Use of antioxidants supplementation on developmental outcomes in children with Down Syndrome - a systematic review and meta-analyses

Sidra Kaleem Jafri (✉ sidra.kaleem@aku.edu)

Aga Khan University <https://orcid.org/0000-0002-3104-6891>

Atiya Iqbal

McMaster University

Anusha Alam

Aga Khan University Hospital

Julia Frei

McMaster University

Zohra Lassi

The University of Adelaide Robinson Institute: The University of Adelaide Robinson Research Institute

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Abstract

AIM To evaluate the published literature on the use of antioxidants in improving developmental outcomes in DS children **METHODS** The systematic review included interventional studies (randomized controlled trials (RCTs) and quasi-RCTs (q-RCTs)) on children between the ages of 0 to 18 years diagnosed with DS and used antioxidants for improving developmental outcomes, and compared with similar children given placebo or no supplementation. Aim was to have a true randomized population for intervention, thus RCTs and quasi-RCTs were included. Studies were excluded if those were non-randomized controlled studies (including observational studies, systematic and narrative review articles, editorials and commentaries) with no random allocation and no comparison group. We also excluded studies if data from participants with DS was included as part of a larger group of participants (e.g. children with intellectual disability) and could not be separated for subgroup analysis, and studies that did not assess developmental domains and only assessed biochemical markers. The review included children with other conditions associated with developmental disability, only when data for children with DS were separately reported. **RESULTS** Eleven RCTs and q-RCTs fulfilled the eligibility criteria with 683 participants with Down syndrome, with age range of newborns to 17 years of age. Eight studies did not show any statistically significant clinical benefit of using antioxidants. A meta-analysis was applied to compare changes in the effect sizes (post and pre-intervention) in GDQ between the intervention and control groups. The forest plot including the three studies doesn't demonstrate a statistically significant improvement. **INTERPRETATION** This review concludes that the use of antioxidants does not improve the developmental outcomes in pediatric population with DS.

Background

Down syndrome (DS) is the most common genetic cause of intellectual disability worldwide. Every year, about 6,000 babies are born with DS in the United States, which is about one in every 700 babies born. (1) The neurocognitive phenotype of an individual with DS is characterized by the global involvement encompassing motor, language, cognitive, self-care, and personal-social dimensions; (2) however, there is a disproportionate impairment in certain cognitive domains such as language and memory in comparison to other intellectual disabilities. Research studies have shown that cognitive dysfunction is due to the differences in the functional neural connectivity even *in utero* secondary to the Trisomy 21 affecting the embryogenesis and organogenesis. (3) Additionally, brains of individuals with DS also undergo premature aging with early onset Alzheimer's disease. (4)

Beside the distinct neurocognitive phenotype, differences in the metabolic profiles have been observed in individuals with DS. (5–7) Additionally, the involvement of reactive oxygen species (ROS) has been observed in some of the DS pathologies. The gene coding for Cu/Zn-superoxide dismutase (also known as SODL) is found on chromosome 21 in humans. All individuals with DS have a partial or complete extra copy of chromosome 21. It has been proposed that increased gene expression of SODL may contribute to the premature aging and/or intellectual disability seen in DS. (8) Overproduction of Beta Amyloid Precursor protein and subsequent increased cellular oxidation, also causes neuronal degeneration. (9)

Oxidative stress has been implicated as the neuropathological mechanism and several markers demonstrating free radical damage have been identified through *in vitro* studies. (10) Mitochondrial dysfunctions have also been studied in correlation with the pathogenesis of DS and there is evidence to show increased oxidative stress in DS cells. Hence it is likely that an extra demand of antioxidants is required in these individuals. Thus, the proposed therapeutic interventions have focused on agents which can mitigate the production of ROS and may improve the cognition in DS patients. (11–14)

There has been increasing advocacy by parents who were seeking the use of different non-conventional therapeutic agents such as antioxidants to improve the development in children with DS. Simultaneously, there has been a growing body of pharmaceutical intervention focused research in the past few years aiming at the impact of cognitive enhancers targeting specific brain networks. Many of these supplements are available over-the-counter without a prescription. This not only has financial implications for the parents as well as potential adverse effects for the children. Subsequently, there is growing literature on their use; however, there has been heavy reliance on anecdotes and low-quality evidence. For health care professionals, putting efforts to practice evidence-based medicine, this presents as a challenge. Therefore, the goal of this systematic review is to evaluate the use of antioxidants in improving developmental outcomes in children with DS. The last systematic review carried out in 2002, thus making this review vital. (15)

Method

We have used Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to report this systematic review (Supplementary File 1). (16) The title has been registered with PROSPERO: CRD42020206011.

