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EXPLORING PRENATAL RISK FACTORS AMONG HISPANIC CHILDREN WITH AUTISM SPECTRUM DISORDERS. – A PILOT STUDY IN THE RIO GRANDE VALLEY

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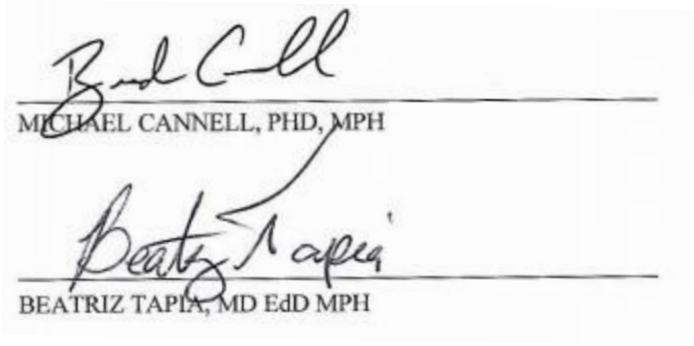

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Background: Peer-reviewed studies suggest that a hostile gestational environment is associated with delayed neurological development in infants. Autism spectrum disorder (ASD) is estimated to affect 1 in 59 children in the United States; however, few studies have specifically investigated risk factors for autism in Hispanic children. **Purpose**: The University of Texas RGV Hispanic Autism Research Center (HARC) studied Hispanic children with ASD and their biological mothers to identify potential environmental exposures and prenatal risk factors that could impact the risk for ASD through preliminary research in the Rio Grande Valley. **Methods**: This pilot study recruited 25 Hispanic mothers and their biological children with autism and 25 Hispanic control mothers and children. Participants completed a twelve-section survey that included specific prenatal risk factors, such as illnesses and medications taken during pregnancy. The goal was to evaluate if specific maternal prenatal risk factors are associated with a higher prevalence of ASD among Hispanic children living on the US-Mexico border. **Results**: Descriptive analysis showed a higher proportion of vaginal bleeding and urinary tract infections (UTIs) during gestation in

mothers of children with autism than control mothers. We also found evidence of an association between ASD in children and mothers who experience vaginal bleeding, UTIs, and allergies. No significant inferences can be drawn of maternal medicines taken during gestation and ASD in the child. **Conclusion**: This pilot study adds to the body of research suggesting maternal inflammation contributes towards increasing autism risk. The results are consistent with peer-reviewed studies that included a varied racial/ethnic study population, suggesting no difference in risk factors between Hispanic women and women of other races.

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BACKGROUND

Literature Review

Autism

Autism spectrum disorder (ASD) is a neurodevelopmental disability that can cause significant social, communication, and behavioral challenges (CDC, 2018). Symptoms are typically detected during childhood and sustain throughout life. The condition was initially called “autism”, but identification of the disorder was put on a spectrum in the early 1990s when it became apparent that autism could not be defined by a specific set of symptoms or severity (CDC, 2018). Symptoms range from communicational and social deficits and repetitive behaviors, such as a child not responding when their name is called, avoiding eye contact, or trouble adapting to routine changes (CDC, 2018). The prevalence of ASD is currently estimated at 1 in 59 United States children, affecting approximately 4 times as many males as females (Baio, Wiggins, & Christensen, 2018). The causes of ASD are still unknown, but it is hypothesized to have moderate genetic heritability in combination with environmental factors (Hallmayer, et al., 2011).

Autism among Hispanic people

White non-Hispanic children have ASD prevalence rates 1.2x higher than Hispanic children Invalid source specified.. While genetics and environmental exposures have not been excluded as possible causes for the disparity, the most likely cause is underdiagnosis of ASD in Hispanic children (Palmer, Walker, Mandell, Bayles, & Miller, 2010). Hispanic families are more likely to be of lower socioeconomic status and lack health insurance or

financial resources to seek medical attention (Palmer, Walker, Mandell, Bayles, & Miller, 2010). Outside of economic determinants however, gaps in education and cultural differences may additionally contribute to the underdiagnosis of autism spectrum disorder in Hispanic children (Zuckerman, et al., 2017) (Ijalba, 2016).

