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# The Role of Antioxidants in the Treatment of Congenital CMV-Related Hearing: A Case-Control Study

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## Abstract

*Objective.* Antioxidants have been used as a therapeutic measure for several causes of hearing loss, and this study aims to examine the use of antioxidants in children with congenital cytomegalovirus (cCMV)–related hearing loss.

*Study Design.* Case-control study.

*Setting.* Academic pediatric hospital.

*Subjects and Methods.* A retrospective chart review of pediatric patients with cCMV-related hearing loss treated with and without antioxidants (vitamins A, C, and E and magnesium, known as ACE-Mg) was completed. The primary end point was the mean change in hearing thresholds for the right and left ears after therapy. An evaluation of the mean change in thresholds was evaluated at the following frequencies: 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz. A 2-sample *t* test and multiple linear regression were used to evaluate the data.

*Results.* A total of 78 children with cCMV-related hearing loss were included in the study, of whom 10 were treated with antioxidants. The average amount of time in which antioxidants were taken was 387 days. When comparing cases and controls, there was no differences in the mean change of hearing thresholds at each frequency for both the right and left ears ($P > .05$). Length of antioxidant therapy and age at which therapy was initiated had no effect on hearing scores ($P > .05$).

*Conclusions.* Oxidative stress plays a role in the pathogenesis of cCMV-related hearing loss. ACE-Mg is a safe adjuvant therapy for the treatment of hearing loss in children; however, this study demonstrates no hearing-related benefit from ACE-Mg antioxidant therapy.

## Keywords

CMV, congenital hearing loss, sensorineural hearing loss, infection, pediatric otolaryngology

Reactive oxygen species (ROS) have been found to mediate several types of hearing loss, including age or noise related, genetic, ototoxic, or infectious.[1] The pathophysiology of this disorder stems from disruption of normal mitochondrial function, with generation of ROS that slowly impair the mitochondria, which ultimately damages biological molecules (including DNA).[2] Elevated levels of ROS cause subsequent apoptosis of cells through several pathways, including oxidative phosphorylation dysfunction, increased pro-ROS enzyme activity, and decreased anti-ROS activity.[1] Antioxidants have been proposed as an approach to scavenge these ROS, which can protect hair cells from ototoxicity. Hatano et al[3] found that antioxidant treatment with vitamins C and E improved hearing outcomes among patients with idiopathic sensorineural hearing loss, with a statistically significant hearing gain and recovery rate. In the setting of acute inflammatory conditions, like meningitis, antioxidant therapy has been found to be highly otoprotective. A study by Klein et al[4] evaluated the use of antioxidants among rats that were induced with pneumococcal meningitis, where antioxidant use was found to reduce acute and long-term hearing loss. However, the true benefits of antioxidants for hearing loss have been

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**Table 1.** Dose of Antioxidants Administered Daily Based on Age.

| Age | Tri-Vitamin Drops, mL | Vitamin E Soft Gel | Magnesium + Soy Protein Tablet, mL | Liquid Vitamin C Supplement, mL |
|---|---|---|---|---|
| 0-11 months | 0.6 | 0.1 mL | 2.5 | — |
| 1-3 years | 0.6 | ¾ of 200-IU capsule | 2.5 | 1.8 |
| 4-8 years | 1.3 | 200-IU capsule | 5 | 3.5 |
| 9-13 years | 2 | 400-IU capsule | 15 | 5.3 |

—, no dose administered.

inconsistent across studies, with some data suggesting there is no protective effect.[5]

The use of antioxidants among infants and children with congenital cytomegalovirus (cCMV)–induced hearing loss has not been studied. CMV is a leading cause of pediatric hearing loss, accounting for up to 20% of all pediatric hearing loss.[6,7] The unborn fetus acquires the infection either from a mother developing the infection for the first time or from a seropositive mother who develops reactivation or becomes infected from another strain of the virus.[8] The purpose of this study was to determine the effect of antioxidant therapy on hearing in CMV-infected children.