The systematic review included interventional studies (randomized controlled trials (RCTs) and quasi-RCTs (q-RCTs)) on children between the ages of 0 to 18 years diagnosed with DS and used antioxidants for improving developmental outcomes, and compared with similar children given placebo or no supplementation. Aim was to have a true randomized population for intervention, thus RCTs and quasi-RCTs were included. Studies were excluded if those were non-randomized controlled studies (including observational studies, systematic and narrative review articles, editorials and commentaries) with no random allocation and no comparison group. We also excluded studies if data from participants with DS was included as part of a larger group of participants (e.g. children with intellectual disability) and could not be separated for subgroup analysis, and studies that did not assess developmental domains and only assessed biochemical markers. The review included children with other conditions associated with developmental disability, only when data for children with DS were separately reported. The developmental outcome of interest for this review are the following as defined by the study author and ascertained by at least one standardized measure of developmental functioning.

- Global developmental age,
- Developmental quotient,
- Intelligence quotient,
- Motor proficiency,
- Visuo-motor integration,
- Receptive and expressive language,
- Articulation proficiency,
- Tests of learning aptitude,
- Test of psycholinguistic abilities,
- Behavioural rating scale, and
- Academic achievement assessment

Electronic databases such as OVID Medline (1946 to August 2020), EMBase (1974 to August 2020), Psycinfo (1806 to August 2020), PubMed, (1966 to August 2020), CINAHL (Nursing Citation Index) (1982 to August 2020), the Cochrane Central Register of Controlled Trials (1996–August 2020), WHO International Clinical Trials Registry Platform ICTRP (2005 to August 2020) and Clinical trials.gov were searched for potential studies using the search strategy prepared in consultation with senior librarian from McMaster University (JP). Search strategy comprehensively covered three main concepts along with synonyms for each i.e. Down Syndrome, children, and antioxidants (Supplementary File 2). The last date of search was 21st August, 2020 and the searches were limited to papers published in English language. Mendeley bibliographical software was employed to download the search results and remove the duplicates. Bibliographies of included studies were also manually searched to identify any eligible study.

Two reviewers (SK and AI) independently performed the title/abstract and full-texts screening using pre-set inclusion and exclusion criteria; and a third independent reviewer (JF) assessed articles where there was a disagreement. Two reviewers (SK and AI) independently performed data extraction, and methodological quality assessment of all included studies. The data extraction form retrieved the following information from all included studies:

Study characteristics: Study design, objectives, outcome, intention to treat analysis, examination of generalization. Participant characteristics: source of participants, the number of participants with DS (randomized at the start, at the end, analyzed), age, gender, and level of intellectual functioning. Intervention characteristics: including type of anti-oxidant used, dose of anti-oxidant, duration, frequency per week, outcomes measured (developmental domain function), statistical analyses, adverse events, period to assessments, duration of follow-up, number lost on follow-up, outcome scale used and the type of outcome assessors. Outcome characteristics: benefit observed, and not observed (Supplementary File 3).

The methodological quality of included studies was assessed using the Cochrane's Risk of Bias (ROB) assessment tool (17). We used Review Manager 5.4 to meta-analyze the outcomes (18). We have reported standard mean difference (SMD) with 95% confidence intervals (CI) for the continuous outcomes where the units of measurement used were different. For the categorical outcomes, we planned to report risk ratio (RR) with 95% CI; however, we could not pool any categorical outcomes.

For the measure of heterogeneity, we have used I^2 test and P value of χ^2 test. We considered low heterogeneity when values of I^2 were less than 25% and P value of χ^2 was < 0.1 . For clinically homogenous measures, we have used fixed-effects model. We planned to assess the publication bias when the outcomes have 10 or more studies. We also planned on performing secondary analyses comparing the outcome

in studies where different types of antioxidants were used; however, we could not perform sub-group analysis because of limited number of studies in an outcome.

