THE EFFECT OF NEUROTOXINS IN VACCINES AND PREGNANCY FACTORS ON AUTISM SPECTRUM DISORDER

Tyler Youn

ABSTRACT: This study proposal aims to evaluate the relative strength of associations of the neurotoxin exposure from vaccinations and the parental health factors with the risk of autism spectrum disorder in offspring. The prevalence of autism spectrum disorder has recently increased in the U.S. Infants aged up to 6 months old in the U.S. have 14.7 to 49 times greater neurotoxin exposure than the U.S. safety limits from parental aluminum-adjuvanted vaccines. The thimerosal exposure from vaccines for healthy infants younger than 7 months increased from 75 μg in 1990 to 187.5 μg in 1999 and healthy children younger than 2 years had thimerosal exposure increased from 100 μg in 1990 to 237.5 μg in 1999. A 74% increased risk of autism spectrum disorder in offspring was found from pre-gestational diabetes. Children of overweight mothers indicated a 28% higher risk of autism spectrum disorder and children of obese mothers showed a 36% higher risk relative to children of mothers at normal weight based on body mass index. An 18% higher risk of autism spectrum disorder in offspring for every ten year increase in maternal age and a 21% higher risk for increase in paternal age were found. A case-control study design will be implemented evaluating non-probability convenience sampling from three hospitals in Washington State. A sample of 120 children with autism spectrum disorder and 180 children without autism spectrum disorder will be studied. Autism spectrum disorder is mostly acquired as early as 2 years of age and children between 2 and 5 years of age will be included in the study. Controls will be matched to cases on gender, birth weight, and residence in order to accurately measure the associations of the parental health factors and neurotoxin exposure with autism spectrum disorder for public health planning and implementation of autism etiology.

Introduction

Autism spectrum disorder (ASD) is a group of developmental disorders that involves repetitive patterns of behavior, cognitive dysfunction, and social impairment (Mohamed et al., 2015). Fombonne (2009) found that the prevalence of ASD in the U.S. has increased significantly in recent decades. The number of recommended pediatric vaccinations in the U.S. increased from 10 shots in the late 1970s to 32 shots in 2010 (Tomljenovic, 2011). As the prevalence of ASD and the number of recommended vaccinations has increased during the same period, the causal relationship between vaccines and ASD has been debated over time.

Aluminum, a commonly used vaccine adjuvant in hepatitis B vaccine, is a neurotoxin that may cause abnormalities during brain development in infants and children (Tomljenovic & Shaw, 2011b). Higher risk of ASD diagnosis was found among male neonates that received hepatitis B vaccines in the U.S. (Gallagher & Goodman, 2010). Similar to aluminum adjuvants, thimerosal preservatives in measles-mumps-rubella (MMR) vaccine was intensively debated due to neurotoxicity of thimerosal. However, many studies have revealed no significant association between MMR vaccination and ASD (Mrozek-Budzyn, Kiełtyka, & Majewska, 2010).

On the other hand, studies indicated that pregnancy factors may increase the prevalence of ASD. Maternal diabetes may play a significant role in ASD in offspring (Xu, Jing, Bowers, Liu, & Bao, 2014). An increased risk of ASD in offspring was reported for overweight and obese mothers (Wang, Tang, Xu, Weng, & Liu, 2016). An increase of ASD prevalence was found in advanced parental age groups (Wu et al., 2016).

Washington State had a pertussis epidemic in 2011 and 2012 with one of the highest vaccine exemption rates in the U.S. largely influenced by parents' fear of vaccine adverse effects such as ASD (Wolf, Opel, Dehart, Warren, & Rowhani-Rahbar, 2014). Therefore, this proposal aims to provide mixed research findings about neurotoxin exposure and describe how pregnancy factors play a role in prevalence of ASD. This proposal's research question is how are neurotoxin exposure and pregnancy factors associated with ASD in Washington State? The hypothesis is that a combined influence of neurotoxin exposure and pregnancy factors are positively associated with ASD in Washington State.

