Examining Serum Levels of Zinc and Copper in Individuals with Chronic Problematic Tinnitus

Reference

This is an original study conducted by myself, Valerie Ingalls, as well as Dr. Ishan Bhatt, PhD, member and PI, respectively, of the Audiogenomics Lab, part of the Department of Communication Sciences and Disorders at the University of Iowa. Data for the study are taken from the National Health and Nutrion Examination Survey (NHANES), which has its data published publically available at their website.

Introduction

Tinnitus, or the phantom perception of sound in abscence of external stimulus, which is commonly but not exclusively experienced as ringing in the ears, affects an estimated 10% of US adults each year. This high prevalance makes it a very common disorder. The rate increases with age, with nearly 5 million total individuals in their 50's experiencing tinnitus during a given year. There are numerous differences between tinnitus phenotypes that individuals experience. Tinnitus may manifest only in the short term, which is known as acute tinnitus, or it may be experienced for multiple years, which is known as chronic tinnitus. Even among chronic tinnitus patients, some people perceive tinnitus consistently, while for others it is only present or discernible intermittently. A significant portion of individuals with tinnitus report that it presents at least a moderate problem in their life, and over 1 million U.S. adults report it is a big problem. As a common disorder with a notable negative impact on those who have it, the importance of treatment for tinnitus is clear. Those with chronic, problematic tinnitus represent the population of greatest clinical interest.

While noise exposure is known to be the causal trigger for the manifestation of tinnitus, the exact biological origins of the disorder yet remain unclear. Moreover, it is not yet fully clear why, when two individuals are exposed to the same loud noise, it is possible that only one of them develops tinnitus in response. One leading theory is that tinnitus is connected to damage within the cochlea, the primary structure of the inner ear that is responsible for transduction of sound into the electrical signal that is passed to the brain. Zinc and copper are known to prevent free radical damage in the cochlea over time by neutralizing the superoxide radical. It is theorized that these molecules may have a protective effect against noise-induced cochlear damage, and therefore tinnitus. It follows that their deficiency may result in tinnitus susceptibility. Previous studies examining this hypothesis have showed some promising results, but have largely been underpowered and lacking statistical significance. Further, several studies that attempted to use zinc for treating tinnitus neglected to examine pre-existing differences in zinc levels and rates of deficiency between individuals with and without tinnitus. With this project, we hold the goal of completing a large-scale analysis of differences in the levels of zinc and copper in individuals with and without tinnitus, as well as the rates of deficiency, in order to determine whether there are population-level variations that could explain tinnitus susceptibility.

Example Figure

As this is an original study, I do not have a figure to replicate. Additionally, the previously mentioned studies did not use the type of visualization that I am planning to use, because their sample sizes were too small for a histogram to be an effective visualization. As such, I have pulled a somewhat similar example and will describe the differences that I plan for my final version.