Results

The electronic database search yielded 1611 results, of which 534 references were reviewed. A total of 12 articles were included for full text review as the remaining 522 looked at different aspects of DS. Eleven studies fulfilled the eligibility criteria (20-30) and one study was excluded as it demonstrated the difference in the biochemical markers rather than reporting the developmental outcome. (19) One study had mixed population with five children with DS (20). Study characteristics of the included studies are presented in Tables 1 and 2.

Study characteristics: Six studies were carried out in North America (20-25,30) while two each in France (21-22) , and the UK (23,24). Majority of the studies (8/11) were conducted in the 1980s (20,24-30), and only three trials were conducted after the year 2000 (21-23). Most of the studies from the 1980s were q-RCTs (7/11), (19-24,28,29) while remaining were RCTs (21-23). A total of 683 participants with a male preponderance (male to female ratio of 1.2:1) were present. The age range of included participants was between newborns to 17 years.

Intervention: Intervention type, dosage, and mode of administration is outlined in Table 1. The most common intervention used was Harrel's formula (A compound comprising of Vitamin A palmitate 15,000 IU (reduced to 5000 U for months 5 through 8), Vitamin D (cholecalciferol) 300 IU, Thiamine mononitrate 300 mg, Riboflavin 200 mg, Niacinamide 750 mg, Calcium pantothenate 490 mg, Pyridoxine hydrochloride 350 mg, Cobalamin 1,000 µg, Folic acid 400 µg, Vitamin C (ascorbic acid) 1,500 mg, Vitamin E 600 IU, Magnesium (oxide) 300 mg, Calcium (carbonate) 400 mg, Zinc (oxide) 30 mg, Manganese (gluconate) 3 mg, Copper (gluconate) 1.75 mg, Iron (ferrous fumarate) 7.5 mg, Calcium phosphate 37.5 mg, Potassium Iodide 0.15 mg) which was used in three studies (20,25,26), while three studies used Folinic acid (Calcium leucovorin) (21-23). The remaining studies employed pyridoxine (27), other compounding of antioxidants (24,28), and other multivitamins (29-30). All studies compared the intervention with the placebo. (20-30)

Quality assessment of included studies: The overall quality of included studies and the ROB was variable with qualitative deficits (**Figure 2 and 3**). The reviewers observed that the three studies with low risk of bias (21,22,23) were carried out in 2008 and 2010s, with up to date methods, with the exception of the study by Lonsdale et al. showing low risk of bias and carried out in 1986(29). However, the majority of the studies in the review demonstrate high risk of bias. (19) Key concerns for ROB included potential selection bias in the form of lack of random sequence generation (n=5) (19,24-27), allocation concealment (n=5) (19,23,24,28,29), blinding of outcome assessors (n=5) (20,22,24,25,26), and selective reporting (n=2) (19-20).

Effect of intervention: In terms of outcomes reported and their measurement scales, study design, source population, participant's age, and anti-oxidants used, quantitative analyses could not be performed as the trials were heterogeneous. Twelve different domains have been assessed and fourteen outcome measures were used (Table 2 matrix) with the outcomes summarized in the Table 3. Global Developmental Quotient (GDQ) using Griffith's Mental Development Scale was the most common outcome measure employed in three studies (21-23). Additionally, a high proportion of studies were assessed to have a high risk of bias (7/11), thus the meta-analysis was carried out using these three studies.

A meta-analysis was applied to compare changes in the effect sizes (post and pre-intervention) between the intervention and control groups. The forest plot including the 3 studies (Figure 4) does not demonstrate a statistically significant improvement. Of the 11 included studies, eight studies did not show any statistically significant clinical benefit of using antioxidants (22-25,27-29). Harrel et al. showed improvement in 3/5 children with DS, however, this study had a mixed population of children with intellectual impairment (19). The second study by Blehaut et al. in the intention to treat analysis did not show any increased benefit of using leucovorin versus placebo, however, on the as per protocol analysis, there was demonstration of improvement in the developmental age (leucovorin=53.1% and placebo=44.4%, p value= 0.031) (20). The third study was by Lonsdale et al. where 5/22 showed increased IQ with the use of Thiamine Tetra hydrofurfuryl Disulfide (28).

