Status of Vitamin D in Children with Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS)

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Executive Summary:

Numerous studies have indicated an association between vitamin D deficiency and the immune dysregulation and the pathogenesis of autoimmunity. An increased prevalence of vitamin D deficiency has been demonstrated in several pediatric autoimmune diseases, including autoimmune thyroid disease and Type-1 diabetes mellitus. This study was undertaken to determine the prevalence of vitamin D deficiency in children with PANS, an autoimmune disorder characterized by abrupt-onset neuropsychiatric symptoms. A retrospective chart review of 122 pediatric PANS patients at a single treatment center was performed. Data collected included 25-hydroxy vitamin D level, age, gender, and age of diagnosis. Vitamin D status was categorized as deficient (0-<20 ng/ml) , insufficient (20-<30 ng/ml) and sufficient (30-100 ng/ml). Vitamin D status was evaluated by prespecified demographic data, including age of PANS onset or diagnosis, gender, and deficiency range. 8% of patients were demonstrated to be deficient, 24% were insufficient, and 68% were sufficient. 32% of all patients were either deficient or insufficient. The odds ratio of vitamin D deficiency was 0.843505 [95% confidence interval (CI): -2.91 to 4.59] in PANS patients, when compared to deficiency, insufficiency, and sufficiency rates in the general US pediatric population. Vitamin D deficiency may be a risk factor for PANS in children. Supplementation of vitamin D may play a role in management or treatment of PANS.

Business Context:

Industry Description:

- This internship is categorized under the pediatrics and pharmaceutical industries. A pediatrician is a medical doctor that specializes in providing medical care to children. They manage the physical, behavioral, and mental care for children from birth until age 18. They are also trained to diagnose and treat a broad range of childhood illnesses. Concerning pharmacology, the pharmaceutical industry discovers, develops, manufactures, and markets drugs or pharmaceuticals for use as medications to be administered to patients, with the aim to cure them, vaccinate them, or alleviate the symptoms. As a whole, the industry is led by the drug discovery process in which professionals identify possible new drugs by examining their targets and corresponding effects.

Company Description:

- MASA Service Cooperation (SC) and MASA Food Allergy Center for Treatment is a small healthcare company that was founded in 2013. It has principal locations in Bloomington-Normal and Springfield, along with outreach offices that serve Central Illinois. MASA specializes in health care services and drug development in clinical trials within the medicinal industry. MASA offers services such as environmental and food allergy testing, allergy action plans, asthma care, sinusitis treatment, immunology, treatment for hives and contact dermatitis, etc. SWIA Clinical Research is the sister company of MASA and has been in business for over 25 years while conducting nearly 1000 research trials. It focuses on full-time clinical research in all phases of drug and medical device development.

Department/Product Description:

- As stated previously, this Internship is related to the pediatrics industry. The specific autoimmune disease that I am collecting data on is Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS) or Pediatric Acute-onset Neuropsychiatric Syndrome (PANS). As a reference, PANDAS is considered a subset of PANS. In general, PANS is often diagnosed with obsessive-compulsive disorder (OCD), eating disorder, and tic symptoms, but the sudden onset of symptoms separates PANS from these other disorders. Specifically, PANDAS also incorporates strep infections, such as strep throat or scarlet fever. PANDAS is diagnosed when the aforementioned symptoms happen overnight or out of the blue. Both PANDAS and PANS are immunological diseases associated with autoimmunity. For this internship, I am reviewing the association between vitamin D deficiency and the occurrences of PANDAS autoimmune disease in children.

Business Project Description:

Leading up to the internship, I researched peer-reviewed case studies and reports as background literature to develop my familiarity with the topic I am researching. As a student intern, I analyzed the status of vitamin D in children with PANS. In order to examine this phenomenon, the research method that I used over the course of the internship was the MASA patient database which included hundreds of patients that ranged from various backgrounds. At the beginning of the internship, a typical I-Day consisted of me examining the MASA patient database to find information including 25-hydroxy vitamin D levels, age, gender, race, and age of diagnosis. I then populated a master spreadsheet with this data. After collecting and stratifying the data into graphs, I began my analysis by calculating an odds ratio (OR) by cross-analyzing my data with a similar case-control study. An example of a spreadsheet that I have worked on is shown below:

Age PANDA Dx	Gender	Vitamin D, 25-hydroxy (Before supplementation)	Vitamin D Supplement (Yes/No)	Age Category	Vitamin D Category
5	M	43, nl	Yes	11 and under	Sufficient
9	F	19.5, Lo	Yes	11 and under	Deficient
10	M	21(>20), nl Lo	Yes	11 and under	Insufficient
11	F	45.5	No	11 and under	Sufficient
3	F	68.9	No	11 and under	Sufficient
9	M	30.5	No	11 and under	Sufficient
16	F	36.5	No	Age 12-17	Sufficient
16	M	21.7(30-100), Lo	No	Age 12-17	Insufficient
11	F	26.3 (30-100) Lo	Yes	11 and under	Insufficient
17	M	92	Yes	Age 12-17	Sufficient
5	M	34	Yes	11 and under	Sufficient
7	M	54	No	11 and under	Sufficient
11	F	94	Yes	11 and under	Sufficient
12	F	32, nl	No	Age 12-17	Sufficient
7	F	46.3(30-100)	Yes	11 and under	Sufficient
6	M	20, Lo	No	11 and under	Insufficient
7	F	27.2(30-100), Lo	Yes	11 and under	Insufficient
9	F	40(30-100)	No	11 and under	Sufficient
10	M	33 (30-100)	No	11 and under	Sufficient
10	F	32.5 ng/ml	No	11 and under	Sufficient
6.5	F	34.8(30-100)	No	11 and under	Sufficient
8	F	54	Yes	11 and under	Sufficient
5.8	M	31	Yes	11 and under	Sufficient
11.8	M	54	Yes	Age 12-17	Sufficient
10.5	M	30	No	11 and under	Sufficient
3	M	33	No	11 and under	Sufficient
10.5	M	33.5	Yes	11 and under	Sufficient
5	M	22.8, Lo (it is no	Yes	11 and under	Insufficient
9	M	52.1(30-100), hi	No	11 and under	Sufficient
7	F	37 ng/ml	No	11 and under	Sufficient

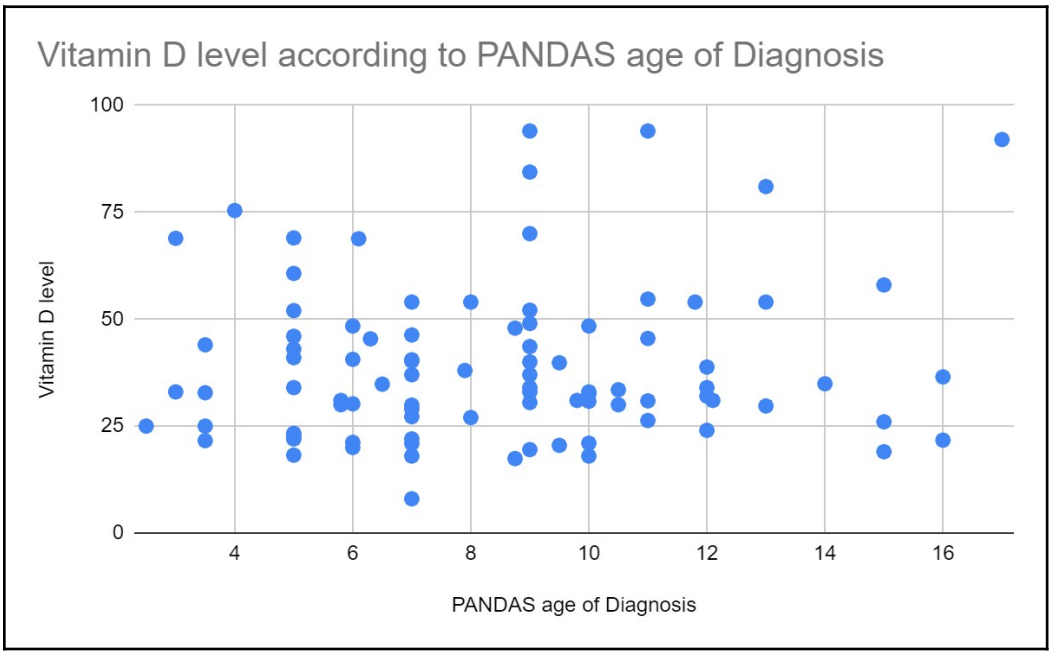
Demographic data of 122 total patients was collected. Data from 91 patients is displayed in the following graphs as either vitamin D 25-hydroxy levels for 31 patients could not be found or the patients exceeded the age limit.