## Methods

A case-control study of pediatric patients with cCMV-related hearing loss was completed. Patients were identified in a database kept by the senior author, who has followed patients infected with cCMV, and multiple outcomes were collected from these patients, who were seen in our pediatric otolaryngology clinics at Primary Children's Hospital from 2010 to 2018. All patients with cCMV were confirmed to have CMV infection through urine or throat-swab specimens within the first 3 weeks of life or dried-blood spot testing if the child was older than 3 weeks. There were both symptomatic and asymptomatic patients with cCMV included in the study, where symptomatic cCMV was defined as having 1 or more of the following conditions: thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth restriction, hepatitis, microcephaly, intracranial calcifications, abnormal cerebrospinal fluid indexes, and chorioretinitis. Asymptomatic patients with CMV had no clinical findings at birth but tested positive for the virus. Both symptomatic and asymptomatic patients had hearing loss associated with their disease at the time of enrollment in the study. However, approximately 40% of patients in both the case and control arms of the study had normal hearing upon enrollment.

A subset of our pediatric cCMV-related hearing loss patients was provided the antioxidant ACE-Mg (vitamins A, C, and E and magnesium) (cases). The doses of antioxidants were stratified based on the age of the patients (**Table 1**). These cases were matched with controls based on the following factors: age, sex, presence of symptomatic disease, having progressive hearing loss, central nervous system (CNS) findings, level of hearing loss, and if they were treated with valganciclovir therapy. A baseline auditory brainstem response (ABR) was performed on both cases and controls prior to the initiation of any therapeutic intervention. Patients were then followed with regular audiologic assessments after any therapeutic intervention was made. The primary end point was the mean change in hearing thresholds after antioxidant therapy was completed. An evaluation of the mean change in hearing was completed at the following thresholds: 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz. Secondary end points included the effect of age and duration of antioxidant therapy on hearing outcomes and differences in clinically significant worsening of hearing between cases and untreated controls. Clinically significant worsening hearing in the best ear was defined as the occurrence of (a) 10-dB or greater increase in minimum response level (MRL) at both 2 and 4 kHz, (b) 15-dB or greater increase at either frequency, or (c) cochlear implantation. Cochlear implantation was used as a separate indication for worsening hearing, as this is a clinically significant indicator of progression. None of these patients included in the study underwent cochlear implantation for any other reason than their CMV disease. A Pearson's $\chi^2$ test and a paired sample $t$ test were used to compare baseline differences between cases and untreated controls. A 2-sample $t$ test was used to evaluate changes in the mean differences before and after therapy, while a multiple linear regression was used to determine the effect of length of therapy and age on our primary outcome of interest. The institutional review board (IRB) at the University of Utah approved our study.

## Results

A total of 78 patients were included in the study, of whom 10 patients were treated with a course of antioxidants and 68 patients were not treated with antioxidants. Among the patients included in the study, 30.8% had symptomatic CMV (n = 24) and 69.2% had asymptomatic CMV (n = 54). The average age of participants was 5.2 years (range, 1-18 years). A total of 39 males and 39 females were included in the study. A significant proportion of participants (44.8%) had CNS manifestations of CMV, with 40% of participants receiving antioxidants had significant CNS disease. CNS disease was defined as having findings on imaging, including ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI). Twelve participants (15.4%) were found to have hearing loss at the time of their initial presentation. Among the participants who received antioxidants, the average duration of therapy was 387 days (range,