Background

Neurotoxin Exposure

Thimerosal preservatives that contain a neurotoxin, ethyl mercury, were commonly used in vaccines including MMR vaccine; the U.S. Public Health Service agreed to remove thimerosal preservative from pediatric vaccinations in 1999 (Schechter & Grether, 2008). Madsen et al. (2002) demonstrated that no positive association between MMR vaccination and ASD was found. Furthermore, Mrozek-Budzyn et al. (2010) found a lower risk of ASD among children vaccinated with MMR compared to children not vaccinated with MMR. On the other hand, there are studies that indicated possible evidences for the association of ASD with aluminum- adjuvanted vaccines such as hepatitis B vaccine.

Cumulated aluminum exposure from pediatric vaccines and ASD prevalence in the U.S. had a remarkably positive linear correlation from 1991 to 2008 (Tomljenovic & Shaw, 2011b). Tomljenovic and Shaw (2011b) showed that ASD prevalence was also highly correlated with the number of aluminum-adjuvanted vaccines given to infants aged 3 to 18 months old in Western countries including U.S., UK, Canada, Australia, Sweden, Iceland, and Finland. A significantly lower blood aluminum concentration was found in typically developed children compared to autistic children in Jamaica (Rahbar et al., 2016). Similarly, research revealed a lower level of aluminum in the hair of typically developed children compared to children with ASD (Mohamed et al., 2015).

Research indicated that newborns and infants are highly vulnerable to neurotoxins during early development of the central nervous system (CNS) (Tomljenovic & Shaw, 2011a). Based on the U.S. immunization guideline, infants aged up to 6 months old in the U.S. have 14.7 to 49 times greater aluminum exposure than the U.S. Food and Drug Administration safety limits from parental aluminum-adjuvanted vaccines such as hepatitis B (Tomljenovic & Shaw, 2011a). Gallagher and Goodman (2010) found that male neonates in the U.S. who received hepatitis B vaccines during the first month of life had a three times higher risk of an ASD diagnosis compared to boys who did not receive it. However, research not only suggested that the benefits of vaccination outweigh the risks of ASD but it also did not find the causal relationship between hepatitis B vaccine and ASD due to an insufficient sample size and missing vaccination records (Gallagher & Goodman, 2010).

Cumulative dose of thimerosal exposure from pediatric vaccines in the U.S. continued to increase and was greater than safety standard by 1999 (Schechter & Grether, 2008). The thimerosal exposure from recommended vaccines for healthy infants younger than 7 months increased from 75 μg in 1990 to 187.5

μg in 1999 (Schechter & Grether, 2008). Similarly, healthy children younger than 2 years had thimerosal exposure that increased from 100 μg in 1990 to 237.5 μg in 1999 (Schechter & Grether, 2008). After thimerosal was removed from or reduced in all pediatric vaccines, healthy infants younger than 7 months had less than 17.9 μg in 2004 while healthy children younger than 2 years had less than 40.2 μg (Schechter & Grether, 2008). Despite the exclusion or reduction of thimerosal in pediatric vaccines, the prevalence of ASD in 3- to 5-year-old children continued to increase from 1990 to 2004 (Schechter & Grether, 2008).

Pregnancy Factors and Confounders on ASD

Xu et al. (2014) revealed a significant association between maternal diabetes and ASD in offspring. Research found a 74% increased risk of ASD for pre-gestational diabetes and a 43% increased risk of gestational diabetes respectively (Xu et al., 2014). Research suggested that exposure to hyperglycemia caused by maternal diabetes may cause hypoxia in the fetus (Eidelman & Samueloff, 2002). As a result, oxygen supply for fetus may be insufficient and may cause a higher risk of ASD in offspring (Burstyn, Sithole, & Zwaigenbaum, 2010).

