The Relationship Between Vitamin D Levels and Brain Volume in Children with Demyelinating Diseases of the Central Nervous System

- Yug Yadava
Table of Contents

I. Abstract .............................................................................................................(2)

II. Introduction ....................................................................................................(3)

III. Objective .......................................................................................................(5)

IV. Methodology ..................................................................................................(5)
    A. Patients and Groups ......................................................................................(5)
    B. Timeline ..........................................................................................................(5)
    C. Vitamin D Months ..........................................................................................(5)
    D. Brain Volume Z-Scores ...................................................................................(6)
    E. Python and Excel ............................................................................................(6)
    F. Statistical Analysis ...........................................................................................(6)

V. Results .............................................................................................................(7)

VI. Discussion .......................................................................................................(10)

VII. Conclusion .....................................................................................................(14)

VIII. Acknowledgements ......................................................................................(14)

IX. References .....................................................................................................(15)

X. Figures .............................................................................................................(19)

XI. Appendix .......................................................................................................(30)

XII. Python Code ................................................................................................(33)
Abstract

Multiple sclerosis (MS) is an autoimmune disease that targets the central nervous system, specifically the myelin, a sheath that insulates the nerves (National Multiple, 2019). Myelin Oligodendrocyte Glycoprotein (MOG) is a component of myelin, and antibodies toward it are responsible for a distinct demyelinating disease, which shares some similarities with MS. Higher Vitamin D levels have been associated with less autoimmune responses and reduced disease activity in adult MS. Patients (n = 59) from the Canadian Pediatric Demyelinating Disease Study (CPDDS) were split in three groups: Pediatric MS (n = 12), Pediatric MOG Positive (MOG+) (n = 21), and Pediatric MOG Negative (MOG-) (n = 26). Age and gender in the three groups were consistent with previous studies. Vitamin D levels were lower in pediatric MS patients than in MOG positive and MOG negative patients. Three Kruskal–Wallis Tests revealed no significant difference between the three groups in Serum 25(OH)D levels at presentation and Brain Z-scores at presentation and at 1 year of follow up. A Dunn test performed on Vitamin D levels revealed no significance between any combination of the three groups. Six Linear Regression tests revealed no significant correlation between 25-hydroxyvitamin D concentrations and brain volumes among the three groups. Studies with more patients and follow ups along with investigating lesion volume to onset Vitamin D levels will yield more precise conclusions.

-Yug Yadava
Introduction

Multiple sclerosis (MS) is an autoimmune disease that targets the central nervous system, specifically the myelin: a protein that insulates nerve fibers (National Multiple, 2019) (Figure 1). Patients will experience one of the four main types of multiple sclerosis if diagnosed. The cause of MS is unknown but could be linked to factors such as genetics and the environment. Countries such as Canada and the United Kingdom have the highest rates of multiple sclerosis per 100,000 people (Figure 2). The lesion-damaged tissue-causing disease occurs mainly in adults but as many as 10,000 children in total are diagnosed with it in the United States, a majority being females (Figure 3) (Weatherspoon, 2018). Within pediatric multiple sclerosis, a vast majority of cases report to have relapsing-remitting form of the disease, meaning that there are periods of time when the immune system does not attack the nerves and thus there may be no progression of the disease (Figure 24) (John Hopkins Medicine, 2020).

In the past few decades, treatments for MS have skyrocketed. Consisted of medications such as Avonex, Copaxone, and Rituxan have shown effectiveness in small studies (National Multiple, 2019). Oral treatments such as Tecfidera and Aubagio have been studied and recently approved for new treatments for MS, making the process easier. Hospitals will also advise family and friends on how to seek other treatment for the child such as tutors and psychologists to make the transition as easy as possible for everyone involved. Pediatric patients also can go to regular visits to physical therapists to maintain or even increase their physical strength.

The Myelin Oligodendrocyte Glycoprotein (MOG) is a protein that is expressed on the myelin sheath. MOG antibodies are responsible for the demyelination of the optic nerve but can also affect the brain and spinal cord. Despite some similarities between MS and MOG disease, the two demyelinating diseases are distinguished from one another. In recent years, many studies have been pointing to the MOG antibody as a way to distinguish between MS, MOG disease, etc (MayoClinic, 2018). Patients may also observe a case of a monophasic demyelinating event, meaning that children have lost some coating of the myelin sheath during a one time attack by the immune system.

One of the factors that could be influencing patients that have MS, MOG disease, etc, is Vitamin D levels. Vitamin D comes from many sources including UVB rays from the sun, fish -
especially salmon - and whole milk (Table 2) (USDA, 2020). When Vitamin D3 is absorbed in the human body, it is transferred into the liver to be converted over into a serum called 25-hydroxyvitamin D [serum 25(OH)D] in order organs to use. This serum can in accordance to the metric system, patients below 50 nanomoles over liters (nmol/L) experience insufficient levels of vitamin D, 50 nmol/L - 75 nmol/L are sufficient though not all experts agree, 75 nmol/L - 125 nmol/L concentration is sufficient and anything above 125 nmol/L is too much serum 25(OH)D (Table 1) (Sullivan, 2017). 25-hydroxyvitamin D levels have been found to be lower in value when it comes to MS patients than other distinguishable demyelinating diseases (Alharbi, 2015). Lower serum 25(OH)D levels - specifically below 50 nmol/L - have also been associated with increased autoimmunity and susceptibility to infection.