Table 1. Prevalence of vitamin D status by age and gender.

	Deficient	Insufficient	Sufficient	Total (n=91)
Child (<12 yo)	6 (8%)	18 (24%)	51 (68%)	75
Adolescent (12-17 yo)	1 (6.3%)	4 (25%)	11 (68.8%)	16
Male	4 (8.3%)	12 (25%)	32 (66.7%)	48
Female	3 (7%)	10 (23.3%)	30 (69.8%)	43
Total	**7 (7.7%)**	**22 (24.2%)**	**62 (68.1%)**	**91**

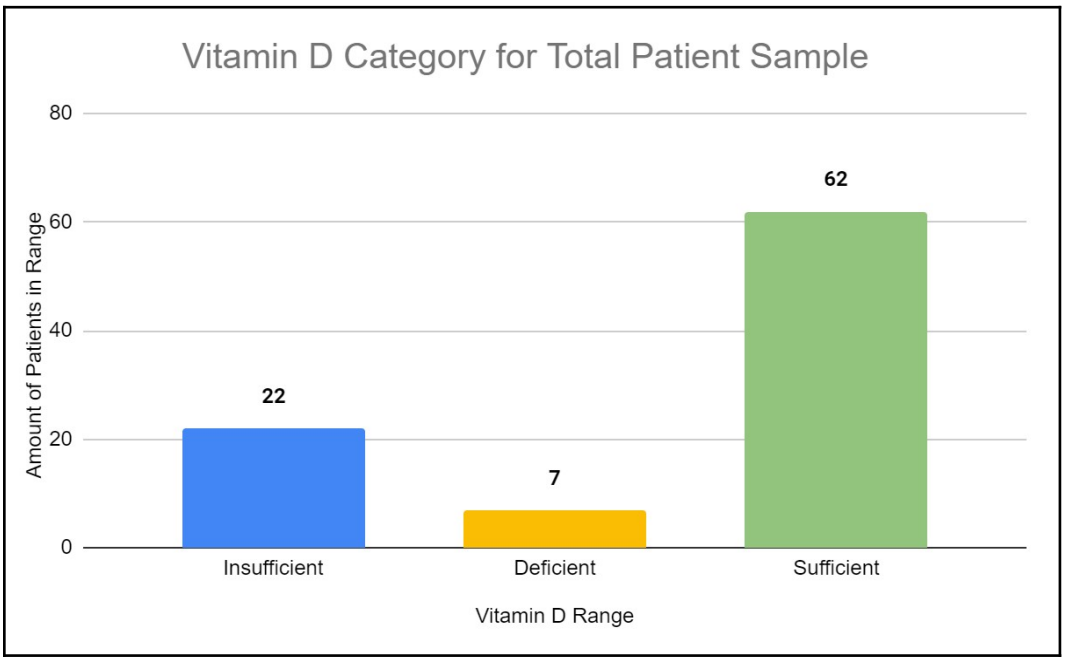
This table showcases the vitamin D status by age and gender. 24.2% of my sample is categorized as insufficient, 7.7% as deficient, and 68.1% as sufficient.

Figure 1. Age at PANS/PANDAS diagnosis vs. 25-hydroxy vitamin D.



This scatter plot showcases the age at which the patients were diagnosed with PANDAS in relation to their current vitamin D level.

Figure 2. Vitamin D status of PANS/PANDAS pediatric population



This graph showcases the number of patients and their respective vitamin D categories. 22 patients were determined to be insufficient, 7 were determined to be deficient, and 62 were determined to be sufficient.

Table 2. Prevalence of vitamin D status in the PANS/PANDAS cohort compared with the prevalence in a recently studied in a general US pediatric population (Kumar 2009 *Pediatrics*).