Based on body mass index (BMI), Wang et al. (2016) found a 28% higher risk of developing ASD among children of overweight mothers and a 36% higher risk for children of obese mothers compared to children of mothers at normal weight; in contrast, research showed no evidence for the association between maternal underweight and risk of ASD. Additionally, Anderson et al. (2005) suggested that maternal obesity may increase neurodevelopmental disorders associated with the CNS birth defects in offspring from abnormal metabolism due to insulin resistance and hyperinsulinemia. Wu et al. (2016) revealed a positive association between advanced parental age and risk of ASD through meta-analysis. Research found an 18% higher risk of ASD in offspring for every ten- year increase in maternal age and a 21% higher risk for increase in paternal age (Wu et al., 2016). Younger mothers had a 10% reduced risk of ASD in offspring, while younger fathers had a 20% reduced risk of ASD in offspring (Wu et al., 2016). Durkin et al. (2008) indicated that mothers over 35 years old and fathers over 40 years old are more likely to have children with ASD. Additionally, older mothers had a 41% increased risk of ASD in offspring and older fathers had a 55% increased risk of ASD in offspring (Wu et al., 2016). Higher rate of abnormal fetal growth and preterm birth in advanced maternal age was associated with increased risk of ASD (Abel et al., 2013).

Mrozek-Budzyn et al. (2010) indicated potential confounders on ASD. Mothers of children with autistic symptoms took medication such as antibiotics and antihypertensive drugs more often during pregnancy compared to mothers of typically developed children (Mrozek-Budzyn et al., 2010). Autistic children had significantly more prenatal injuries than children without ASD (Mrozek-Budzyn et al., 2010). Gestation time less than 38 weeks was found significantly more often among autistic children (Mrozek-Budzyn et al., 2010).

Concerns of Early Childhood Vaccination in Washington State

Henrikson et al. (2017) found that 42.2% of mothers at their baby's birth and 33.8% of mothers of toddlers at 24 months in Washington State were concerned about severe side effects from early childhood vaccination. In 2015, 34.2% of mothers of newborns and 24.9% of mothers at their children age 24 months in Washington State were concerned about safety of early childhood vaccination (Henrikson et. al., 2017). Research indicated that 27.4% of mothers of newborns and 22.8% of mothers of children age 24 months were concerned whether a vaccine would be effective in preventing the disease (Henrikson et al., 2017).

It appears that the public's distrust of vaccinations in Washington State caused low vaccination rates in past years. Also, studies indicated that there are pregnancy factors and potential confounders associated with ASD. Therefore, this research aims to address how neurotoxin exposure and pregnancy factors are associated with ASD in Washington State through a case-control study design.

Methods

Study Design

This proposal will conduct a case-control study with a group of children diagnosed with ASD and a group of children without diagnoses of ASD and other disorders with symptoms similar to ASD. This research design will provide an adequate assessment of ASD prevalence in children by evaluating parental health factors and the level of neurotoxin exposure from childhood immunizations.

Participants and Sample Design

A state-wide sample of 120 children diagnosed with ASD and 180 children without any symptoms or diagnoses of ASD in Washington State between 2 and 5 years of age will be studied since most cases of ASD are acquired as early as 2 years of age (Martínez-Pedraza & Carter, 2009). ASD signs or symptoms before 2 years of age will be considered congenital dysfunction and those participants will be excluded from the study in order to accurately measure the outcome potentially influenced by neurotoxin exposure from vaccines. The parental health factors in both cases and controls will be evaluated and compared with neurotoxin exposure to determine the relative strength of associations with ASD. The sampling method will be non-probability convenience sampling from two hospitals in King County and one hospital in Snohomish County. Cases and controls will be recruited from those three hospitals and controls will be matched to cases on gender, birth weight, and residence. Those three hospitals will be contacted for data collection.