In 2014, Dr. Alberto Ascherio of the Harvard T.H. Chan School of Public Health and fellow colleagues utilized the BENEFIT study conducted from 2002-2003 to see if Vitamin D levels affected the prognosis of adult multiple sclerosis. The individuals in the study were from mostly European descent and two main groups were created based on their Vitamin D levels - those below 50 nmol/L at onset and those at or above 50 nmol/L at onset. The research conducted analysis of multiple variables including lesion and brain volume (Figure 23). The study concluded that the percentage loss of brain volume from one year after onset to five years after onset was lower in those adult MS patients that had a initial Serum 25(OH)D level at or above 50 nmol/L than their peers who had serum levels below 50 nmol/L (Ascherio et al., 2014).

With higher Vitamin D levels being associated with less autoimmune responses and showing lower brain volume loss in adult MS, could patients that have pediatric multiple sclerosis, MOG disease monophasic, and MOG negative monophasic have higher brain volumes? In this study, 59 patients from three different groups - MS, MOG+, and MOG- - will be analyzed to investigate their Vitamin D levels at onset, Brain Z-scores at onset (0-3 Months) and after 12 months (10-14 months), along with comparing the patients vitamin D levels to the correlated Brain Z-score to find any significance between the two fields.
Objective

To determine if higher Vitamin D levels - being associated with the regulation of the immune system - will indicate a higher brain volume in pediatric MS, MOG positive (MOG+), and MOG negative (MOG-) patients compared to their peers with lower Serum 25(OH)D levels.

Methodology

Patients and Groups

Retrieving 59 patients from the Canadian Pediatric Demyelinating Disease Study (CPDDS) at the Children's Hospital of Philadelphia (CHOP), 12 patients were diagnosed with pediatric multiple sclerosis, 21 were diagnosed with MOG disease but had monophasic demyelination, and 26 patients were tested negative for the MOG antibody but did exhibit monophasic demyelination. The study sites include multiple locations in Canada and the United States. Originally, the proposal called for two determined by the Serum 25(OH)D level - below 50 nmol/L and above 50 nmol/L - however due to the amount of patients in this research project, the two subgroups were eliminated.

Timeline

The proposal for this project was presented to the Children’s Hospital of Philadelphia Department of Neurology in August of 2019 and approval was granted by the department and later the IRB in late September of 2019. The analysis of the data collected from the CPDDS was concluded in early March of 2020 and results were presented to the board of Neurology at CHOP with final comments and edits made to this specific research project.

Vitamin D Months

Vitamin D levels were collected at the onset of the disease or attack in MS, MOG+, and MOG- patients and were measured in nano moles over liters (nmol/L). The month in which Serum 25(OH)D levels were collected was not predetermined and therefore was an independent variable when considered for further analysis.
Brain Volume Z-Scores

A Z-Score in statistical terms is a determination of how far a value is from the mean of a certain population (StatisticsHowTo, 2020). When determining brain volume, doctors and researchers use a Z-score to get a mean brain volume from all socioeconomic backgrounds of a particular age and compare this to a patient’s brain volume. If the Z-Score is negative, that indicates a patient's brain volume is lower than the mean of the mainstream population of that age. A positive Brain Z-score indicates that the patient’s brain volume is higher than the mean of the mainstream population of that age.

Python and Excel

Python is one of the main languages to computer programming and is being utilized by the general public to produce a multitude of graphs and run statistical analysis. Python was used to create histogram charts to find normal distribution in the three groups - pediatric multiple sclerosis, MOG disease positive, and MOG disease negative - and to run a Dunn test on Vitamin D levels at onset. Microsoft Excel was utilized to produce the scatter plots and box plots.

Statistical Analysis

A Kruskal–Wallis Test with Dunn was utilized to analyze Serum 25(OH)D levels between MS, MOG+, and MOG- groups to indicate any substantial difference between one another. Another two Kruskal–Wallis Tests (the equivalent of a One-Way ANOVA) were used to see whether Brain Z-Scores from onset and after twelve months among the three groups - MS, MOG+ and MOG- - had a significant difference with one another. Significance of all three Kruskal–Wallis Tests was defined by an alpha value of 0.05 or less. From this, six Linear Regression tests, utilizing the 95% confidence level, were conducted in order to determine the relationship of Vitamin D levels at onset versus Brain Volume at onset (0-3 months) and after 12 months (10-14 months). R-squared values and trendlines from these six tests were graphed on scatter plots to identify any correlation between the given parameters.
Results

There were a total of 59 patients (n = 59) with twelve being diagnosed with multiple sclerosis (n = 12), twenty-one were diagnosed with MOG disease but all were monophasic (n = 21), and twenty-six tested negative for MOG disease but had a monophasic demyelination (Figure 5). In the MS group, there were a total of 8 females and 4 males (Figure 5). In the MOG+ group, there were 11 females and 10 males (Figure 5). In the MOG- group, there were 13 females and 13 males (Figure 5). Due to age not being normally distributed, the first, median, and third quartiles were reported (Figure 6). For the multiple sclerosis set of patients, the median age was reported to be 13.51 years with the first quartile being 11.84 years and the third being 15.12 years (Figure 7). In the MOG+ set of patients, the median age was reported to be 8.97 years with the first quartile being 6.73 years and the third being 10.33 years (Figure 7). In the MOG- set of patients, the median age was reported to be 12.02 years with the first quartile being 9.62 years and the third being 13.54 years (Figure 7).