**Table 2.** Characteristics of Participants at Baseline.

| Characteristic | cCMV Treated (n = 10) | cCMV Untreated Control (n = 68) | *P* Value |
|---|---|---|---|
| Age, mean (range), y | 4.9 (3-18) | 7.2 (1-18) | >.05 |
| Sex, No. (%) | | | >.05 |
| Male | 3 (30) | 36 (53) | |
| Female | 7 (70) | 32 (47) | |
| Symptomatic CMV, No. (%) | 3 (30.0) | 21 (30.8) | >.05 |
| Asymptomatic CMV, No. (%) | 7 (70) | 47 (69.2) | |
| Progressive hearing loss, No. (%) | | | >.05 |
| Yes | 2 (20) | 12 (17.6) | |
| No | 8 (80) | 56 (82.4) | |
| CMV with CNS findings, No. (%) | | | >.05 |
| Yes | 4 (40) | 29 (42.6) | |
| No | 6 (60) | 39 (57.4) | |
| Level of hearing loss, No. (%) | | | >.05 |
| Normal | 4 (40) | 27 (39.7) | |
| Mild | 0 (0) | 4 (5.8) | |
| Moderate | 1 (10) | 8 (11.8) | |
| Moderately severe | 0 (0) | 2 (3) | |
| Severe | 1 (1) | 3 (4.4) | |
| Profound | 4 (40) | 24 (35.3) | |
| Treatment with valganciclovir, No. (%) | | | >.05 |
| Yes | 6 (60) | 31 (45.6) | |
| No | 4 (40) | 37 (54.4) | |

Abbreviations: cCMV, congenital cytomegalovirus; CMV, cytomegalovirus; CNS, central nervous system.

325-435 days). Approximately 48% of patients were treated with valganciclovir therapy (n = 38), for an average duration of 77.4 days. All patients underwent a baseline and follow-up ABR, with an average follow-up duration of 19.4 months (range, 3.8 months to 7.4 years). However, among patients who received antioxidant therapy, the average follow-up was 15.3 months. There were no significant differences between baseline characteristics of cases and untreated controls. Patient characteristics are included in **Table 2**.

Baseline mean hearing thresholds prior to the initiation of antioxidant therapy were not statistically different when comparing the treated group and untreated controls ($P > .05$ at all frequencies for the right and left ears). The proportion of patients who developed hearing loss among the treated cases was 60%, while 60.3% of untreated controls developed hearing loss ($P > .05$). In addition, there were no differences in the proportion of patients who developed profound hearing loss between cases and untreated controls, where 40% of cases and 39.7% of untreated controls developed profound hearing loss ($P > .05$). This suggests that an equal proportion of these patients would qualify for a cochlear implant. There was not a statistically significant difference appreciated when comparing the mean changes in hearing thresholds among cases and untreated controls for the right ear, including 500 Hz (95% CI, –5.3 to 26.3, $P > .05$), 1000 Hz (95% CI, –17.3 to 21.9, $P > .05$), 2000 Hz (95% CI, –17.3 to 41.7, $P > .05$), and 4000 Hz (95% CI, –18.5 to 26.5, $P > .05$) (**Figure 1**). Mean changes in hearing thresholds for the left ear were again not statistically significant at 500 Hz (95% CI, –2.6 to 39.9, $P > .05$), 1000 Hz (95% CI, –15.6 to 13.1, $P > .05$), 2000 Hz (95% CI, –15.1 to 44.4, $P > .05$), and 4000 Hz (95% CI, –23.9 to 27.4, $P > .05$) (**Figure 2**). There was no difference in the proportion of patients who had clinically significant worsening of hearing between cases and untreated controls for both the right and left ears ($P > .05$). The length at which antioxidant therapy was provided was not found to have a

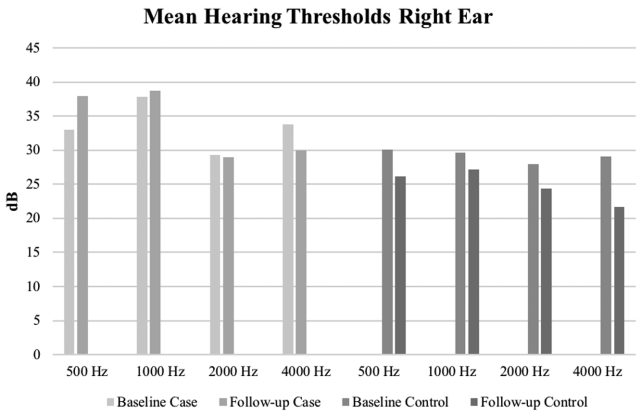


**Figure 1.** Mean changes to hearing thresholds for the right ear from the baseline auditory brainstem response (ABR) to the follow-up ABR for both cases and untreated controls.