Intelligence quotient (IQ) was the outcome used in three studies employing Stanford Binet (20,25-26), while two studies used Wechsler Intelligence Scale for Children (WISC) (28,29). Other outcomes include Language skills (23,26,28,30) Visuomotor integration (25,28) Social Maturity (27,30) articulation proficiency (26), academic achievement (26), learning aptitude (26), behavior rating (26), psycholinguistic skills (26). To report language skills, three different tools were used, such that MacArthur communicative development inventory (M-CDI) has been used by Ellis et al. (23), Peabody Picture Vocabulary Test (PPVT) by Smith and Bennet et al. (26,28), Receptive Expressive Emergent Language Scale (REELS) by Pueschel et al (30). The difference between the intervention and control group was not statistically significant for any of these developmental outcomes. (23-28,30)

Adverse events were observed in nine patients which include nocturnal enuresis (25), gastrointestinal symptoms (20,22-24), skin manifestations (23-24), infantile spasms (21,26), and sudden death (21).

Discussion

For the past several decades, there has been an ongoing search for an ideal supplement which can dramatically improve the development in children with DS and ultimately improve the long-term outcomes. In this current systematic review, we have synthesized evidence for a common caregiver question about the use of antioxidants in improving the development of children with DS. Since varying patterns of oxidative stress have been observed in individuals with DS and association being seen with early aging with plausible linkages to the neurocognitive phenotype, efforts have been made to develop strategies that could mitigate it. Literature is inundated with both pharmacological and non-pharmacological interventions.

Early institution of the antioxidants has been shown to attenuate the systemic oxidative damage, a lack of antioxidant efficacy in terms of little or no benefit in the developmental outcomes has been observed in clinical trials included in this systematic review. Thus, this review provides evidence that the supplementation of antioxidants does not improve the neurocognitive outcome. It is a compilation of randomized or qRCTs over a period of almost 40 years. There could be questions regarding the heterogeneous nature of the antioxidant combinations and outcomes assessed, and the high risk of bias in the initial studies which were carried out in the 1980s (19–24,28–29); however, trials in 2000s with low ROB (25–27) have demonstrated similar findings. An earlier review from 2002 that included other pharmacological agents showed comparable results. (15) The findings of this review can be generalized to the pediatric population with DS as it covers the entire age range and includes children from three countries.

Limitations of this systematic review include: firstly, being restricted to literature being published in English. Additionally, despite using a carefully developed search strategy, potentially beneficial research and unpublished or grey literature could have been missed. Secondly, the number of studies included was limited with quality varying from studies with low risk of bias to high risk. Thirdly, development is an all-encompassing concept and different developmental domains/standardized tools have been employed. Thus, the studies included were heterogeneous, making comparisons difficult. Ultimately, of 11 studies, only three were included for meta-analysis. No publication bias was investigated as there were only 3 articles in the meta-analysis.

Conclusion

With the better understanding of the developmental profiles of children with DS and the potential role of oxidative stress mechanisms in the cognitive dysfunction, multiple anti-oxidant substances have been studied with no proven success. The evidence from our systematic review which considers 4 decades of experimental studies using antioxidants, does not suggest that the use of antioxidants improves the developmental outcomes in pediatric population with DS. Therefore, the exploration of other intervention modalities (pharmacological and non-pharmacological) is the way forward along with using strategies already known to make a positive impact, that is a collaborative life centered approach using education, and community-wide advocacy based interventions.

Declarations

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COMPETING INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

All authors have consented for publication

AVAILABILITY OF SUPPORTING DATA

Supplementary files

AUTHORS' CONTRIBUTIONS

SKJà conception, data collection, manuscript writing, the research at all points, and reviewed the manuscript.

AA, AI, JF, ZLà were involved in the data collection, analysis, manuscript writing and final review.