25-hydroxyvitamin D levels were collected at the onset of the demyelinating attack. Due to the data revealing no normal distribution for all three groups, the first quartile, the median, and third quartile were reported (Figure 8). In the pediatric multiple sclerosis population, Vitamin D levels had a median value of 43.65 nmol/L with the first quartile reporting 27.50 nmol/L and the third quartile reporting 66.43 nmol/L (Figure 9). In the MOG positive population, Serum 25(OH)D levels had a median value of 56.00 nmol/L with the first quartile reporting 34.30 nmol/L and the third quartile reporting 77.87 nmol/L (Figure 9). In the MOG negative population, Vitamin D levels had a median value of 58.60 nmol/L with the first quartile reporting 45.73 nmol/L and the third quartile reporting 73.30 nmol/L (Figure 9). At the time these Vitamin D levels were taken, the MS population demonstrated no skewness to either summer months, from April - September, or winter months, from October - March. For the MOG+ population, there was no skewness to either the summer or winter months (Figures 10 & 11). However for the MOG- population, 17 out of the 26 patients Serum 25(OH)D levels were taken during the winter months (Figure 12).

Brain Z-Scores were taken at two separate occasions: one at onset (0-3 months) and another after 12 months (10-14 months). Due to the data revealing no normal distribution for all
three groups at either onset or 12 months, the first quartile, the median, and third quartile were reported (Figures 13 & 14). For the MS group at onset, Brain Z-Scores revealed a median value of -0.77 with the first quartile being -1.11 and the third quartile being -0.18 (Figure 15). For the MOG+ group at onset, Brain Z-Scores revealed a median value of -0.72 with the first quartile being -1.41 and the third quartile being -0.22 (Figure 15). For the MOG- group at onset, Brain Z-scores revealed a median value of -0.58 with the first quartile being -1.21 and the third quartile being 0.10 (Figure 15). In the multiple sclerosis population after 12 months, Brain-Z scores indicated a median value of -0.90 with the first quartile reporting -1.30 and the third quartile reporting -0.04 (Figure 16). In the MOG positive population after 12 months, Brain Z-Scores indicated a median value of -0.79 with the first quartile reporting -1.33 and the third quartile reporting -0.15 (Figure 16). In the MOG negative population after 12 months, Brain Z-Scores indicated a median value -0.55 with the first quartile reporting -0.96 and third quartile reporting 0.21 (Figure 16).

A Kruskal–Wallis Test with Dunn was performed on initial Vitamin D levels among the MS, MOG+, and MOG- group. This revealed a P-value of 0.3848, indicating no significance in the Serum 25(OH)D levels among the three groups (Figure 9). The Dunn test performed on this Kruskal-Wallis test revealed a P-value of 0.92 for MS versus MOG+, a P-value of 0.16 for MS versus MOG-, and a P-value of 0.85 for MOG+ vs MOG- (Figure 9). All three P-values indicate no significance between either combination of groups in terms of Vitamin D levels at onset. Another two Kruskal-Wallis tests were performed on Brain Z-Scores at onset and after 12 months among the three groups. The Kruskal-Wallis test for the onset Brain Z-score values revealed a P-value of 0.6670, indicating no significance in this regard (Figure 15). The Kruskal-Wallis test for the 12 month follow up Brain-Z Scores revealed a P-value of 0.4991, indicating no significance in this regard (Figure 16).

Six Linear Regression Tests were conducted to compare Serum 25(OH)D levels to Brain Z-Scores at onset and the 12 month follow up for the MS, MOG+, and MOG- groups. The first linear regression test compared initial Vitamin D levels to Brain Z-Scores at onset in the MS group which revealed a P-value of 0.9715, indicating no significant relationship (Figure 17). The R-squared value of this specific test yielded a value of 0.0001 which indicates a weak
correlation. Once graphed in a scatter plot, the trendline revealed to have a positive correlation. The second linear regression test compared initial Serum 25(OH)D levels to Brain Z-scores at onset in the MOG+ group which revealed a P-value of 0.1627, indicating no significant relationship (Figure 18). The R-squared value of this test yielded a value of 0.0999 which indicates a weak correlation. Once graphed in a scatter plot, the trendline revealed to have a positive correlation. The third linear regression test compared initial 25-hydroxyvitamin D levels to Brain Z-Scores at onset in the MOG- group which revealed a P-value of 0.3870, indicating no significant relationship (Figure 19). The R-squared value of this test yielded a value of 0.0313 which indicates a weak correlation. Once graphed in a scatter plot, the trendline revealed to have a positive correlation. The fourth linear regression test compared initial Vitamin D levels to Brain Z-Scores at the 12 month follow up in the MS group which revealed a P-value of 0.9071, indicating no significant relationship (Figure 20). The R-squared value of this test yielded a value of 0.0014 which indicates a weak correlation. Once graphed in a scatter plot, the trendline revealed to have a positive correlation. The fifth linear regression test compared initial Serum 25(OH)D levels to Brain Z-Scores at the 12 month follow up in the MOG+ group which revealed a P-value of 0.4815, indicating no significant relationship (Figure 21). The R-squared value of this test yielded a value of 0.0264 which indicates a weak correlation. Once graphed in a scatter plot, the trendline revealed to have a positive correlation. The final linear regression test compared initial 25-hydroxyvitamin D levels to Brain Z-Scores at the 12 month follow up in the MOG- group which revealed a P-value of 0.2066, indicating no significant relationship (Figure 22). The R-squared value of this test yielded a value of 0.0656 which indicates a weak correlation. Once graphed in a scatter plot, the trendline revealed to have a positive correlation.
Discussion

