Gestational vitamin A deficiency: A novel cause of sensorineural hearing loss in the developing world?

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Abstract

Hearing loss is a substantial public health problem with profound social and economic consequences in the developing world. The World Health Organization (WHO) estimates that there are 360 million people living with disabling hearing loss globally, and 80% of these individuals are from low- and middle-income countries. The epidemiology of hearing impairment remains poorly defined in most impoverished societies. Middle ear infections in childhood are a key determinant; however, congenital anomalies may also comprise an important etiology and may arise from gestational malnutrition.

While evidence exists that preventable vitamin A deficiency exacerbates the severity of ear infections and, consequently, hearing loss, antenatal vitamin A deficiency during sensitive periods of fetal development may represent an etiologically distinct and virtually unexplored causal pathway. Evidence from multiple animal systems clearly shows that fetal inner ear development requires adequate vitamin A nutriture to proceed normally. Inner ear malformations occur in experimentally imposed maternal vitamin A deficiency in multiple species in a dose–response manner. These anomalies are likely due to the loss of retinoic acid-dependent regulation of both hindbrain development and otic morphogenic processes.

Based on *in vivo* evidence in experimental animals, we hypothesize that preventable gestational vitamin A deficiency, especially during early stages of fetal development, may predispose offspring to inner ear malformations and sensorineural hearing loss. As vitamin A deficiency affects an estimated 20 million pregnant women globally, we hypothesize that, in undernourished settings, routine provision of supplemental vitamin A at the recommended allowance throughout pregnancy may promote normal inner ear development and reduce risk of an as yet unknown fraction of sensorineural hearing loss. If our hypothesis proves correct, gestational vitamin A

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None declared.

deficiency would represent a potentially preventable etiology of sensorineural hearing loss of substantial public health significance.

Introduction

Hearing loss is a public health problem throughout the developing world. The World Health Organization (WHO) estimates that there are 360 million people living with disabling hearing loss globally, and 80% of these individuals are from low and middle income countries [1]. Hearing loss is the 12th highest contributor to the global disease burden based on disability-adjusted life years [2].

The disability that results from hearing loss has profound social and economic consequences. The hearing–impaired are often socially isolated and experience stigma as a result of their disability [1]. Adults with hearing loss are frequently unemployed or underemployed. Income of the hearing–impaired has been shown to be 40–45% less than that of the general population [3]. As a result, most affected individuals are found in the lower income groups around the world [3].

More concerning is the impact of hearing loss on children. Because auditory stimulation is critical for development of speech and language, children born with hearing impairment are at substantial risk for irreversible speech, language, and cognitive delays [4–6]. In developed countries, deaf children enrolled in school at the same age as their hearing-peers were shown to be an average of 1.5 years behind in reading comprehension by the 3rd grade and 7.5 years behind by the age of 15 [2]. These trends are intensified in low-income countries where school enrollment for hearing impaired children is often delayed until 5 years of age or older [7]. Enrollment delays may frequently be a result of delayed detection of the hearing loss itself, a common occurrence when routine hearing screening for infants and children is unavailable [2]. Furthermore, there are few specially-equipped schools for the hearing impaired in low resource settings, resulting in hearing impaired children often having little to no access to formal education [7]. These educational discrepancies may go on to have an impact on employment and social interactions as these children grow to adulthood, which may result in life-long disability and inequality [8,9].

A parallel burden that is rarely associated with hearing loss is vitamin A deficiency, which is estimated to affect 190 million children in the developing world [10,11]. Vitamin A deficiency is the leading cause of preventable childhood blindness, due to xerophthalmia, and is an established cause of increased severity of infections and mortality in the preschool years [12]. Vitamin A deficiency is estimated to also be widely prevalent in the school-aged years and adolescence across Southern Asia [13] and likely persists into the reproductive years with health consequences [10,11]. In rural Nepal, prolonged provision of supplemental vitamin A before, during, and following pregnancy improved maternal vitamin A status, reduced risk of night blindness (a clinical symptom of vitamin A deficiency), lowered mortality related to pregnancy by ~40% [14,15], and improved development of offspring, evident by enhanced lung function [16]. In Bangladesh, although maternal mortality was not affected [17], supplemental antenatal vitamin A lowered risk of bacterial vaginosis [18], revealing multiple possible health effects of assuring vitamin A adequacy during pregnancy.

The long described synergism between vitamin A deficiency and infection [19] extends to the ear, in which animal studies [20,21] and sporadic epidemiological reports [22] have documented increased risk of otitis media with vitamin A deficiency. The association appears to be causal, given recent evidence that preschool vitamin A supplementation can reduce risk of hearing loss due to otitis media. Schmitz et al. demonstrated in Nepalese young adults that periodic vitamin A supplementation of children during their preschool years decreased risk of hearing loss attributed to early childhood ear infections [23].

