\bullet-}$, ONOO-, HOCL) are higher for the thiolate anion (RS−) compared to RSH. Due to NAC having a significantly higher pKa value of 9.5 compared to Cys (8.3) or GSH (8.7), the concentration of the active RS− form at physiological pH is notably lower for NAC. At a pH level of 7.4, the RS− form of NAC is only present in 0.8%, while Cys is observed at 12.6%. However, the higher pKa value of the SH group in NAC gives it an advantage in effectively neutralizing the highly hazardous hydroxyl radical ($^{\bullet}OH$) through hydrogen abstraction.[69]

The protective properties of NAC are enhanced by binding harmful substances to its highly reactive SH group. This extends to a wide range of other reactive electrophilic species, such as quinones and epoxides.[66] The process of neutralizing highly reactive electrophilic species, especially potent Michael acceptors, serves as a crucial defense mechanism with significant potential across various applications. Additionally, an excess of electrophiles capable of interacting with sulfhydryl groups and subsequently hindering the function of antioxidant enzymes can result in elevated levels of ROS.[8] In certain cases, NAC administration effectively inhibits ROS generation by addressing the root cause.[70] Despite its limited direct reaction with ROS, NAC demonstrates significant antioxidant activity, attributed to a newly proposed mechanism involving the generation of hydropersulfides (R-S-SH).[69] These persulfides, known for their heightened reactivity towards oxidants and electrophiles, are formed from NAC through deacetylation to produce Cys, followed by subsequent enzymatic reactions.[27]

Thiol groups are highly susceptible to oxidation in laboratory settings, and their interaction with oxidants is energetically favorable.[71] As time has passed, it has become increasingly clear and widely recognized that thiol oxidation encounters significant kinetic barriers, requiring specific catalytic pathways for efficient thiol-based oxidant scavenging within the cellular environment.[72,73] For instance, the rate of H_2O_2 reduction by NAC is 104–108 times slower compared to the reduction of H_2O_2 by thiol peroxidases.[74] Hypochlorite, an oxidizing agent produced by activated phagocytes, shows a higher level of reactivity toward thiols compared to peroxides or superoxide. However, when it comes to thiol reactivity, NAC does not surpass endogenous low-molecular-weight thiols like Cys, GSH, or methionine. Specifically, NAC exhibits a 12-fold lower reactivity than Cys, a fourfold lower reactivity than GSH, and similar effectiveness to methionine.[75]

It is unlikely that under normal circumstances, intracellular NAC concentrations would exceed the endogenous levels of GSH, Cys, and/or methionine. In human RBC, the highest reported intracellular NAC concentration following intravenous administration was 200 μM. This concentration is notably higher than the intracellular levels of Cys (approximately 15 μM) and methionine (around 25 μM) yet considerably lower than the intracellular concentration of GSH (approximately 1500 μM).[76–78] It is also unlikely that the observed cytoprotective effects of NAC are a direct result of its interaction

with ROS. The idea of NAC acting as a direct scavenger of ROS goes against chemical kinetics and lacks substantial support in the literature. While NAC demonstrates antioxidative effects within cells, it is more likely that this occurs indirectly, possibly through conversion into more reactive species or by supporting enzymatic scavenging mechanisms.[79]

NAC demonstrates superior disulfide-reducing capabilities compared to Cys and GSH due to its enhanced nucleophilicity. Replenishing GSH is particularly critical when excessive oxidants lead to a significant decline in cellular GSH levels, which typically range from 3 to 10 mM. The negative feedback mechanism governing GSH biosynthesis makes it challenging to raise GSH levels above their normal range using NAC. However, it is conceivable that restoring pathologically low GSH levels to a normal state could yield beneficial outcomes beyond safeguarding against electrophiles and xenobiotics. GSH serves as a cofactor for glutaredoxins, participating in the enzymatic reduction of disulfides,[80] and for GPx, engaging in the enzymatic reduction of peroxides, such as lipid peroxides.[81] Several studies have shown that a decrease in GSH levels corresponds to increased levels of malondialdehyde, a byproduct of lipid peroxidation. NAC has been found to be effective in reducing these levels.[82–84]

Furthermore, the overall concentration of GSH affects its redox potential. Restoring normal levels of GSH could enhance enzymatic activities involved in disulfide reduction and oxidant scavenging, which are typically associated with NAC.[85] Some studies have suggested that NAC has a beneficial impact on cells through pathways unrelated to GSH production. However, none of these investigations have fully elucidated the exact cytoprotective mechanism of NAC treatment. There has been some evidence that NAC functions as an oxidant scavenger,[86] while others have contested this idea.[87] An unexplored mechanism that is responsible for its GSH-independent protective properties exists.

4.4 | Limitations

The conclusion may be touched by the inadequacy of the included studies, as assessing the NAC's antioxidant characteristics among β–thalassemia cases is less studied. Besides, our study was confined to the Scopus, PubMed, Web of Science, Trip, and CENTRAL databases, potentially impacting the inclusivity of our findings. The scope of the current study was limited to the β-thalassemia population, excluding evaluation of other hemoglobinopathies. Current work lacked statistical approaches, such as meta-analysis, sensitivity analysis, and quality analysis. Likewise, only two studies addressed the side effects in TDT patients receiving NAC monotherapy. As a result, the current evidence needs to be evaluated more to establish the safety of NAC monotherapy in these patients. Given the high risk of bias in most studies and the limited number of included studies, caution is advised when interpreting the findings, thereby reducing the generalizability of our study.

4.5 | Perspectives

Incorporating large sample sizes and well-conducted studies that administrate treatment for extended periods offers a more precise assessment of the effectiveness and adverse effects of NAC monotherapy. When the study follows a double-blind, placebo-controlled crossover design, the evaluation of patients yields more valuable clinical insights. Besides, it is essential to consider NAC monotherapy as a potential treatment option for defining its role for β-thalassemia patients. Exploring NAC's antioxidant traits according to types of iron chelators is also encouraged in upcoming clinical trials. Controlling such potential confounders as the intensity of iron excess and systemic inflammation, as well as using alcohol and cigarette smoking, will allow future studies to determine the net antioxidant effects of NAC on oxidative status in this population.

5 | CONCLUSION

Using NAC may be sufficient in alleviating oxidative stress in individuals with β-thalassemia major. Three-month duration appears to be adequate for demonstrating the antioxidant properties of NAC in this population. However, it is essential to promote larger, well-designed clinical trials before establishing a meta-analysis on oxidative stress biomarkers in the future. Individuals with diagnosed β-thalassemia may consider NAC supplementation to help reduce oxidative indicators, particularly TOS, TAC, and OSI biomarkers, although current clinical evidence possesses a high risk of bias.

AUTHOR CONTRIBUTIONS

Mobin Ghazaiean, Hadi Darvishi-Khezri, Aily Aliasgharian, and Mohammad Mohsen Ghasemi performed searching the database, data extraction phase, and preparing the manuscript draft. Mobin Ghazaiean, Hadi Darvishi-Khezri, Aily Aliasgharian, Hossein Karami, and Mohammad Mohsen Ghasemi were responsible for critically appraising and reviewing the manuscript. Mobin Ghazaiean and Hadi Darvishi-Khezri edited the final manuscript. All authors contributed to the interpretation of data and critical revision of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article (and/or) its Supporting Information.

TRANSPARENCY STATEMENT

The lead author Hadi Darvishi-Khezri affirms that this manuscript is an honest, accurate, and transparent account of the study being

reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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