	PANS/PANDAS Cohort	US Child Cohort
Vitamin D Deficiency	7.7%	9%
Vitamin D Insufficiency	24.2%	61%

Odds Ratio: 0.843505 **95% CI (-2.91 to 4.59)**

The Prevalence of vitamin D status is shown in the PANS/PANDAS cohort compared with the prevalence in a recently studied in a general US pediatric population (Kumar 2009 *Pediatrics*). The odds ratio comparing the two cohorts is noted, indicating a lower rate of vitamin D deficiency or insufficiency in the PANS/PANDAS cohort studied, versus the general US pediatric population.

Business Project Research:

As mentioned earlier, my business research was driven by the question to determine if there is an association between vitamin D and children with PANS. There is much literature already surrounding the relationship between vitamin D deficiency and the development of autoimmune diseases. A comprehensive review of the implication of vitamin D and autoimmunity even indicated that "increasing evidence demonstrates a strong association between vitamin D signaling and many biological processes."[1]A recent study has even discovered that vitamin D supplementation with calcitriol could prevent both the initiation and progression of autoimmune diseases such as experimental autoimmune encephalomyelitis, collagen-induced arthritis, and experimental models of multiple sclerosis and rheumatoid arthritis.[2] Most children consume less than recommended amounts of vitamin D. In 2009, an observational study assessed the prevalence and association of vitamin D deficiency in children with a nationally representative sample of children aged 1 to 21 years. In this study, the researchers measured serum 25(OH)D deficiency and insufficiency (25[OH]D <15 ng/mL and 15–29 ng/mL, respectively). In terms of the general United States population, 9% (representing 7.6 million US children and adolescents) of the sample were 25(OH)D deficient and 61% (representing 50.8 million US children and adolescents) were 25(OH)D insufficient.[3]

Regarding the methodology used to explore my business research question, a chart review was performed retrospectively from a single PANS/PANDAS treatment center. Data from patients who presented to the specialty clinic for evaluation of PANS/PANDAS was collected.

1 In Annotated Bibliography
2 In Annotated Bibliography
3 In Annotated Bibliography

Additional information collected included 25-hydroxy vitamin D levels, age, gender, race, and age of diagnosis. Vitamin D status was stratified into three groups; deficiency (0 to < 20 ng/ml), insufficiency (20 to < 30 ng/ml), and sufficiency (30-100 ng/ml). Patients were also stratified into two age groups; child (< 12 years old) and adolescent (12-17 years old).

Overall, 24.2% of my sample are categorized as insufficient, 7.7% as deficient, and 68.1% as sufficient. The odds ratio calculation was 0.843505 with a confidence interval from -2.91 to 4.59, indicating a lower rate of vitamin D deficiency or insufficiency in the PANS/PANDAS cohort studied, versus the general US pediatric population. Results indicate that our patient population of children with PANS/PANDAS had a lower risk of vitamin D deficiency and insufficiency status than those of the general US pediatric population regardless of their age or gender. The results are inconsistent with the high prevalence of vitamin D deficiency/insufficiency status reported in children with other autoimmune diseases.

Regarding previous Internship Business Reports, I have been able to increase sample size and completely analyze the data that I collected since I am fortunate enough to have been able to research this phenomenon for a long period of time.

Business Project Key Learnings & Recommendations:

1. Through this experience, I developed an appreciation of data collection and analysis. As all of my findings depended on the source data I gathered from the MASA patient database, it was imperative that I collected the data correctly, accurately, and consistently. As the internship progressed, I had to create multiple spreadsheets with hundreds of rows of data; thus, it was crucial that the data in each spreadsheet correlated with data in past spreadsheets. In order to maintain consistency and accuracy, I learned much about the various formulas that a researcher can use in google sheets. These functions saved me so much time in collecting data. That said, I also learned about time management. Data collection requires sufficient time even for small amounts of data. For example, collected data for 30 rows of cells would usually require two hours to complete. Due to this time requirement, I learned to allocate certain times during my day to simply populate the spreadsheets.

2. Furthermore, I learned that there are multiple factors that can influence the outcome of a scientific research study. Even though myself and my research mentors were surprised by the negative correlation that we found, we understood that there are many variables that contributed to this outcome. In our case, these variables included outside vitamin D supplementation and sample ethnicity.