While compelling evidence of the role of vitamin A in preventing hearing loss mediated by ear infection now exists, few other nutritional causes of hearing loss have been proposed and none, to our knowledge, have been described in association with gestational exposures of public health magnitude. We believe a plausible, yet little appreciated and virtually unexplored, causal pathway exists based on extensive animal evidence demonstrating abnormal fetal inner ear development resulting from maternal vitamin A depletion during pregnancy [24–32]. There is evidence in mice, rats, quail, chicks, and zebrafish that fetal inner ear development requires access to vitamin A to proceed normally, with malformations occurring in deficiency in a dose-dependent manner [24–30,33–35]. In rats, for instance, there appears to be a limit of 125 μg/g retinoic acid in the diet for embryonic inner ear, or otic vesicle, development to proceed normally. One hundred percent of embryos born to vitamin A deficient dams receiving 250 or 125 μg/g retinoic acid had normal otic vesicles, while 70% of embryos in the 50 μg/g group and 100% in the groups fed 25 μg/g or less developed immature and/or ectopic otic vesicles [25]. If the emerging animal evidence extrapolates to humans, gestational vitamin A deficiency would represent a potentially preventable etiology of sensorineural hearing loss of substantial public health significance in developing world settings where vitamin A deficiency continues to be endemic.

Hypothesis

We hypothesize that, under conditions of endemic vitamin A deficiency, routine antenatal vitamin A supplementation can promote normal inner ear development and reduce risk of sensorineural hearing loss in offspring via a previously undescribed etiology.

Basis for the hypothesis

Mammalian inner ear development is immensely complex, involving over 50 genes variably contributing to its formation [30]. However, a handful of genes and diffusible factors are considered master regulators because of their ability to broadly affect development [30]. Vitamin A, in the form of its active metabolite retinoic acid (RA), is considered a "master differentiating factor" and has been shown to be indispensible for inner ear development [24,36]. The role of retinoic acid synthesizing molecules, RA's influence on hindbrain development, and the downstream factors regulated by RA demonstrate the essentiality of vitamin A in inner ear formation.

Retinoic acid synthesizing molecules

Retinoic acid is produced in mammalian embryos by the retinaldehyde dehydrogenase-2 (Raldh2) enzyme. This enzyme is required for survival and morphogenesis of mouse

embryos [25]. Homozygous mutant *Raldh2−/−* embryos die at midgestation with multiple anomalies, including precursors of the inner ear, or otocysts, that are severely hypoplastic and located farther from hindbrain neuroepithelium than normal [25]. These changes are believed to be due to alterations in expression of retinoic-acid regulated genes in the ear, including fibroblast growth factor 3 (fgf3). *Fgf3* is normally expressed in hindbrain rhombomeres 5 and 6 and is known to stimulate otocyst development, but in *Raldh2−/−* mutant mouse embryos *Fgf3* expression is weak and spread over a broader area than its expected location in the posterior rhombomeres of the hindbrain [25].

Retinaldehyde dehydrogenase (Raldh) synthesizing enzymes are present in the inner ear of multiple animal models and are often balanced with metabolizing enzymes, suggesting that these enzymes work to precisely regulate the amount of RA present in the inner ear during various stages of development. Raldh2 and 3 are upregulated in the mouse inner ear during the developmental window corresponding to inner ear morphogenesis, and the metabolizing enzyme cyp261A is upregulated at the end of this developmental window [37]. Retinol dehydrogenase-10 (Rdh10) is another RA synthesizing enzyme found in the mouse inner ear during development and is co-localized with Raldh2 and 3 [33]. Analogs of the Raldh synthesizing enzymes are found in zebrafish and chicks as well, both with localized expression in the developing otocyst [34,35]. Importantly, the phenotype of *Raldh2−/−* mutant embryos is similar to the defects seen in vitamin A deficiency, and the anomalies can be partially rescued with maternal administration of retinoic acid [25,26,37].

Retinoic acid and hindbrain development

In addition to the synthesizing enzymes, retinoic acid also affects inner ear development by influencing hindbrain formation. In quail, retinoic acid deficient embryos exhibit disruption of the posterior segments, or rhombomeres, of the hindbrain, and abnormal otic vesicle development [27]. Rhombomere disruption is explained by the presence of retinoic acid response elements (RAREs) upstream in the Hox genes, allowing RA to directly regulate expression of these genes that define the rhombomeres of the hindbrain [27]. Inner ear patterning is disrupted in vitamin A deficient quail embryos lacking posterior hindbrains. This phenotype can be rescued, however, with RA administration prior to 30 hours of development [28].