Out of the total of 59 patients (n = 59) in this study, 12 had pediatric multiple sclerosis, 21 were tested positive for MOG disease but were monophasic, and 26 that tested negative for MOG but exhibited monophasic demyelination (Figure 5). While male and female patients in the MOG+ and MOG- group exhibited even consistency, 11 females and 13 females respectively, the MS group demonstrated that 8 patients were female. This can be inferred by the fact that as many as 4 females have multiple sclerosis to every male that has the demyelinating - the loss of myelin - disease (Purdy, 2014). Researches are still investigating the cause of the disparity in the sex ratio among multiple sclerosis patients but a new study has found that a protein called sphingosine-1-phosphate receptor 2 (S1PR2) that controls the permeability of the blood brain barrier is more prevalent in female multiple sclerosis patients than their male counterparts when analyzing mice and autopsies of deceased MS patients (Cruz-Orengo et al., 2014). The median age for MS, MOG+, and MOG- patients was 13.51 years, 8.97 years, and 12.02 years respectively (Figure 7). In regards to pediatric MS patients, this age of onset - the first signs of symptoms - corresponds to recent reports displaying that only 3-5% of people living with MS are diagnosed before the age of 16 but after the age of 10 (Weatherspoon, 2018). In MOG+ and MOG- patients, all of them had a monophasic demyelinating event. The median age of 8.97 years matches the interquartile range in a recent study on pediatric MOG+ patients that had a range of 6.2 - 13.9 years (Waters et al., 2019). The median age of 12.02 years matches most children diagnosed with a monophasic event as a study noted the age of its participants in the range between 3.8–16.5 years old (Aubert-Broche et al., 2017).

Pediatric Multiple Sclerosis patients reported a median 25-hydroxyvitamin D level of 43.65 nmol/L while patients that tested positive for MOG disease and negative for MOG disease reported close Serum 25(OH)D levels of 56.00 nmol/L and 58.60 nmol/L respectively (Figure 9). Observing lower vitamin D values in MS patients than in other demyelinating diseases is supported by evidence noting that in past studies, patients with multiple sclerosis observed lower vitamin D values than controls (Alharbi, 2015). Higher Serum 25(OH)D levels in multiple sclerosis patients have been associated with inhibiting the role of activated myelin reacting CD4+ T cells which could limit the number of relapses a patient observes (Aranow, 2011).
Yadava 11

Pediatric MS patients \((n = 12)\) did not have any skewness towards the winter or summer months when analyzing Vitamin D levels at onset and the same holds true to the patients that tested positive for MOG disease \((n = 21)\) (Figures 10 & 11). By the even consistency of measurements throughout the year when patients had their first attack, the first to third quartile vitamin D levels reported are most probable to be around the same if 25-hydroxyvitamin D concentrations are taken at any point in the year. However, 17 MOG negative patients \((n = 26)\) Vitamin D levels were taken during the duration of October through February (Figure 12). During winter months, especially in northern latitude countries, the human population observed lower Serum 25(OH)D count than in the summer months (Figure 4) (Bjarnadottir, 2017). If MOG- patients vitamin D levels at onset had an even spread in which months their serum count was analyzed or had a skewness towards the summer months, the median value of 58.60 nmol/L would most likely be higher, giving evidence that lower vitamin D count is a possible risk factor when diagnosing patients with pediatric multiple sclerosis.

Brain Z-Scores observed at onset in MS patients reported a median value of -0.77. Similarly, MOG+ and MOG- patients observed onset median Brain Z-scores of -0.72 and -0.58 respectfully (Figure 15). In pediatric onset-MS patients, Brain Z-scores in one study found an average value of \(-1.09 \pm 1.49\) (Kerbrat et al., 2012). Negative Brain Z-scores have also been noted for patients that tested both positive and negative for the MOG disease in several studies. Median Brain Z-Scores observed after 12 months in MS patients was -0.90 compared to MOG+ and MOG- patients Brain Z-scores after 12 months which were -0.79 and -0.55 respectively (Figure 16).

A Krushal-Wallis test revealed no significance in Vitamin D values among MS, MOG+, and MOG- patients. A Dunn tested followed to reveal no significance in Serum 25(OH)D levels in MS vs MOG+, MS vs MOG-, and MOG+ vs MOG- (Figure 9). Reduced vitamin D levels have been associated with the risk of developing pediatric multiple sclerosis as seen with the results (Banwell et al., 2011). Following this, higher vitamin D levels at onset were exhibited in monophasic patients than their recurrent Central Nervous System (CNS) disease counterparts (Mealy et al., 2012). However, there have been no specific studies to date conducted on the analysis of vitamin D levels in patients with MOG disease (Koduah, Paul & Dörr, 2012). Further
evidence on MOG disease patients can help enhance the explanation for no significance in Vitamin D levels. Two further Krushal-Wallis tests were conducted on Brain Z-scores at onset and after 12 months and both tests indicated no significance among pediatric multiple sclerosis, MOG positive, and MOG negative groups (Figures 15 & 16). While median Brain Z-Scores in this study found -0.77 at onset and -0.90 at the 12 month follow up, recent research has found pediatric multiple sclerosis patients having Global Brain Z-Scores one standard deviation lower than normal healthy participants (Waldman et al., 2014). Negative Global Brain Z-scores have also been found in MOG+ and MOG- patients. Brain atrophy is one of the classical signs of monophasic demyelination, MOG disease, and multiple sclerosis (Aubert-Broche et al., 2017).