In one study, Latinos with limited English proficiency disproportionately experienced barriers to seeking ASD diagnosis and treatment compared to Non-Latino Whites and Latinos with English proficiency (Zuckerman, et al., 2017). While all study groups reported the barrier "stress of the diagnostic process" most frequently, "parent knowledge about ASD" was the most common barrier in Latino families regardless of English proficiency. "Parent trust in providers" was a statistically significant more common barrier among Latinos with limited English proficiency than in Non-Latino Whites (Zuckerman, et al., 2017). This study demonstrates that English proficiency is a crucial indicator of overcoming barriers to seeking ASD diagnosis and treatment.

Several studies have found a lower level of trust in health institutions by racial/ethnic minority groups, specifically African Americans and Hispanic Americans, compared to Non-Hispanic Whites (Schwei, Kadunc, Nguyen, & Jacobs, 2014). Hispanics are three times as likely as non-Hispanic whites to lack a regular health care provider and 83% of Hispanic participants reported obtaining health information from media, with television being the dominant source (Pew Research Center, 2008). Some reasons for health institution distrust by Hispanics include a distrust in Western medicine, lack of cultural competency by medical providers, and fear of judgement by medical providers towards Hispanic culture and lifestyle (Julliard, et al., 2008). Zuckerman et al. stated, "families with higher maternal education and

ASD knowledge have better access to ASD care regardless of ethnicity". However, many Hispanic families originate from other countries with little awareness of ASD or few resources devoted to children with disabilities (Ijalba, 2016). A study found that all fourteen Hispanic mothers of children with ASD believed their child's condition was temporary (Ijalba, 2016). Therefore, a history of little ASD education combined with general distrust in Western providers contribute as barriers to seeking ASD diagnosis and treatment (Ijalba, 2016) (Zuckerman, et al., 2017) (Julliard, et al., 2008).

Additionally, cultural beliefs in the Hispanic community are believed to play an important role in the underdiagnoses of ASD in children, as evidenced in the Ijalba study that interviewed fourteen Hispanic mothers of children with ASD (Ijalba, 2016). The Hispanic community has a very strong relationship with the Catholic faith, and for this reason many mothers in the study relied on their faith or divine intervention to cure their child rather than seeking medical attention (Ijalba, 2016). Other Hispanic beliefs, such as *susto* and *familismo*, played roles in a mother not seeking treatment for her child. *Susto* is the Mexican belief that an acute traumatic or fearful event can cause illness (Ijalba, 2016). While a *susto* event can be obvious and physical, other events may be apparent only to the child. In the Ijalba study, four out of fourteen mothers reported believing an event of *susto* caused their child's ASD, with the results being immediate. One mother describes that her 18-month-old was developing normally until one day, "Suddenly, he turned to the window and saw something, he covered his eyes and cried and would not stop" (Ijalba, 2016). Upon waking up the next day, the child did not speak anymore and would eventually be diagnosed with ASD (Ijalba, 2016). *Familismo* is a defined as "the strong sense of interdependency and shared decision

making in many Hispanic families" and is an important cultural norm in many Hispanic communities (Ijalba, 2016). Mothers of children with ASD sought the advice of family members, such as grandmothers, who would assure that the child comes from a family history of delayed speech or insist that the mother wait longer before seeking professional attention. In the study, seven of the fourteen interviewed mothers were advised that "children can be late with language" or "father was a late talker" by family members (Ijalba, 2016).

Language barriers, lack of ASD education, and cultural beliefs all play a role in preventing Hispanic mothers from seeking medical attention for their child with ASD (Ijalba, 2016) (Zuckerman, et al., 2017). We will now delve into a hypothesis concerning the biological pathway maternal inflammation may play a role in ASD development in the child.

Maternal Inflammation

Maternal infection during gestation is a known risk factor for autism and has also been linked as a risk factor to other neurological disorders such as schizophrenia and cerebral palsy (Patterson, Immune involvement in schizophrenia and autism: Etiology, pathology and animal models, 2008). A proposed mechanism is that a maternal immune response to infection causes an elevation of pro-inflammatory cytokines, which activate a fetal inflammatory response that may interrupt proper neurodevelopment (Burd, Balakrishnan, & Kannan, 2012). Because the fetal immune system is underdeveloped and vulnerable to infection, the fetus begins gestation with an increased concentration of inflammatory proteins for protection that decrease with gestational age (Burd, Balakrishnan, & Kannan, 2012). Any imbalance of these inflammatory proteins could play a role in injuring a developing brain (Burd, Balakrishnan, & Kannan, 2012).