Ethical Considerations

Participation in this proposal will be voluntary and freedom of choice will be assured for all participants. More importantly, children are a vulnerable population and informed consent forms will be provided to parents of all participants to ensure beneficence and confidentiality. Consent forms will be reviewed and signed by parents prior to participation and any questions regarding this study will be thoroughly explained to parents along with the review of consent forms. Additionally, all researchers and assistants in this proposed study will receive certified Health Insurance Portability and Accountability Act (HIPAA) training prior to conducting research. All information and collected data will be confidential and strictly used for the purpose of this research. This proposal will be reviewed by the Institutional Review Board from hospitals and funders.

Measures and Variables

This proposal aims to determine the relative strength of associations of the level of neurotoxin exposure from vaccination and the presence of parental health factors with prevalence of ASD in young children. Immunization records of cases and controls will be evaluated and ASD symptoms or diagnoses will be assessed by reviewing healthcare providers' comments in participants' medical records. Parental health factors will be assessed by reviewing parents' medical records during pregnancy. The diagnoses of diabetes, BMI, age and medication history of parents will be evaluated to determine pregnancy factors. This proposal has a multivariate analysis with independent, dependent, and other important variables. The independent variables are neurotoxin exposure from vaccines and pregnancy factors while the dependent variables are ASD symptoms or diagnoses. There are other important variables to consider such as neurotoxin exposure from parents' occupation and participants' residence areas. Parents will be contacted via phone and emails for HIPAA authorization for surveys

regarding occupation information. Parents will be followed up via phone and emails and surveys will be provided through emails or delivered to parents' healthcare providers' offices for physical pick up. Surveys will be collected electronically via emails, by mail, or at healthcare providers' offices. Surveys will have an open-ended question such as, "Could you please describe your job title and duties?" Any responses associating with potential risks of neurotoxin exposure, such as a factory manager position with duties of manufacturing heavy metal equipment, will be considered to have influences on the outcome. Data regarding participants' residence areas will be assessed by reviewing participants' addresses in medical records. Geographical data collected such as zip codes will be used to indicate any potential neurotoxin exposure from environment such as nuclear facilities in the area.

Procedures and Analysis

This proposal will have multivariate analysis with aforementioned variables. The medical record departments of all three hospitals will be contacted via phone, email, and in-person visits. Upon approval by the hospitals, participants' medical records will be examined. Based on participants' age, this research will assess 2 to 5 years of medical records up to 10 times within 6-month intervals for each assessment. The level of neurotoxin exposure from each vaccine will be assessed by analyzing the list of the vaccine excipient provided by the Centers for Disease Control and Prevention (cdc.gov, 2011). The total amount of accumulated neurotoxin in each participant will be measured and this data will be added to pregnancy factors of corresponding participant to indicate which independent variable is more positively associated with dependent variables. A combination of both quantitative and qualitative analyses will be used in this proposal. The majority of medical record reviews will be quantitative analysis, and the review of healthcare providers' and parents' comments regarding ASD symptoms and diagnoses will be qualitative analysis.

Discussion

Significance

Thimerosal preservatives and aluminum adjuvants in vaccines can be toxic to CNS and potentially contribute to various forms of disorders. While thimerosal preservatives were mostly removed from vaccines, aluminum adjuvants are continuously used in many different types of vaccines. Due to neurotoxicity of aluminum adjuvants that may associate with neurological complications, the continued use of aluminum adjuvants in pediatric vaccines may be considered of great concern.

Pregnancy complications such as advanced parental age, maternal diabetes and obesity may also have a positive influence on ASD. Therefore, it is important to study a combined influence of pregnancy factors and neurotoxin exposure from vaccinations and aforementioned environmental factors. The findings in this research will help provide a more in-depth understanding of rapidly increasing ASD prevalence for healthcare providers and parents. This research will further establish better patient education and vaccine safety in public.