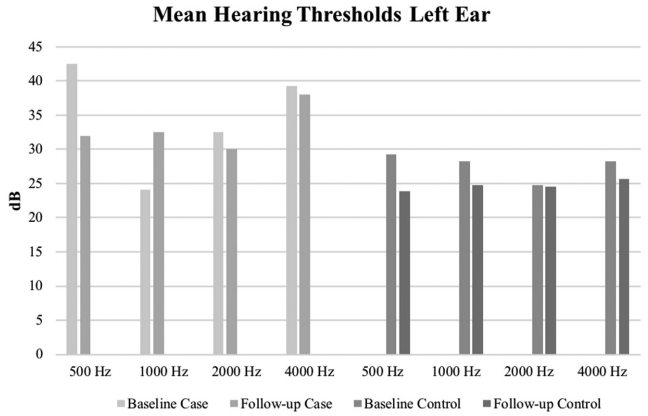


**Figure 2.** Mean changes to hearing thresholds for the left ear from the baseline auditory brainstem response (ABR) to the follow-up ABR for both cases and untreated controls.

statistically significant effect on hearing scores among cases for the right ear (95% CI, –0.05 to 0.02, $P > .05$) and left ear (95% CI, –0.04 to 0.08, $P > .05$). Furthermore, there was no effect on age at which therapy was initiated on hearing scores among cases for the right ear (95% CI, –1.2 to 1.8, $P > .05$) and left ear (95% CI, –1.7 to 2.5, $P > .05$). No serious adverse effects were reported in the patients treated with antioxidants, including gastrointestinal (GI) events such as emesis and diarrhea or non-GI events such as fever, chills, or sore throat. All patients were able to take the antioxidants for almost 1 year.

## Discussion

In this study, we found that the antioxidant ACE-Mg does not improve hearing outcomes in children with cCMV-related hearing loss. We evaluated the proportion of patients who developed hearing loss, the proportion who developed clinically significant worsening of hearing, the number eligible for cochlear implantation, and the mean change in hearing thresholds by frequency for each group. None of these measures indicated any beneficial therapeutic effect from the use of ACE-Mg.

We have recently reported that a high proportion of symptomatic cCMV-infected infants treated with the antiviral drug valganciclovir will develop progressively worsening hearing if followed long enough.[9] Fourteen of 16 patients were found to have a clinically significant worsening of their hearing thresholds. Lanzieri et al[10] reported that delayed-onset and progressive sensorineural hearing loss occurs commonly in asymptomatic CMV-infected children throughout adolescence. Ninety-two asymptomatic CMV-infected children underwent surveillance hearing testing, with 65% developing progressive hearing loss. These results highlight the need for long-term hearing testing and the need for novel therapies to ameliorate future progressive loss. Le Prell et al[11] reported that the antioxidant agents, vitamins A, C, and E, act in synergy with magnesium to effectively prevent noise-induced trauma in guinea pigs. Neither antioxidant agents nor magnesium prevented noise-induced hearing loss when delivered alone. In combination, however, they were highly effective in reducing hearing loss and inner and outer hair cell loss. The same group reported otoprotective effects from ACE-Mg in guinea pigs exposed to gentamicin, a potent ototoxic aminoglycoside.[12] Thatcher et al[13] also presented a case with connexin 26–related hearing loss who experienced progressive hearing loss over 7 years of observation. He was given ACE-Mg daily for 3 years, during which time his progressive hearing loss was ameliorated, which provides some rationale for further study that there is a role for the use of antioxidant therapy among children with hearing loss.