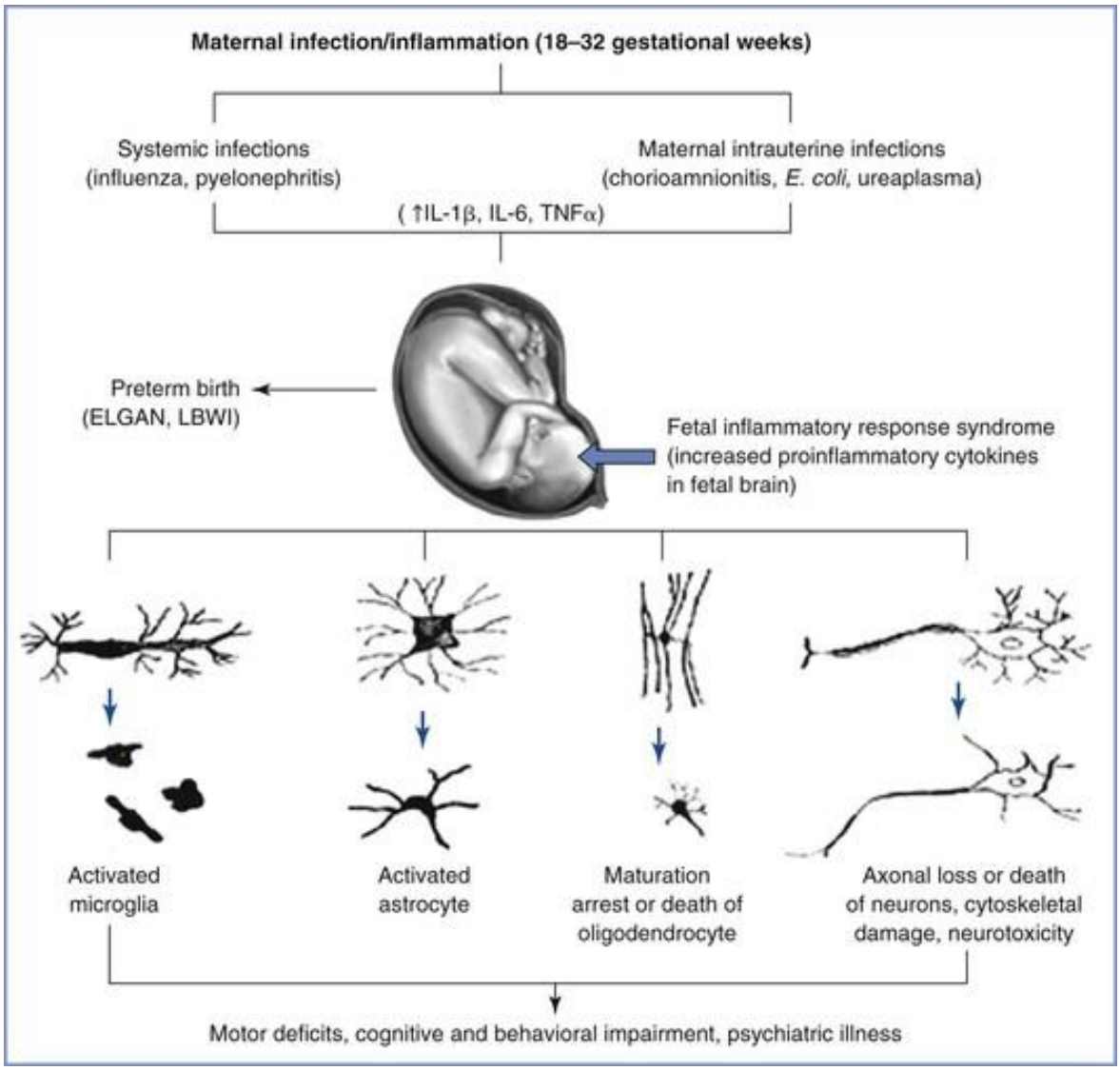
Scientists have identified key indicators of immune dysregulation in the brains of children with autism, including upregulation of cytokines and activated microglia and astrocytes. These indicators are found in the brains of children, suggesting an early initiation, as well as adult brains, which suggests a permanent state (Burd, Balakrishnan, & Kannan, 2012). Cytokines possibly act on the fetus through three different pathways: transplacental passage of maternal cytokines, placental production and secretion of cytokines, or fetal production of cytokines (Burd, Balakrishnan, & Kannan, 2012). Animal experiments have been able to demonstrate the effects of a "maternal inflammation risk factor" with cytokine releasing treatments that activate an immune response in mice, even in the absence of a pathogen (Patterson, 2011). Offspring of activated mice exhibited behavioral abnormalities consistent with ASD, including deficits in communication social interaction and elevated anxiety (Patterson, 2011).