Three Linear Regression Tests were performed to compare Brain Z-scores at onset with 25-hydroxyvitamin D concentrations collected at onset in the three groups: pediatric multiple sclerosis, MOG positive, and MOG negative. All three linear regression tests revealed no significance between higher Vitamin D levels to higher Brain Z-Scores in MS, MOG+, and MOG- patients (Figures 17-19). Serum 25(OH)D levels have shown no meaningful association to brain volume in multiple sclerosis patients (Table 3) (Mowry et al., 2018). However, other studies have found that after a year or more from the initial Vitamin D count, brain volume was higher in those that had serum levels above 50 nmol/L than those with less than 50 nmol/L (Ascherio et al., 2014). Another three linear regression tests were performed to compare Brain Z-scores after 12 months with Serum 25(OH)D concentrations collected at onset in the three groups: pediatric multiple sclerosis, MOG positive, and MOG negative. All three linear regression tests revealed no significance between higher Vitamin D levels to higher Brain Z-Scores in MS, MOG+, and MOG- patients (Figures 20-22). From the University of Buffalo, only 18.3% with Relapsing Remitting MS (RRMS) were sufficient in Serum 25(OH)D levels and those with Vitamin D deficiency had less brain atrophy compared to their healthy and RRMS but Serum 25(OH)D sufficient peers (University at Buffalo, 2010).

One major limitation in this research came about in the total and group level n values - number of patients. The study only consisted of 59 patients of which 12 were diagnosed with pediatric multiple sclerosis, 21 with MOG disease, and 26 that had monophasic demyelination but tested negative for MOG antibody (Figure 5). This low number of patients, especially in the
pediatric multiple sclerosis group, could have influenced results when it came time to run linear regression tests comparing 25-hydroxyvitamin D concentrations to Brain Z-scores at onset and after 12 months. More patients in all three groups could have led to possibly stronger associations to the hypothesis of higher Serum 25(OH)D levels leading to higher brain volumes due to the fact that positive correlations were noticed in all six linear regression tests.

In the future, more recruitment of patients can assist in further analysis of pediatric multiple sclerosis, MOG positive, and MOG negative Brain Z-scores compared to Vitamin D levels collected at onset. While the Brain Z-scores after 12 months provided vital information when it came time to analyze the three groups with the onset Serum 25(OH)D levels, further follow ups can provide in even more detail of how Vitamin D levels at onset can predict Brain Z-Scores in MS, MOG+, and MOG- pediatric patients. When the next opportunity arrives to conduct a similar study, more patients and further analysis of the effects of onset Vitamin D levels on brain volume will yield more accurate conclusions. The next phase after suggestions are made will be to look at onset 25-hydroxyvitamin D concentrations compared to lesion volumes in MS, MOG+, and MOG- patients to investigate if Vitamin D levels can serve as a predictor to lesion volume at onset and at several follow ups.
Conclusion

A conducive analysis concludes that ages and population dynamics were consistent in previous studies. Vitamin D levels were lower in pediatric multiple sclerosis patients than in MOG positive and MOG negative patients and MOG negative patients observed a skewness towards the winter months when it came time to analyze onset Vitamin D levels suggesting that the 58.60 nmol/L observed is at the lower end than normally observed. However, a Kruskal-Wallis Test with Dunn revealed no significance in Serum 25(OH)D levels among the three groups and between one another. Another two Kruskal-Wallis Tests revealed no significance in terms of Brain Z-scores at onset and after 12 months among MS, MOG+, and MOG- patients. The six linear regression tests that compared 25-hydroxyvitamin D concentrations to Brain Z-scores at onset and after 12 months in all three groups revealed no significance but positive trends were observed. In the following days, more patients and follow ups along with investigating lesion volume to onset Vitamin D levels will yield precise conclusions.

Acknowledgements

A big thank you to the Children’s Hospital of Philadelphia (CHOP) Department of Neurology for allowing me access to analyze part of the Canadian Pediatric Demyelinating Disease Study (CPDDS) and for assisting in IRB approval. Thank you to my advisors and teachers for providing support, resources, and extra time to complete my assignments while I was conducting this project at CHOP. Thank you to the parents of all participants in the study for entrusting the staff at CHOP and in turn giving me the privilege of using this data to help in the betterment in diagnosis and next steps in getting closer to finding a cure to demyelinating diseases like pediatric multiple sclerosis and MOG disease. Finally, thank you to my own parents, brother, intermediate and extended family members for always giving me advice and support throughout the duration of this project.
References


USDA. (2020). Appendix 12. Food Sources of Vitamin D. Retrieved from


Figures

Figure 1: The diagram comes from Villa Medica that demonstrates which parts of the body are affected by multiple sclerosis and specifically goes into which part of the nerves are affected. Organs like the Optic Nerve and Spinal Curve are examples of areas multiple sclerosis targets. Vision impairment, muscle atrophy, slurring, fatigue and even depression are symptoms of the demyelinating disease.

Figure 2: From the World Health Organization (WHO) in 2008, the world map indicates the prevalence of multiple sclerosis around the world per 100,000 people. Blue indicates no information, green is 0-5 people, yellow is 5-20 people, orange is 20-60 people, red is 60-100
people, and dark red indicates over 100 people. Some countries with the highest MS rates include Canada, the United States, Germany, Australia, etcetera.