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Tables

Table 1: Characteristics of the included studies: Description of the Participants

Study, Source of population and country	Study design & duration	Sample size	Age and Gender	Intervention (Dosage/Frequency per week/Mode of administration)	Baseline level of developmental functioning
Bennet 1983 Seattle, Washington, USA	Quasi Randomized Controlled Trial 8 months	Total: 20 Intervention: 10 Control: 10	Age range: 60 – 156 months Male/Female: 10/10	Genetotropic Concept Formula used by Harrel et. al.¥ (Daily/Orally)	Mean pre-treatment IQ of treatment group (range): 53.1 months (40-76) Mean pre-treatment IQ of control group (range): 45.2 months (26-61)
Bidder 1989 South-east Wales Down's Association, UK	Cross-over Randomized Controlled Trial 7 months	Total: 13 Intervention: 13 Control: 13	Age range: 8 – 63 months Male/Female: 9/15	Bidder compound§ (Daily for 7 months/Orally)	Mean developmental age in Intervention first group: 24 ± 10 months Mean developmental age in Placebo first group: 19 ± 6 months
Blehaut 2010 Institut Jérôme Lejeune in Paris, France	Randomized Controlled Trial 12 months	Total: 113 Intervention (Folinic Acid): 43 Control: 44	Age range: 3 – 30 months Male/Female:60/53	Pentahydrated calcium salt of leucovorin (5 mg/Daily for 12 months/Orally)	Developmental Age of Intervention group: 63.4 ± 13.0 months (29.1-91.3) Developmental Age of Placebo group: 63.7 ± 13.8 months (29.5-97.8)
Coleman 1985 Georgetown University, Washington, USA	Quasi Randomized Controlled Trial 36 months	Total: 19 Intervention: 10 Control: 9	Age: <2 months Male/Female: 11/8	Pyridoxine (25 mg/kg for the first 6 months and 35 mg/kg thereafter/Daily for 3 years/ Orally)	Not mentioned
Ellis 2008 Midlands, Greater London, and the south west of England, UK	Randomized Controlled Trial 22 months	Total: 156 Antioxidants and Folinic Acid: 37 Antioxidants only: 40 Folinic Acid only: 36 Control: 33	Mean corrected age 4.15 months Male/Female: 89/67	4 intervention groups 1. Antioxidants: Selenium 10 µg, Zinc 5 mg, Vitamin A 0.9 mg, Vitamin E 100 mg, Vitamin C 50 mg 2. Folinic Acid (0.1 mg) 3. A combination of the same doses of antioxidants and Folinic Acid 4. A placebo (Mannitol, Maltodextrin, and a natural food colour) 100% of the recommended daily allowance for Vitamin E, Zinc, and Selenium and 200% of the recommended daily allowance for Vitamin C and Folinic acid. The dosage was increased by 30% after the child's first birthday	Not mentioned
Harrel 1981 Near Norfolk, Virginia, USA	Two phased Quasi Randomized Controlled Trial 8 months	Group I received supplements during both 4-month periods. Group II received placebos the first months and supplements during the second phase. Total with Down Syndrome: 5 Intervention: 5 Control: 10	Age range: 60 to 120 months Male/Female: 2/3	Harrel formula¥ (Daily for 8 months/Orally)	Mean + standard error of the mean IQ Group 1: 46.3 ± 3.6 months Group 2: 48.5 + 5.2 months
Lonsdale 1986 USA	Quasi Randomized Controlled Trial 12 months	Total: 22 Intervention: 11 Control: 11	Age range: 96 – 192 months Male/Female: 11/11	Thiamine Tetrahydrofurfuryl Disulfide (Daily for 12 months/Orally)	Not mentioned