There continues to be ongoing interest in the use of antioxidants for the treatment of hearing loss and other neurological issues. A recent systematic review by Ibrahim et al[14] in 2018 examined the use of antioxidants in sudden sensorineural hearing loss. This type of hearing loss could be viewed in a similar fashion as CMV-induced hearing loss as there is a sudden insult to hearing that requires treatment in a timely fashion to ensure some resolution in hearing. Furthermore, both CMV-induced hearing loss and sudden sensorineural hearing loss are proposed to be caused by inflammation. This review found that the use of antioxidant therapy as an adjuvant therapy to the standard of care (corticosteroids) led to an increased success in therapy and improved hearing outcomes.[14] These results are encouraging; however, our study did not reveal a similar benefit from the use of antioxidant therapy. Ongoing research regarding inflammation-related hearing loss will continue to improve future therapies that could potentially benefit hearing loss with several etiologies.

Several limitations to our study could ultimately affect the outcomes and the lack of benefit to hearing outcomes among patients who received antioxidant therapy. One limitation may be patient compliance. Although participants reported good compliance during the treatment period, it is certainly possible that there may have been missed doses. A treatment log may have provided better insight into patient compliance. Another possibility is the failure to achieve an adequate therapeutic dose. Doses were determined based on the daily recommended intake and below the tolerable upper intake level for each agent. We also extrapolated based on the doses used in Thatcher et al.[13] The doses were stratified based on patient age, as seen in **Table 1**. Future studies should incorporate blood samples to confirm plasma levels of the active agents to assess compliance and bioavailability. Importantly, no side effects from the use of ACE-Mg were seen in this study. Antioxidants are reported to have few or minimal side effect; however, patients can experience mild gastrointestinal symptoms, nausea, or skin irritation from the use of antioxidant therapy.[15]

Another limitation of this study was the sample size of 78 patients with cCMV, with only 10 of these patients being treated with antioxidants. While having a great number of patients on antioxidant therapy would have improved our ability to compare it to traditional CMV therapy, there are several reasons there were not more patients enrolled, including parent preference or cost. The cost of a year of

ACE-Mg therapy is approximately $100, which may be a deterrent for some families. Antioxidant therapy was offered to patients; however, for unseen reasons, only 12.8% decided to pursue this therapy. The number needed to treat (NNH) for this study is 41.7 based on the incidence of progressive hearing loss; however, this study is an important first step in discussing the role of antioxidant therapy in the treatment of CMV-related hearing loss. The retrospective study design resulted in children being started on antioxidants at varying times after their diagnosis of CMV. A unified standard protocol that initiates the use of antioxidants within a certain timeframe after the diagnosis of CMV would improve our methodology for examining the use of antioxidants. It would also be important to clarify how the age that therapy is initiated affects hearing results. Furthermore, this study was designed as a case-control study. A study design that uses a randomized controlled trial would improve our ability to evaluate the true effect of antioxidants among children with cCMV. Regardless of these limitations, we were unable to prove that the use of antioxidants improves hearing outcomes among children with cCMV compared to untreated controls. These results highlight the continued efforts to evaluate novel therapies that can improve the long-term hearing outcomes of children infected with cCMV.

## Conclusion

Our study evaluated the use of ACE-Mg in the treatment of children with cCMV at a single institution. Our primary outcome measure, which examined the mean change in hearing in the right and left ears after administration of antioxidants, was not statistically significant between the treated and untreated groups. We also found no effect in mean change in hearing loss based on the age antioxidants were started or duration of therapy. Antioxidant therapy is a safe adjuvant therapy that should not yet be considered in the treatment of cCMV hearing loss. Future studies into optimizing the dose and other antioxidants will need to be done.

### Author Contributions

**Hilary McCrary**, design of the work, analysis and interpretation of the data, drafting and revision manuscript, final approval of draft; **Veronica del Calvo**, data analysis and collection, drafting and revision of the manuscript, final approval of draft; **Jeremy Purser**, data analysis and collection, drafting and revision of the manuscript, final approval of draft; **Geoff Casazza**, data analysis and collection, drafting and revision of the manuscript, final approval of draft; **Albert Park**, design of the work, analysis and interpretation of the data, drafting and revision manuscript, final approval of draft.

### Disclosures

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