**Figure 3:** The infographic from Healthline displays some of the risk factors and certain populations multiple sclerosis (MS) can affect. The ratio of Multiple Sclerosis patients is almost four females to every one male. As of right now, scientists do not know the reason behind the gender disparity but recent studies on the blood brain barrier could finally solve this mystery. Nearly 15% of people have one or more relatives with MS and if an identical twin has MS, there is a 33% chance the other twin will also develop multiple sclerosis.

**Figure 4:** From “Vitamin D and multiple sclerosis: An update”, the global map indicates the amount of sufficient UVB ray exposure during the year. Countries in the Northern latitude do not experience as much sufficient UVB rays than those near the equator, providing evidence to Vitamin D deficiency and possible development of multiple sclerosis.
Figure 5: In the study, there were a total of 59 patients (n = 59). In the pediatric multiple sclerosis group (n = 12), there were 4 males, light orange, and 8 females, dark orange. In the MOG disease group (MOG+) (n = 21), there were 10 males, light blue, and 11 females, dark blue. In the MOG negative group (MOG-) (n = 26), there were 13 males, light green, and 13 females, dark green.

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>MOG+</th>
<th>MOG-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>4</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 6: Using Python, density curves were applied to the histograms to find normal distribution among the ages in the three groups: Pediatric Multiple Sclerosis (orange), MOG Positive (blue), and MOG negative (green). None of the groups demonstrated normal distribution in regard to ages. Due to this, the Interquartile Range (IQR) - the first quartile, median, and third quartile - were reported.
Figure 7: Due to the data not being normally distributed, the Interquartile Range for ages was reported. The Pediatric MS group, orange, had a median age of 13.51 years with the first quartile being 11.84 years and third quartile being 15.12 years. In the MOG+ group, blue, the median age was 8.97 years with the first quartile reporting 6.73 years and the third quartile reporting 10.33 years. The MOG- group, green, reported a median age of 12.02 years with the first quartile being 9.62 years and the third being 13.54 years.

Figure 8: Using Python, density curves were applied to the histograms to find normal distribution among Vitamin D levels - measured in nanomoles over liters (nmol/L) - in the three groups: Pediatric Multiple Sclerosis (orange), MOG Positive (blue), and MOG negative (green). None of the groups demonstrated normal distribution in regard to Serum 25(OH)D levels. Due to this, the Interquartile Range (IQR) - the first quartile, median, and third quartile - were reported.
Figure 9: Due to the data not being normally distributed, the Interquartile Range for Vitamin D levels in nmol/L was reported. The Pediatric MS group, orange, had a median Serum 25(OH)D concentration of 43.65 nmol/L with the first quartile being 27.50 nmol/L and the third quartile being 66.43 nmol/L. In the MOG+ group, blue, the median 25-hydroxyvitamin D concentration was reported to be 56.00 nmol/L with the first quartile reporting 34.30 nmol/L and the third quartile reporting 77.87 nmol/L. The MOG- group, green, reported a median Vitamin D level of 58.60 nmol/L with the first quartile being 45.73 nmol/L and the third being 73.30 nmol/L. A Kruskal-Wallis Test was performed and revealed a P-value of 0.3848, indicating no significance. A Dunn test was performed to find significant relationships between groups. MS vs MOG+ revealed a P-value of 0.9200, MS vs MOG- revealed a P-value of 0.1600, and MOG+ vs MOG- revealed a P value of 0.8500, each of them indicating no significant relationship.

Figure 10: In the pediatric multiple sclerosis group (n = 12), the month of measurement for Vitamin D levels was collected at the onset of the disease. The data presented had an even spread between summer months and winter months with Serum 25(OH)D levels being collected in January for 3 patients, April for 1 patient, May for 4 patients, June for 3 patients, and October for 1 patient.
**Figure 11:** In the MOG positive disease group (n = 21), the month of measurement for Vitamin D levels was collected at the onset of the disease. The data presented had an even spread between summer months and winter months with Serum 25(OH)D levels being collected in January for 2 patients, February for 1 patient, March for 2 patients, May for 3 patients, June for 1 patient, July for 2 patients, August for 2 patients, October for 4 patients, and November for 4 patients.

**Figure 12:** In the MOG negative disease group (n = 26), the month of measurement for Vitamin D levels was collected at the onset of the disease. The data presented did not have even spread between summer months and winter months with Serum 25(OH)D levels being collected in January for 7 patients, February for 2 patient, April for 1 patient, May for 1 patient, June for 2 patients, July for 3 patients, September for 2 patients, October for 6 patients, and November for 2 patients. In the October - February timeframe, 17 out of 26 patients 25-hydroxyvitamin D concentrations were collected suggesting that the median value of 58.60 nmol/L could be higher if the months were normally distributed.
Figure 13: Using Python, density curves were applied to the histograms to find normal distribution among Brain Volume Z-Scores at onset (0-3 months) in the three groups: Pediatric Multiple Sclerosis (orange), MOG Positive (blue), and MOG negative (green). None of the groups demonstrated normal distribution in regard to Brain Volume Z-Scores. Due to this, the Interquartile Range (IQR) - the first quartile, median, and third quartile - were reported.