3. I also learned to collaborate with professionals in the field and hone my presentation skills. Fortunately, my paper was published in abstract form in the February 2022 online supplement of the Journal of Allergy and Clinical Immunology. Additionally, I was chosen to present my research at the American Academy of Allergy Asthma and Immunology annual conference to professionals in the pharmaceutical industry and beyond in Phoenix, Arizona in February.

4. I only have one small recommendation for my business mentor. Even though I am unfamiliar with data collection and analysis in general, the collection overall would have been easier in Excel rather than google sheets since Excel offers a multitude of more in-depth functions. Despite this minute reservation, I am very satisfied with this internship because it has given me experience in meeting deadlines, time management, and collaborating with professionals as explained previously.

Annotated Bibliography

Yang, C. Y., Leung, P. S., Adamopoulos, I. E., & Gershwin, M. E. (2013). The implication of vitamin D and autoimmunity: a comprehensive review. *Clinical reviews in allergy & immunology*, *45*(2), 217–226. https://doi.org/10.1007/s12016-013-8361-3

The authors of this study provide further evidence to validate the idea that vitamin D plays a pivotal role in gene regulation and expression and the development of autoimmune diseases. The authors examine the relationship between vitamin D and several autoimmune diseases including Multiple Sclerosis, Type 1 Diabetes, and Systemic Lupus Erythematosus. Based on this research, the authors found that vitamin D can regulate gene expression and further exert its immunomodulatory effects on immune cells due to its unique binding capability.

Dankers, W., Colin, E. M., van Hamburg, J. P., & Lubberts, E. (2017). Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential. *Frontiers in immunology*, *7*, 697. https://doi.org/10.3389/fimmu.2016.00697

The authors of this study also provide further evidence to validate the idea that vitamin D
plays a pivotal role in gene regulation and expression and the development of autoimmune diseases. As well as examining the relationship between vitamin D and autoimmune diseases, this study goes in-depth to examine the relationship between vitamin D and cellular functions including the molecular pathways used by vitamin D in order to find new potential therapeutic targets.

Kumar, Juhi et al. “Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004.” *Pediatrics* vol. 124,3 (2009): e362-70. doi:10.1542/peds.2009-0051

This study was used in the cross-analysis with my data as it provides vitamin D deficiency statistics of the US children population. It found that nearly 9% (representing 7.6 million US children and adolescents) of the US population were 25(OH)D deficient and 61% (representing 50.8 million US children and adolescents) were 25(OH)D insufficient.

Appendices:

An odds ratio (OR) was used to compare the relative odds of vitamin D deficiency in the general United States population given the exposure to the PANDAS/PANS autoimmune diseases. An OR was chosen instead of a risk ratio (RR) as this study reviews existing data and examines the exposure to an intervention factor in relation to a predetermined outcome.

The formula used to calculate the OR is as follows where:

a = Number of exposed cases

b = Number if exposed non-cases

c = Number of unexposed cases

d = Number of unexposed non-cases

$$¿ = \frac{a/c}{b/d} = \frac{ad}{bc}$$

$$¿ = \frac{(n)\,exposed\ cases / (n)\,unexposed\ cases}{(n)\,exposed\ non-cases / (n)\,unexposed\ non-cases}$$

$$¿ \frac{(n)\,exposed\ cases \times (n)\,unexposed\ non-cases}{(n)\,exposed\ non-cases \times (n)\,unexposed\ cases}$$

Upper and lower confidence intervals were calculated to determine the range that the OR can fall between. These intervals indicate the accuracy of the calculated OR.

The formula used to calculate the confidence intervals is as follows:

$$Upper\ 95\%\ CI = e\text{^}[ln\ (OR) + 1.96\sqrt{\ (1/a + 1/b + 1/c + 1/d)}]$$
$$Lower\ 95\%\ CI = e\text{^}[ln\ (OR) - 1.96\sqrt{\ (1/a + 1/b + 1/c + 1/d)}]$$

In the case of this study, the variables mean the following:

a = Patients with vitamin D deficiency/insufficiency and with PANDAS

b = Patients without vitamin D deficiency/insufficiency and with PANDAS

c = General population with vitamin D deficiency/insufficiency and without PANDAS

d = General population without vitamin D deficiency/insufficiency and without PANDAS