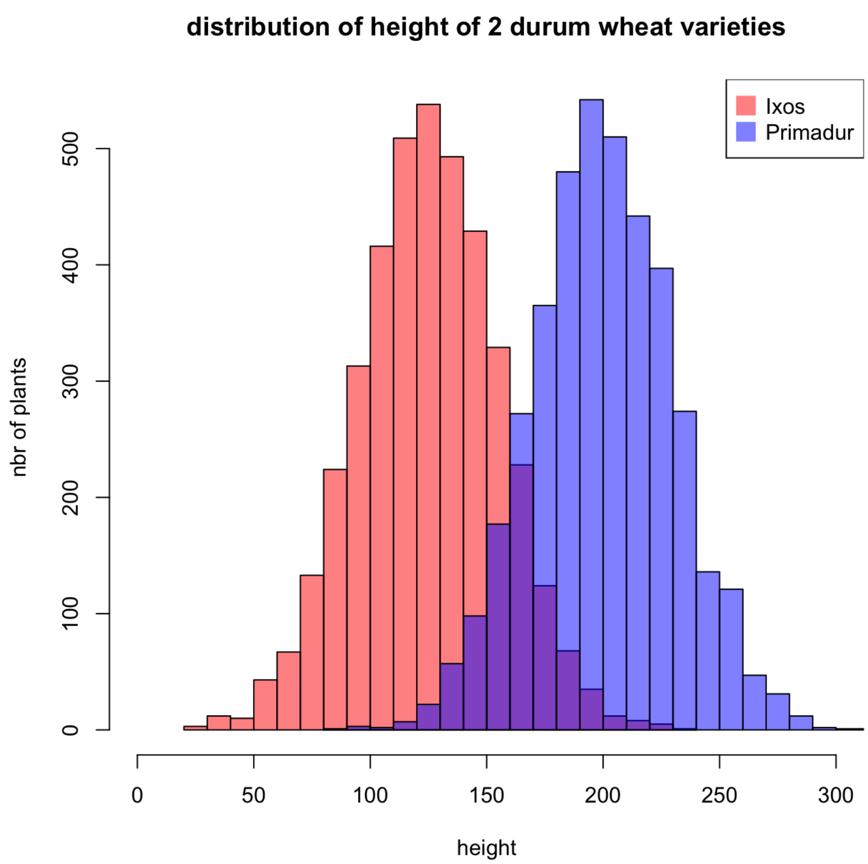

Figure 1: An example histogram

As you can see from this example, the goal is to create a plot with multiple histogram distributions on the same axis. I will be making two figures with the same underlying design, the only difference being whether the figure is for copper or zinc. Due to differences in normative serum levels of copper and zinc, it is likely impractical to measure both metals on the same figure. On the x-axis for each figure will be level of serum copper (or zinc), while the y-axis will be density (preferable to a count y-axis in this case because our generalized sample size is in the millions). One of the distributions will be for the no tinnitus control group, one distribution will be for individuals with chronic problematic tinnitus, while a third distribution will be for individuals with other tinnitus phenotypes (either acute or unproblematic). I will also add a vertical line at the clinical cutoff value for deficiency in those metals. In this way, I will visualize the difference in zinc and/or copper in those with chronic problematic tinnitus compared to the control groups of those with no tinnitus and those with clinically non-significant tinnitus, while simultaneously showing the proportion of individuals in each population who have a deficiency in that metal.

Materials and Methods

This is a large-sample epidemiological study using data from the National Health and Nutrition Examination Survery (NHANES).

NHANES Data Collection

The NHANES is a nationally representation health survey that is unique in that it makes use of both questionnaires and physical examinations to gather a robust set of health data. It began several decades ago with a series of multi-year surveys, the NHANES I, II, and III. The modern NHANES, known as the continuous NHANES, is a series of survey cycles, each lasting for two years. Individual cycles are constructed to be fully representative of the United States population; researchers are still encouraged to combine multiple cycles in order to increase the robustness of their dataset. The NHANES utilizes a complex stratified survey design in order to increase its representativeness. In summary:

- Subpopulations of particular public health interest are identified (eg non-white Hispanic individuals)
- The total population is broken up into strata based on location, often but not always by county
- Strata are assigned a weight that increases their likelihood of being randomly selected for sampling based on the proportion of their population that includes the targeted subpopulation(s). Some strata are weighted so heavily as to be guaranteed for selection
- Weighted randomized selection of strata for the survey occurs
- A random selection of individuals from within those strata are actually surveyed

- Weights and strata information are then included in the NHANES data so that it is possible to accurately extrapolate results to the population level. All data are publicly available on the NHANES website.

Data Selection and Processing

Data from the NHANES are stored in .XPT format and are accessible via the website. In order to preserve metadata and avoid data corruption in the present study, we acquired data via CLI using the wget command in a bash shell. Two cycles, 2011-2012 and 2015-2016 were selected for this study, as the only ones to have full audiological datasets available alongside serum metal levels.