Figure 14: Using Python, density curves were applied to the histograms to find normal distribution among Brain Volume Z-Scores after 12 months (10-14 months) in the three groups: Pediatric Multiple Sclerosis (orange), MOG Positive (blue), and MOG negative (green). None of the groups demonstrated normal distribution in regard to Brain Volume Z-Scores. Due to this, the Interquartile Range (IQR) - the first quartile, median, and third quartile - were reported.
Figure 15: Due to the data not being normally distributed, the Interquartile Range for Brain Volume Z-Scores at onset was reported. The Pediatric MS group, orange, had a median Brain Volume Z-Score of -0.77 with the first quartile being -1.11 and the third quartile being -0.18. In the MOG+ group, blue, the median Brain Volume Z-Score was reported to be -0.72 with the first quartile reporting -1.41 and the third quartile reporting -0.22. The MOG- group, green, reported a median Brain Volume Z-Score -0.58 with the first quartile being -1.21 and the third being 0.10. A Kruskal-Wallis Test was performed and revealed a P-value of 0.6670, indicating no significance.

Figure 16: Due to the data not being normally distributed, the Interquartile Range for Brain Volume Z-Scores after 12 months was reported. The Pediatric MS group, orange, had a median Brain Volume Z-Score of -0.90 with the first quartile being -1.30 and the third quartile being -0.04. In the MOG+ group, blue, the median Brain Volume Z-Score was reported to be -0.79 with the first quartile reporting -1.33 and the third quartile reporting -0.15. The MOG- group, green, reported a median Brain Volume Z-Score -0.55 with the first quartile being -0.96 and the third being 0.21. A Kruskal-Wallis Test was performed and revealed a P-value of 0.4991, indicating no significance.
Figure 17: The correlation between Brain Z-Scores at onset to initial Vitamin D levels in the pediatric multiple sclerosis group (n = 12) from the CPDDS at the Children’s Hospital of Philadelphia. The trendline between these two variables had an R-squared value of 0.0001, indicating a positive yet weak correlation. The regression test following the plotting of the data revealed a P-value of 0.9715, suggesting no significant relationship between Brain Volume and Serum 25(OH)D levels.

Figure 18: The correlation between Brain Z-Scores at onset to initial Vitamin D levels in the pediatric MOG positive group (n = 21) from the CPDDS at the Children’s Hospital of Philadelphia. The trendline between these two variables had an R-squared value of 0.0999, indicating a positive yet weak correlation. The regression test following the plotting of the data revealed a P-value of 0.1627, suggesting no significant relationship between Brain Volume and Serum 25(OH)D levels.
**Figure 19:** The correlation between Brain Z-Scores at onset to initial Vitamin D levels in the pediatric MOG negative group \((n = 26)\) from the CPDDS at the Children’s Hospital of Philadelphia. The trendline between these two variables had an R-squared value of 0.0313, indicating a positive yet weak correlation. The regression test following the plotting of the data revealed a P-value of 0.3870, suggesting no significant relationship between Brain Volume and Serum 25(OH)D levels.

**Figure 20:** The correlation between Brain Z-Scores at the 12 month follow up to initial Vitamin D levels in the pediatric multiple sclerosis group \((n = 12)\) from the CPDDS at the Children’s Hospital of Philadelphia. The trendline between these two variables had an R-squared value of 0.0014, indicating a positive yet weak correlation. The regression test following the plotting of the data revealed a P-value of 0.9071, suggesting no significant relationship between Brain Volume and Serum 25(OH)D levels.
**Figure 21:** The correlation between Brain Z-Scores at the 12 month follow up to initial Vitamin D levels in the pediatric MOG positive group (n = 21) from the CPDDS at the Children’s Hospital of Philadelphia. The trendline between these two variables had an R-squared value of 0.0264, indicating a positive yet weak correlation. The regression test following the plotting of the data revealed a P-value of 0.4815, suggesting no significant relationship between Brain Volume and Serum 25(OH)D levels.

**Figure 22:** The correlation between Brain Z-Scores at the 12 month follow up to initial Vitamin D levels in the pediatric MOG negative group (n = 26) from the CPDDS at the Children’s Hospital of Philadelphia. The trendline between these two variables had an R-squared value of 0.0656, indicating a positive yet weak correlation. The regression test following the plotting of the data revealed a P-value of 0.2066, suggesting no significant relationship between Brain Volume and Serum 25(OH)D levels.
Appendix

Table 1: The table comes from the National Institutes of Health (NIH) regarding sufficient and insufficient Serum 25(OH)D levels that are measured in nanomoles over liters (nmol/L) and nanograms over milliliters (ng/mL). Anything below 50 nmol/L or 20 ng/mL is considered inadequate Vitamin D levels that could lead to health related issues such as Rickets. Between 50 nmol/L - 125 nmol/L, 20 ng/mL - 50 ng/mL, sufficient Vitamin D levels are observed and the less likelihood of developing conditions. Above 125 nmol/L or 50 ng/mL is considered to have an excess amount of Serum 25(OH)D and could lead to conditions such as Hypervitaminosis.

| Table 1: Serum 25-Hydroxyvitamin D [25(OH)D] Concentrations and Health* |
|-----------------------------|-----------------|-------------------|
| nmol/L** | ng/mL* | Health status |
| <30 | <12 | Associated with vitamin D deficiency, leading to rickets in infants and children and osteomalacia in adults |
| 30 to <50 | 12 to <20 | Generally considered inadequate for bone and overall health in healthy individuals |
| ≥50 | ≥20 | Generally considered adequate for bone and overall health in healthy individuals |
| >125 | >50 | Emerging evidence links potential adverse effects to such high levels, particularly >150 nmol/L (> 60 ng/mL) |

* Serum concentrations of 25(OH)D are reported in both nanomoles per liter (nmol/L) and nanograms per milliliter (ng/mL).
** 1 nmol/L = 0.4 ng/mL