Limitations

This research has some important limitations to consider. Since most data will be collected and analyzed based on participants' medical records retrospectively, any errors or misrepresentation in medical records will contribute to inaccuracy and unreliability of data analysis. Surveys will have open-ended questions and it may take a greater amount of time and effort to have data comparisons and statistical analysis. Furthermore, this research will have a non-probability convenience sampling method in order to acquire a large number of medical records. Therefore, data may not be fully generalized to the entire population of Washington State. However, this limitation may not significantly affect the overall evaluation of data, since data will be collected from three hospitals in two of the most densely populated counties in Washington State.

Future Research

Neurotoxin and other harmful chemical exposure may come from food consumption, breast milk, and reproductive technologies, but most previous studies did not provide a combined influence of various environmental factors. There are other predictors to consider such as maternal smoking and genetic risk factors. Additionally, cultural and religious factors may play a role in vaccination rates and pregnancy factors. Since this proposed study is solely focused on the population of Washington State, a larger population or different geographical areas are also considered for future research. Therefore, future research could explore the aforementioned potential risk factors and a larger population with broader social aspects to provide a more in-depth analysis for ASD.

References

Abel, K. M., Dalman, C., Svensson, A. C., Susser, E., Dal, H., Idring, S., . . . Magnusson, C. (2013). Deviance in fetal growth and risk of autism spectrum disorder. The American Journal of Psychiatry,170(4), 391-398. Retrieved from http://alliance- primo.hosted.exlibrisgroup.com/UW:all:TN_medline23545793

Anderson, J. L., Waller, D., Canfield, M. A., Shaw, G. M., Watkins, M. L., & Werler, M. M. (2005). Maternal obesity, gestational diabetes, and central nervous system birth defects. *Epidemiology*, 16(1), 87-92. Retrieved from http://alliance-primo.hosted.exlibrisgroup.com/UW:all:TN_jstor_archive_2920486004

Burstyn, I., Sithole, F., & Zwaigenbaum, L. (2010). Autism spectrum disorders, maternal characteristics and obstetric complications among singletons born in Alberta, Canada. *Chronic Diseases in Canada*, 30(4), 125-134. Retrieved from http://alliance-primo.hosted.exlibrisgroup.com/UW:all:TN_proquest1112239486

Centers for Disease Control and Prevention. (2011, February 22). *Ingredients of vaccines - fact sheet.* Retrieved from https://www.cdc.gov/vaccines/vac-gen/additives.htm

Durkin, M. S., Maenner, M. J., Newschaffer, C. J., Lee, L., Cunniff, C. M., Daniels, J. L., . . . Schieve, L. A. (2008). Advanced parental age and the risk of autism spectrum disorder. *American Journal of Epidemiology*, 168(11), 1268-1276. Retrieved from http://alliance-primo.hosted.exlibrisgroup.com/UW:all:TN_pubmed_central2638544

Eidelman, A. I., & Samueloff, A. (2002). The pathophysiology of the fetus of the diabetic mother. *Seminars in Perinatology*, 26(3), 232-236. Retrieved from http://alliance-primo.hosted.exlibrisgroup.com/UW:all:TN_sciversesciencedirect_elsevierS0146- 0005(02)80059-3

Fombonne, E. (2009). Epidemiology of pervasivedevelopmental disorders. *Pediatric Research*, 65(6), 591-598. doi:10.1203/pdr.0b013e31819e7203

Gallagher, C. M., & Goodman, M. S. (2010). Hepatitis B vaccination of male neonates and autism diagnosis, NHIS 1997–2002. *Journal of Toxicology and Environmental Health*, Part A, 73(24), 1665-1677. doi:10.1080/15287394.2010.519317

Henrikson, N., Anderson, M., Opel, D., Dunn, J., Marcuse, E., & Grossman, D. (2017). Longitudinal Trends in Vaccine Hesitancy in a Cohort of Mothers Surveyed in Washington State, 2013-2015. *Public Health Reports*, 132(4), 451-454.