NHANES stores data separately by cycle and by subject matter. In the case of this study, we used data from a total of ten different sheets, five from each cycle. The first step in data wrangling was to combine these ten sheets into one master. This was accomplished in R (Version: RStudio 2022.07.2+576 "Spotted Wakerobin" Release (e7373ef832b49b2a9b88162cfe7eac5f22c40b34, 2022-09-06) for Windows Mozilla/5.0 (Windows NT 10.0; Win64; x64) AppleWebKit/537.36 (KHTML, like Gecko) QtWebEngine/5.12.8 Chrome/69.0.3497.128 Safari/537.36), as were all future steps for data wrangling and analysis. Many variables of interest were obtained directly from the data, while others were derived or refactored from the raw NHANES data. As NHANES lists the age of any individuals 85 or older as 85 (in order to protect identities), it was necessary to refactor age into a factor variable, with the upper extreme being "80 or older". Several derived variables were created as well. Tinnitus type was sorted into 3 groups: no tinnitus, chronic bothersome tinnitus, and other tinnitus. "Other tinnitus" includes those individuals who have expereinced tinnitus within the last 12 months, but either **(a)** have not experienced it for at least a total of 1 year **(b)** do not report their tinnitus causing any problem in their life or **(c)** only experience tinnitus as a temporary response to exposure to loud noise. Deficiency was also derived for both zinc and copper levels, using pre-defined clinical cutoff values. Additionally, ethnicity was recoded to "White" and "Non-White". While this coding scheme potentially misses out on some true differences, it has been shown to be an effective representation of population stratification in previous tinnitus studies, and the simplicity has benefits for statistical analysis. Finally, the sample weights are halved for each individual, as is the standard NHANES protocol when combining two cycles together. For this study, we used the laboratory sub sample weights as our serum metal measurements come from this sub sample.

Analysis

The NHANES provides survey design details that allow us to extrapolate our results to the entire US population. In order to make use of the complex survey design in our analysis, we need to use the survey package in R. This package contains functions for regression modeling, t tests, chisq tests, and various forms of graphing, along with other analysis tools. With that package, we can implement the survey design in both our histogram figure as well as any statistical analyses.

For full comparison of the groups we established (no tinnitus, chronic problematic tinnitus, and acute and/or non-problematic tinnitus), we will perform several types of statistical analyses. First, in order to examine stratification within our sample population, we will perform several chi square goodness of fit tests, examining differences in sex, ethnicity, and age across the different tinnitus types. Secondly, we will perform two multiple linear regression analyses, one with serum zinc level as the response variable, and one with serum copper level as the response variable, with sex, age group, ethnicity, and tinnitus type as the predictor variables. This will allow us to examine the effect of tinnitus type on serum metal levels while controlling for these other demographic variables. Finally, we will perform two logistic regression analyses with the same predictor variable, while the response variables will be deficiency in the metals, as defined by pre-established clinical values. This allows us to examine not just the discrete quantity of serum zinc and copper, but also the rates of deficiency across the different tinnitus groups.

Figure Production

These figures were generated using the `ggplot2` package in RMarkdown. geom_density() provides the main distributions for the plot, while geom_vline() is used to show the deficiency cutoff point. The `fill` aesthetic creates the grouping needed to differentaite between tinnitus types, while the `weight` aesthetic allows us to implement the weighting from the complex survey design into these plots. Further aesthetics specify details features that make the graph more visually appealing. A code snippet for one of the graphs is included below for easy reference:

```
ggplot(data = df) +
  geom_density(mapping = aes(x = zinc, weight = weight, fill = tinnitus_type), alpha = .5) -
  geom_vline(xintercept = 61, color = "blue", linetype = 2) +
  scale_fill_discrete(labels = c("Chronic Bothersome Tinnitus", "Other Tinnitus", "No Tinnit
  labs(x = "Serum Zinc Level (\u03BCg/dL)", y = "Density", title = "Distribution of Serum Zi
```

Results

Preliminary results indicate no signficant difference in levels of serum copper or zinc between individuals with chronic bothersome tinnitus, other tinnitus types, or no tinnitus. However, as is clearly visible in these preliminary density plots, the distributions of metal levels are right-skewed. This indicates that it would be most appropriate to apply a transformation to the y-values of our multiple