Further evidence is available in the rat embryo, where abnormalities in otic vesicle formation in the setting of RA deficiency are associated with defects in posterior hindbrain patterning [29]. Rat embryos with a maternal diet above 125 μg/g RA demonstrated normal otic vesicle development, while 70% of otic vesicles were abnormal in the 50 μg/g RA group and 100% were abnormal in the groups fed 25 μg/g RA or less [29]. Abnormalities seen in this study include malformed orthotopic otic vesicles and immature ectopic vesicles [29]. A similar dose-dependent pattern was seen in hindbrain segmentation, with increasing abnormalities corresponding to decreasing maternal RA administration. It was noted that all embryos with absent hindbrain segmentation beyond the border between rhombomeres 3 and 4 had either immature or completely absent otic vesicles [29]. Hindbrain and otocyst-malformed phenotypes could be completely rescued with increased maternal RA supplementation. With this study White et al. demonstrate that RA is necessary for initial

otic vesicle formation, and abnormalities in otic vesicle development occur in a dose-dependent manner with increasing levels of vitamin A depletion.

While hindbrain patterning brings to light the role of retinoic acid response elements (RAREs) located in upstream locations of the Hox genes, evaluation of the retinoic acid receptors (RARs) which bind these RARES is another important element of elucidating the role of vitamin A in inner ear formation. Romand et al. determined the expression pattern of the three RARs (α, β,γ) in the mouse inner ear. RARα/RARγ−/− mice developed severely hypoplastic otocysts with rudimentary inner ear structures, indicating that retinoic acid signaling through either α or γ RARs is necessary for normal otocyst development [31]. The phenotype of RARα/RARγ−/− mice was similar to *Hoxa-1*−/− mice but was actually more severe. This suggests that there are other downstream genes controlled by RA than the Hox genes alone [31].

Retinoic acid-regulated downstream factors

In addition to retinoic acid synthesizing molecules and RA's influence on hindbrain development through Hox genes and retinoic acid receptors, the third pillar of evidence suggesting the vital role of vitamin A in inner ear development is the downstream gene products regulated by retinoic acid. There are a myriad of RAcontrolled gene products that play a role in inner ear development, including factors from the fibroblast growth factor (FGF), transforming growth factor (TGF), bone morphogenic protein (BMP), sonic hedgehod (Shh), and wingless (Wnts) families [30]. The presence of FGF3 and 10 are required for inner ear development in mice, and embryos with mutations in both of these genes exhibit severely hypoplastic otic vesicles and decreased expression or complete absence of other genes critical in otic development such as *Dlx5* and *Pax2* [38].

Some of the most helpful evidence of the impact of vitamin A on these downstream markers in inner ear development comes from vitamin A-induced teratogenesis. Inactivating the *Dlx5* gene results in inner ear defects similar to those seen with excess RA exposure [39]. Liu et al. demonstrated that excess RA disrupts otocyst development in mice by downregulating *Fgf3/10* expression, which subsequently leads to a decrease in expression of the downstream target *Dlx5* [39]. This decrease in *Dlx5* expression, as well as the phenotype associated with RA excess, were rescued by administration of FGF3 and 10 [39]. Retinoic acid deficiency may work through the same *Fgf3/10* and *Dlx5* pathway to cause disturbances in inner ear development.

In analyzing the development of specific inner ear structures, namely the hearing organ or cochlea and the vestibular system critical for balance, RA's downstream products have again been implicated. *Pax2* is necessary for cochlea formation [40], and its expression is absent retinoic acid-deficient mice, such as *Raldh2* homozygous mutants [26]. Raz and Kelley have shown that retinoic acid receptors (RARs and RXRs) are expressed in the developing cochlea, and withholding retinoic acid during development in the mouse leads to both an overall disruption in cochlear development and a decrease in the number of cochlear hair cells, structures that are critical for converting sound waves to electrical energy used by the brain [41]. In further examining the role of *Dlx5*, it was noted that otic induction proceeds

but further morphogenesis is halted in mouse *Dlx5/6−/−* embryos, with no formation of vestibular structures [42,43].

Preliminary human evidence

Although limited to case reports, there is some human evidence that mutations in analogous genes regulated by retinoic acid in animals do produce hearing loss in humans. Homozygous *Fgf3* mutations are associated with inner ear agenesis and accompanied sensorineural deafness [44,45]. Interestingly none of the families in these reports had accompanying mutations in *Fgf8* or *Fgf10*, indicating a difference between humans and mice since murine otic abnormalities require disruption of multiple fibroblast growth factor genes. Secondly, human mutations in *Dlx5* and *Dlx6* are associated with split hand/foot malformation, which includes sensorineural deafness and malformations of the vestibular system [46,47].