Study, Source of population and country	Study design & duration	Sample size	Age and Gender	Intervention (Dosage/Frequency per week/Mode of administration)	Baseline level of developmental functioning
Mircher 2019 Institut Jérôme Lejeune in Paris, France	Randomized Controlled Trial 56 months	Total: 143 Folinic acid: 30 L-Thyroxine: 35 Folinic acid + L-Thyroxine: 38 Control: 40	Age range: 6–18 months Male/Female: 89/65	Four intervention groups 1. Folic Acid placebo and L-Thyroxine placebo 2. Folic Acid placebo and L-Thyroxine 3. Folic Acid and L-Thyroxine placebo 4. Folic Acid and L-Thyroxine Folinic acid (Folinoral®, Therabel) 1 mg/kg/day and L-Thyroxine (Lévothyrox®, Merck) 3 µg/kg/day (or matched placebo)	Global Developmental Quotient at baseline (mean ± standard deviation) Folinic acid: 56.6 ± 8.4 months L-Thyroxine: 53.9 ± 9.8 months Folinic acid + L-Thyroxine: 55.4 ± 9.0 months Placebo: 56.6 ± 10.7 months
Pueschel 1980 Greater Boston area, other parts of Massachusetts, southern New Hampshire and Vermont, northern Connecticut, and Rhode Island, USA	Quasi Randomized Controlled Trial 36 months	Total: 89 Pyridoxine: 23 5-Hydroxytryptophan: 22 5-Hydroxytryptophan Pyridoxine: 24 Control: 20	Newborns Male/Female: 50/39	Hydroxytryptophan and Pyridoxine Hydrochloride Divided doses of 1 to 2 mg/kg/day of 5-Hydroxytryptophan and 5 to 10 mg/kg/day of Pyridoxine Hydrochloride. The initial dosage was low (1 mg/kg/day of 5-Hydroxytryptophan and 5 mg/kg/day of Pyridoxine Hydrochloride). If no side effects were observed, the dosage was gradually increased to its maximum (2 mg/kg/day of 5-Hydroxytryptophan and 10 mg/kg/day of Pyridoxine Hydrochloride). (Daily for 36 months/Orally)	No baseline developmental functioning as criteria for children to partake in study
Smith 1984, USA	Quasi Randomized Controlled Trial 8 months	Total: 56 Intervention: 28 Control: 28	Age range: 84 to 180 months Gender: Not mentioned	12 tablets were orally administered daily throughout the two 4-month period. A daily dose of supplements consisted of Vitamin A palmitate 15,000 IU (reduced to 5000 U for months 5 through 8), Vitamin D (cholecalciferol) 300 IU, Thiamin mononitrate 300 mg, Riboflavin 200 mg, Niacinamide 750 mg, Calcium pantothenate 450 mg, Pyridoxine hydrochloride 350 mg, Cobalamin 1,000 µg, Folic acid 400 µg, Vitamin C (ascorbic acid) 1,500 mg, Vitamin E 600 IU, Magnesium (oxide) 300 mg, Calcium (carbonate) 400 mg, Zinc (oxide) 30 mg, Manganese (gluconate) 3 mg, Copper (gluconate) 1.75 mg, Iron (ferrous fumarate) 7.5 mg, Calcium phosphate 37.5 mg, Potassium Iodide 0.15 mg and Biotin 300 µg.	Developmental age of Intervention group: 54 months ± 20 months Developmental age of Placebo group: 52 months ± 17 months
Weathers 1982 Atlanta Down Syndrome Parents Association, USA	Quasi Randomized Controlled Trial 1.5 months	Total: 47 Intervention: 24 Control: 23	Age range: 6 – 17 years Male/Female: 31/16	Genetotropic Concept Formula used by Harrel et al. ¶	Mean pre-treatment IQ of treatment group (range): 45.6 (30-67) months Mean pre-treatment IQ of control group (range): 45.4 (30-57) months
<p>¶Harrel's Genetotropic Concept Formula: Vitamin A palmitate 15,000 IU (reduced to 5000 U for months 5 through 8), Vitamin D (cholecalciferol) 300 IU, Thiamin mononitrate 300 mg, Riboflavin 200 mg, Niacinamide 750 mg, Calcium pantothenate 490 mg, Pyridoxine hydrochloride 350 mg, Cobalamin 1,000 µg, Folic acid 400 µg, Vitamin C (ascorbic acid) 1,500 mg, Vitamin E 600 IU, Magnesium (oxide) 300 mg, Calcium (carbonate) 400 mg, Zinc (oxide) 30 mg, Manganese (gluconate) 3 mg, Copper (gluconate) 1.75 mg, Iron (ferrous fumarate) 7.5 mg, Calcium phosphate 37.5 mg, Potassium Iodide 0.15 mg</p> <p>§Bidder's Genetotropic Concept Formula: Vitamin A 10,000 IU, Vitamin D (cholecalciferol) 7.5 µg, Thiamine mononitrate 300 mg, Riboflavin 200 mg, Niacin 570 mg, Pantothenic acid 490 mg, Pyridoxine hydrochloride 350mg, Folic acid 400 µg, Vitamin C (ascorbic acid) (mg) 1,500 mg, Magnesium (oxide) 300 mg, Calcium 171 mg, Zinc oxide 30 mg, Copper (gluconate) 1.75 mg, Iron (ferrous fumarate) 7.5 mg, Manganese (gluconate) 3mg, Potassium iodide 0.15 mg, Phosphorus 8.5 mg</p>					