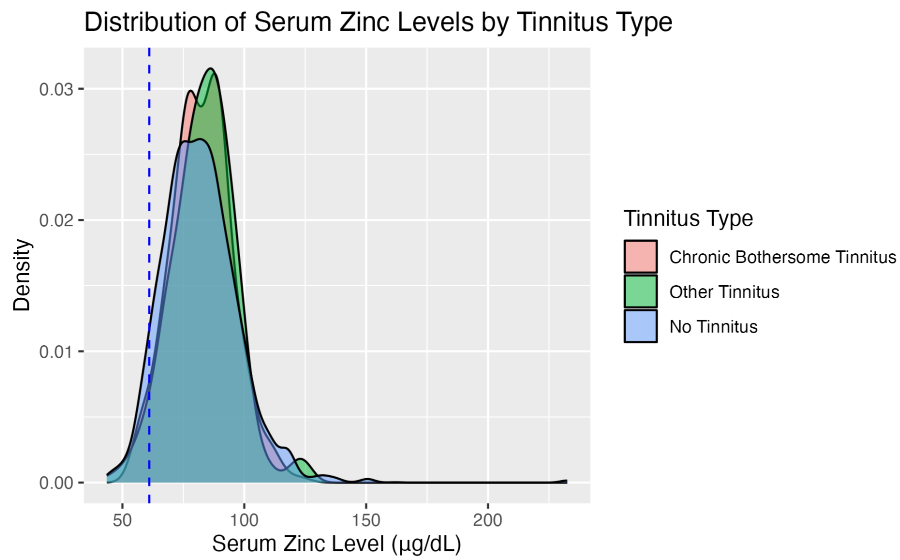

Figure 2: A density plot displaying the distributions of serum zinc levels, differentiated by tinnitus type. A vertical line indicates the cutoff for clinical deficiency.

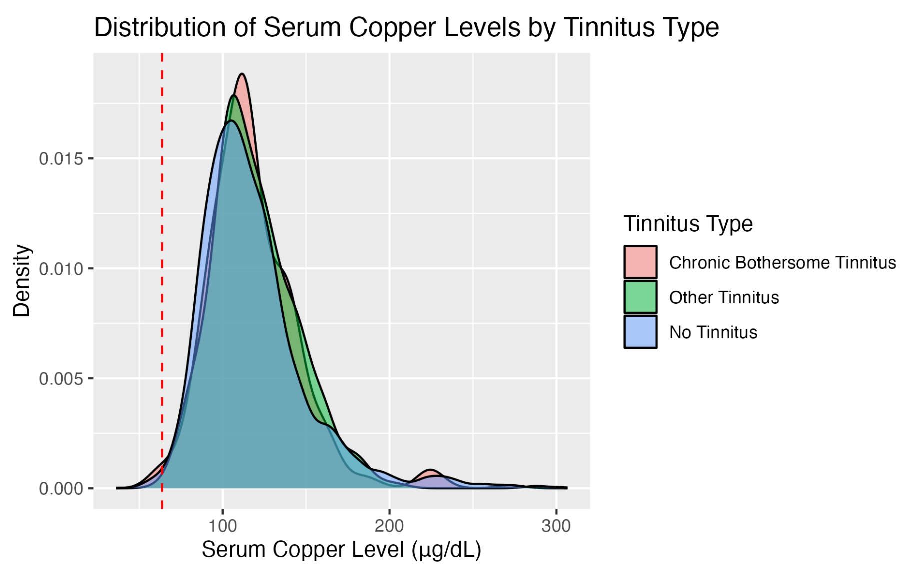

Figure 3: A density plot displaying the distributions of serum copper levels, differentiated by tinnitus type. A vertical line indicates the cutoff for clinical deficiency.

linear regressions, in order to determine whether there is a significant difference when the distributions are more normalized.

Knowing that transformation is the next step in my analysis, I will not go into great detail regarding the preliminary results of statistical analyses, other than to point out a few facts. As mentioned, no significant relationship was found between tinnitus type and either serum copper level or serum zinc level. Additionally, no relationship was found between tinnitus type and rates of clinical deficiency for either metal. Initial chi-square tests showed that there is significant stratification of tinnitus prevalence by both age group and ethnicity, but not by sex. These differences were expected given past epidemiological studies of tinnitus prevalence and do not present a threat to the validity of our current results, as our models control for these factors.