While these case reports confirm that alterations in inner ear development do result from mutations in analogous human genes, they only show two possible outcomes – profound sensorineural deafness with homozygous mutations or normal hearing in heterozygous family members. We believe that the evidence in the animal literature showing dose response of increasing inner ear malformations with increasing severity of retinoic acid depletion is analogous to the human vitamin A deficiency in the developing world and suggests a spectrum of inner ear malformations and resultant sensorineural hearing loss.

Evaluation of the hypothesis

Experimental animal evidence implicates an essential role for vitamin A in inner ear development. Based on this evidence, we believe that routinely providing adequate amounts of vitamin A antenatally in undernourished regions of the world may promote normal inner ear development and reduce the risk of sensorineural hearing loss induced by gestational vitamin A deficiency.

While the amount of supplemental vitamin needed to be consumed in a deficient population to protect ear development is not known, it has been estimated that routine vitamin A intake through diet or supplements at levels that comprise a recommended daily allowance for pregnancy (750–770 μg/day) is safe and effective to maintain adequate vitamin A status of the mother and delivery to the fetus [48]. Adverse effects in pregnant women, such as birth defects or elevated hepatic enzyme levels, are estimated to occur at typically much higher levels, and none have been observed with supplements below 3000 μg/day [48].

Given the low risk of vitamin A supplementation, our causal hypothesis can best be evaluated in a cohort of women living in an undernourished region who were randomly exposed to supplemental vitamin A at levels approximating a recommended allowance or placebo, and who remained under surveillance through pregnancy detection, gestation, and postnatal follow-up. Further, the hypothesis stands the best chance of being confirmed where vitamin A supplementation conferred demonstrable nutritional and health benefits to mothers and children [49]. A study that satisfies all of these conditions was carried out in the rural Terai District of Sarlahi, Nepal from 1994 to 1997, where women were randomized by local community to receive a weekly oral supplement containing a recommended dietary

allowance of vitamin A, either preformed or as beta-carotene, versus placebo prior to, during, and following pregnancy (n = ~22,000 pregnancies). Maternal vitamin A supplementation reduced night blindness (an ocular symptom of vitamin A deficiency), mortality related to pregnancy, and mortality of infants born to mothers prone to night blindness. The trial also demonstrated improvements in lung development and natural immunity among offspring [16,50]. Importantly, this cohort remains intact, is of testable age (18–20 years of age as of 2014), and resides in a population research setting where audiometry and ear health evaluations can be readily performed. While at least two other capable population research sites exist where large, antenatal vitamin A supplementation trials were conducted in recent years [17,51], neither showed discernable effects on maternal and infant survival and thus represent settings with insufficient vitamin A deficiency to test the current hypothesis.

Consequences of the hypothesis

Our hypothesis would be confirmed by a finding that offspring born to women who routinely received a supplement of vitamin A providing a recommended allowance throughout pregnancy, in a setting such as rural Nepal, have a lower risk of sensorineural hearing loss than children born to mothers who received placebo. A new etiology of sensorineural hearing loss of public health consequence will have been elucidated that may be preventable with diet or supplementation.

Understanding the potential public health implications of this etiology requires extrapolation from worldwide epidemiologic and newborn screening data. In developed countries, newborn hearing screening data indicate that sensorineural hearing loss occurs in 0.4–1.1 per 1000 live births [52]. Although data from developing countries is limited, studies from Nigeria and Cote d'Ivoire suggest that the incidence of sensorineural hearing loss is much higher: 5.3 and 5.96 per 1000 live births, respectively [53,54].

The reasons sensorineural hearing loss may be far more frequent in low resource settings have not been elucidated. We believe prenatal malnutrition, particularly vitamin A deficiency during critical periods of fetal development, represents a plausible cause.

UNICEF estimates that 28.3 million births occur annually in the 50 least developed nations in the world [55]. Defining an annual incidence of 1 per 1000 of sensorineural hearing loss in neonates, as observed in developed countries, as a referent, there is approximately 4 per 1000 higher incidence of neonatal sensorineural hearing loss in developing countries. A recent systematic review of the etiology of bilateral sensorineural hearing loss in children concluded that the etiology of approximately 38% of cases is unknown [56]. Applying this 38% unknown fraction to the 4 per 1000 excess incidence, we conservatively estimate that 43,000 annual cases of sensorineural hearing loss may be due to avertable nutritional causes. Addressing the hypothesis represents an extraordinary opportunity to explore and potentially reduce the global burden of hearing loss and consequent disability in the developing world.

Acknowledgments

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