Table 2: Matrix of the developmental outcomes

Study	Developmental age	Developmental quotient	Intelligence quotient	Motor proficiency	Visuo-motor integration	Receptive and expressive language	Articulation proficiency	Tests of learning aptitude	Test of psycholinguistic abilities	Behavioral rating scale	Academic achievement assessment	Socialization
Cher 2019	Revised Brunet-Lezine scale	Griffiths developmental quotient GDQ Brunet-Lezine scale	-	-	-	Griffith's Motor Developmental Scale	-	-	-	-	-	-
2008	-	Griffiths developmental quotient GDQ	-	-	-	MacArthur communicative development inventory	-	-	-	-	-	-
eman 2015	Bayley Scale for Infant Development Stanford Binet test	-	-	-	-	-	-	-	-	-	-	Vineland Social Maturity Scale
nith 2014	-	-	Wechsler Intelligence Scale for Children	-	Beery Visuo-motor integration	Peabody Picture Vocabulary Test	-	-	-	-	-	-
schel 2010	-	Bayleys Scale for Infant Development	-	-	-	Receptive Expressive Emergent Language Scale	-	-	-	-	-	Vineland Social Maturity Scale
sdale 2016	-	-	Wechsler Intelligence Scale for Children	-	-	-	-	-	-	-	-	-
lder 2019	Griffiths developmental Scales	Griffiths developmental Scales	-	-	-	-	-	-	-	-	-	-
haut 2010	Brunet-Lezine scale	-	-	-	-	-	-	-	-	-	-	-
irrel 2011	-	-	Stanford Binet Cattell Infant Scale	-	-	-	-	-	-	-	-	-
ithers 2012	-	-	Stanford-Binet	-	Beery Visuo motor Integration	-	-	-	-	-	-	-
nnet 2013	-	-	Stanford-Binet	-	-	ü	ü	ü	ü	ü	Peabody Individual Achievement Test	-

Table 3: Characteristics of the included studies: Outcomes reported and the adverse effects reported