Table 2: The table comes from the National Institutes of Health (NIH) regarding sufficient foods to maintain or increase Serum 25(OH)D levels. The measurement is displayed in International Units (IU) and the Daily Value (DV) is given in percent (%) to see how much IU a person needs to sustain sufficient 25-hydroxyvitamin D concentrations. Some of the top foods people can take are Cod Liver Oil (1360 IU), Salmon (447 IU), and Milk (115-124 IU).

| Food Sources of Vitamin D |
|---------------------------|-----------------|------------------|
| Food | IU Per Serving | % Daily Value (DV) |
| Cod liver oil, 1 tablespoon | 1,360 | 340 |
| Swordfish, cooked, 3 ounces | 556 | 142 |
| Salmon (sockeye), cooked, 3 ounces | 447 | 112 |
| Tuna fish, canned in water, drained, 3 ounces | 154 | 39 |
| Orange juice fortified with vitamin D, 1 cup (check product labels) | 137 | 34 |
| Milk, nonfat, reduced fat, and whole, vitamin D fortified, 1 cup | 115-124 | 29-31 |
| Yogurt, fortified with 20% of the DV for vitamin D, 6 ounces | 80 | 20 |
| Margarine, fortified, 1 tablespoon | 60 | 15 |
| Sardines, canned in oil, drained, 2 sardines | 46 | 12 |
| Liver, beef, cooked, 3 ounces | 42 | 11 |
| Egg, 1 large (vitamin D is found in yolk) | 41 | 10 |
| Ready-to-eat cereal, fortified with 10% DV of vitamin D, 0.75-1 cup | 40 | 10 |
| Cheese, Swiss, 1 ounce | 6 | 2 |

Table 3: From Dr. Mowry and her colleagues’ study in 2018, the table shows the relationship between brain volume with body mass index BMI and 25-hydroxyvitamin D levels. All tests ran on the relationship between Serum 25(OH)D concentrations and Brain Volume revealed insigniance while Brain Volume and a patient’s BMI yielded significant, suggesting that Vitamin D may not affect brain volume as previously suspected.

<table>
<thead>
<tr>
<th>Table 3 Multivariate models depicting association of vitamin D levels and BMI with brain volume measures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>25-Hydroxyvitamin D level (per 10 ng/mL greater)</strong></td>
</tr>
<tr>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>-0.9 (-2.7 to 1.0) p = 0.36</td>
</tr>
<tr>
<td><strong>BMI (per 1 kg/m² greater)</strong></td>
</tr>
<tr>
<td><strong>Age (per year greater)</strong></td>
</tr>
<tr>
<td><strong>Female sex</strong></td>
</tr>
<tr>
<td><strong>Hispanic ethnicity</strong></td>
</tr>
<tr>
<td><strong>Smoker at baseline</strong></td>
</tr>
<tr>
<td><strong>Use of any DMT</strong></td>
</tr>
</tbody>
</table>

Abbreviations: BMI = body mass index; DMT = disease-modifying therapy; nBPV = normalized brain parenchymal volume; nGMV = normalized gray matter volume; nWMV = normalized white matter volume; SiENAX = Structural Image Evaluation Using Normalization of Atrophy-X. Values in parentheses are 95% confidence intervals.

Figure 23: From Dr. Alberto Ascherio’s study in 2014 that utilized the BENEFIT study to analyze Vitamin D as a predictor of adult multiple sclerosis. From years 1 to 5 following the onset of the disease, the percentage of brain volume loss (Z-Score) was more severe for patients having Serum 25(OH)D levels below 50 nmol/L than patients above 50 nmol/L. A P-value of 0.005 was displayed in the severity of Brain Z-Score loss between the two groups, indicating a significant relationship.
Figure 24: From Healthline, there are four types of multiple sclerosis but the vast majority - 85% - are diagnosed with relapsing-remitting multiple sclerosis (RRMS) at onset. If treated, transition to the more progressive types of MS such as primary progressive MS will take longer and if not, 50% of RRMS patients will go to secondary progressive MS (SPMS) within a decade of onset.
Python Code

```python
import matplotlib.pyplot as plt
import csv
import numpy as np
import pandas as pd
import seaborn as sns

y = '../input/VitD3New.csv'
med_df = pd.read_csv(y)

print(med_df.head())

sns.distplot(med_df['MOGP'], hist=True, kde=True, color='royalblue')
sns.distplot(med_df['MOGN'], hist=True, kde=True, color='limegreen')
sns.distplot(med_df['MS'], hist=True, kde=True, color='darkorange')
plt.title('Histogram of Vitamin D Levels')
plt.xlabel('Vitamin D Levels (nmol/L)')
plt.show()

sns.distplot(med_df['MSZ1'], hist=True, kde=True, color='darkorange')
sns.distplot(med_df['MOGPZ1'], hist=True, kde=True, color='royalblue')
sns.distplot(med_df['MOGNZ1'], hist=True, kde=True, color='limegreen')
plt.title('Histogram of Brain Volumes (0-3 Months)')
plt.xlabel('Brain Z Scores')
plt.show()

sns.distplot(med_df['MSZ2'], hist=True, kde=True, color='darkorange')
sns.distplot(med_df['MOGPZ2'], hist=True, kde=True, color='royalblue')
sns.distplot(med_df['MOGNZ2'], hist=True, kde=True, color='limegreen')
plt.title('Histogram of Brain Volumes (10-14 Months)')
plt.xlabel('Brain Z Scores')
plt.show()
```