Madsen, K. M., Hviid, A., Vestergaard, M., Schendel, D., Wohlfahrt, J., Thorsen, P., . . . Melbye, M. (2002). A population-based study of measles, mumps, and rubella vaccination and autism. *The New England Journal of Medicine*, 347(19), 1477-1482. doi:10.1056/NEJMoa021134

Martínez-Pedraza, F. de L., & Carter, A. S. (2009). Autism spectrum disorders in young children. *Child and Adolescent Psychiatric Clinics of North America*, 18(3), 645-663.doi:10.1016/j.chc.2009.02.002

Mohamed, F. E., Zaky, E. A., El-Sayed, A. B., Elhossieny, R. M., Zahra, S. S., Eldin, W. S., . . . Youssef, A. M. (2015). Assessment of hair aluminum, lead, and mercury in a sample of autistic Egyptian children: Environmental risk factors of heavy metals in autism. *Behavioural Neurology,* 2015, 1-9. doi:10.1155/2015/545674

Mrozek-Budzyn, D., Kiełtyka, A., & Majewska, R. (2010). Lack of association between measles-mumps-rubella vaccination and autism in children: A case-control study. *The Pediatric Infectious Disease Journal*, 29(5), 397-400. doi:10.1097/inf.0b013e3181c40a8a

Rahbar, M. H., Samms-Vaughan, M., Pitcher, M. R., Bressler, J., Hessabi, M., Loveland, K. A., . . . Boerwinkle, E. (2016). Role of metabolic genes in blood aluminum concentrations of Jamaican children with and without autism spectrum disorder. *International Journal of Environmental Research and Public Health*, 13(11), 1095. doi:10.3390/ijerph13111095

Schechter, R., & Grether, J. K. (2008). Continuing increases in autism reported to California's developmental services system: Mercury in retrograde. *Archives of General Psychiatry*, 65(1), 19-24. Retrieved from http://alliance-primo.hosted.exlibrisgroup.com/UW:all:TN_ama482546

Tomljenovic, L. (2011). Aluminum and Alzheimer's disease: After a century of controversy, is there a plausible link? *Journal of Alzheimer's Disease*, 23(4), 567-598.doi:10.3233/JAD-2010-101494

Tomljenovic, L., & Shaw, C. A. (2011a). Aluminum vaccine adjuvants: Are they safe? *Current Medicinal Chemistry*, 18(17), 2630-2637. Retrieved from http://homeoint.ru.91-194-77-203.web10.garmtech.net/pdfs/Aluminum_adjuvants.pdf

Tomljenovic, L., & Shaw, C. A. (2011b). Do aluminum vaccine adjuvants contribute to the rising prevalence of autism? *Journal of Inorganic Biochemistry*, 105(11), 1489-1499. doi:10.1016/j.jinorgbio.2011.08.008

Wang, Y., Tang, S., Xu, S., Weng, S., & Liu, Z. (2016). Maternal body mass index and risk of autism spectrum disorders in offspring: A meta-analysis. *Scientific Reports*, 6(1). doi:10.1038/srep34248

Wolf, E. R., Opel, D., Dehart, M. P., Warren, J., & Rowhani-Rahbar, A. (2014). Impact of a pertussis epidemic on infant vaccination in Washington state. *Pediatrics*,134(3), 456 64. doi:10.1542/peds.2013-3637

Wu, S., Wu, F., Ding, Y., Hou, J., Bi, J., & Zhang, Z. (2016). Advanced parental age and autism risk in children: a systematic review and meta-analysis. *Acta Psychiatrica Scandinavica*, 135(1), 29-41. doi: 10.1111/acps.12666

Xu, G., Jing, J., Bowers, K., Liu, B., & Bao, W. (2014). Maternal diabetes and the risk of autism spectrum disorders in the offspring: A systematic review and meta-analysis. *Journal of Autism and Developmental Disorders*, 44(4), 766-775. doi:10.1007/s10803-013-1928-2