Study, source of population and country	Outcomes Reported	Adverse Events
Bennet 1983 Seattle, Washington, USA	<ul style="list-style-type: none"> Children given vitamins/minerals demonstrated slightly greater gains in the Oral vocabulary assessment, Arizona Articulation Proficiency and Detroit Tests of Learning Aptitude. Illinois Test of Psycholinguistic Abilities: Children given placebo had slightly greater gains on most receptive and expressive subtests. Behavioural rating scale: Both groups demonstrated small average improvements. Academic achievement assessment: The teachers' subjective impressions revealed no group differences or substantial changes in school performance. Stanford-Binet Intelligence Scale: Nine study children (three given vitamins/minerals, six given placebo) showed a slight increase in intelligence test performance (maximum IQ gain 9 points) at the end of the study; nine children (six given vitamins/minerals, three given placebo) showed a slight decrease in test performance (maximum IQ loss 7 points), and two children (one given vitamins/minerals, one given placebo) were unchanged. 	Nocturnal enuresis in a child given treatment occurred which was managed successfully by administering all tablets in the morning. Behavioral change (i.e., more withdrawn, isolated, apathetic) in one control child who was experiencing home disruption due to the marriage of an older sibling; this was managed by discontinuing the study tablets at 6 months.
Bidder 1989 South-east Wales Down's Association, UK	The estimated mean difference is -3.5 Developmental Quotient points ($t = -2.43$, $p < 0.05$), and the 95 percent confidence interval, -6.7 to -0.3, excludes any beneficial effect of active treatment.	Most parental reports of side-effects related to the active preparation, and included flushing, tightness of the skin and occasional vomiting. Furthermore, its poor palatability led to compliance problems.
Blehaut 2010 Institut Jérôme Lejeune in Paris, France	The intention-to-treat analysis (113 patients) revealed no greater positive effect of leucovorin than of placebo on change in global developmental age (52.1% of normal Developmental Age vs. 51.5%, respectively). However, the per protocol multivariate analysis carried out on the 87 patients examined by the same person at the start and end of the trial showed that leucovorin probably enhanced developmental age. The cofactors most strongly linked to the first axis (and thus global Developmental Age) were leucovorin treatment, age at start of treatment, sex and concomitant thyroxine treatment.	DS patients present many common early childhood diseases, all of which were considered adverse events. Thus, For the 113 patients with safety data, a mean of 3.34 adverse events per patient occurred in the treated group, versus 3.84 in the placebo group ($p = 0.05$). No serious adverse effect or drug related adverse effects were observed.
Coleman 1985 Georgetown University, Washington, USA	Psychological testing at 3 years of age indicated no difference between the two groups in any type of testing	One of the infants developed the infantile spasms syndrome during the study
Ellis 2008 Midlands, Greater London, and the south west of England, UK	Study found no evidence for clinically or statistically significant effects of antioxidants or Folinic acid on any of the outcomes measured (Global Developmental Quotient).	More of the children taking antioxidants than taking Folinic Acid alone or placebo stopped taking supplements (15/74, 20% v 2/65, 3%; relative risk 6.5, 95% confidence interval 1.5 to 27). Only children taking antioxidants stopped supplements because of vomiting or distress (10/74 v 0/65; $P = 0.002$).
Harrel 1981 Near Norfolk, Virginia, USA	Statistically significant increase in IQ observed in children receiving the intervention.	None reported
Lonsdale 1986 USA	This study did not reveal any dramatic response in any of the 22 subjects. Although 5 of them did demonstrate some increase in IQ ratings	None reported
Mircher 2019 Institut Jérôme Lejeune in Paris, France	The effects on Global Developmental Quotient were not statistically significant.	<ul style="list-style-type: none"> 5 patients in the Folic Acid arm (11.6%) who discontinued treatment due to hypothyroidism 2 patients discontinued following unrelated events of acute myeloid leukemia and infantile spasms 1 patient in the L-thyroxine arm discontinued due to infantile spasms Sudden death was reported in a female patient treated in the combination arm at 18.5 months. She had normal T4 and TSH levels at her last evaluation and at death, and her death was considered not related to treatment
Pueschel 1980 Greater Boston area, other parts of Massachusetts, southern New Hampshire and Vermont, northern Connecticut, and Rhode Island, USA	No clinically or statistically significant effects of intervention.	None reported
Smith 1984, USA	No evidence that these nutritional supplements enhance any of the specific abilities that contribute to overall global intelligence.	None reported
Weathers 1982 Atlanta Down Syndrome Parents Association, USA	<ul style="list-style-type: none"> Stanford-Binet Intelligence Scale: Differences were not significant Visual Motor Integration: Differences were not significant Behaviour changes: Differences not significant Parents reported subjective improvements in alertness and attention span 	<ul style="list-style-type: none"> 1 case of boils in adolescent boy of experimental group 1 child developed elevation of liver enzymes in experimental group

Figures

Figure 1

PRISMA flowchart for study selection

	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)	Other bias
Bennet 1983	●	●	●	●	●	●	●
Bidder, 1989	?	?	●	?	●	?	●
Blehaut, 2010	●	●	●	●	●	●	●
Coleman 1985	●	?	●	●	●	●	●
Ellis, 2008	●	●	●	●	●	●	●
Harrel, 1981	●	●	●	●	●	●	●
Lonsdale, 1986	●	?	●	●	●	●	●
Mircher 2019	●	●	●	?	●	●	●
Pueschel, 1980	●	?	●	?	●	●	●
Smith, 1984	●	●	●	●	●	●	●
Weathers,1982	●	●	●	●	●	●	●

Figure 2

Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Figure 3

Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Figure 4

Forest plot for Global Developmental Quotient (GDQ)

Supplementary Files

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- [Supplementaryfile2searchstrategy.docx](#)
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