The specific cytokine responsible is still in question. Increased levels of the cytokine IFN-γ were noted in the midgestational serum of mothers of children with ASD, with a 50% increased risk per one-unit increase (on the natural log scale) for development of ASD relative to typically developing controls. IFN-γ is involved in defense against intracellular pathogens, auto-immunity, and allergy (Goines, et al., 2011). This is consistent with studies that associate higher incidence of allergies and autoimmune diseases in mothers of children with ASD than mothers of typically developing children (Croen, Grether, & Yoshida, 2005). Peripheral IFN-γ levels are typically low in healthy pregnancies, and elevated levels have been associated with complications such as preeclampsia (Goines, et al., 2011). Additionally, a maternal immune response could activate the signaling pathway for IL-6, which is one of

few cytokines that may cross the placenta and strongly affect fetal development (Patterson, Immune involvement in schizophrenia and autism: Etiology, pathology and animal models, 2008). Injection of IL-6 alone into mice is enough to generate abnormal behaviors and changes in brain expression in offspring (Patterson, 2011). Conversely, development of these behaviors is prevented when IL-6 is blocked during maternal immune activation, suggesting direct correlation between IL-6 expression and behaviors similar to ASD (Patterson, 2011).

Regardless of which cytokine is directly responsible for ASD in offspring, there are several proposed pathways of action causing injury to neural cells (Burd, Balakrishnan, & Kannan, 2012). Probable mechanisms by pro-inflammatory cytokines may include direct injury to oligodendrocytes and neurons, secondary injury through activation of microglial cells, or a combination of both direct and secondary injury (Burd, Balakrishnan, & Kannan, 2012). Burd et al. created a diagram demonstrating possible mechanisms of fetal brain injury in response to maternal inflammation (Figure 1).

Figure 1: *Possible mechanisms for fetal brain injury by maternal inflammation* ***(Burd, Balakrishnan, & Kannan, 2012)***



Public Health Significance

Autism spectrum disorder is currently considered one of the most common child morbidities (Wang, Geng, Liu, & Zhang, 2017). The public costs of supporting individuals

with ASD are a multi-billion-dollar endeavor (Buescher, Cidav, & Knapp, 2014). The cost of supporting a child with ASD during his or her lifespan in the United States is $2.4 million if the child has an intellectual disability and $1.4 million if the child is without intellectual disability (Buescher, Cidav, & Knapp, 2014). The largest cost components for children were special education services and parental productivity loss (Buescher, Cidav, & Knapp, 2014). Assuming 40% prevalence of intellectual disability in children with ASD, the aggregate national cost in the United States is $61 billion per year (Buescher, Cidav, & Knapp, 2014). When calculating for adulthood, the highest cost components were supporting living accommodations and individual productivity loss. Assuming 40% prevalence of intellectual disability in adults with ASD, the aggregate national cost is $175 billion per year in the United States (Buescher, Cidav, & Knapp, 2014). Medical bills to care for adults with ASD were higher than for children with ASD (Buescher, Cidav, & Knapp, 2014).

The causes and consequences of ASD in Hispanic populations in the United States is incompletely understood. However, the previously discussed literature about the potential impacts of commonly held cultural beliefs in many Hispanic communities on knowledge about ASD and distrust of medical professionals warrants further study (Ijalba, 2016) (Schwei, Kadunc, Nguyen, & Jacobs, 2014). More specifically, the border communities that reside on the Texas/Mexico border suffer from lack of access to quality healthcare. The PCP to patient ratios in Hidalgo County and Cameron County of the Rio Grande Valley are 2,230:1 and 2,200:1, respectively, while the state average is 1,670:1. Both counties have uninsured rates of 30% (Torres, 2018). Additionally, Hispanic mothers often turn to family or cultural beliefs before seeking professional medical attention when their child exhibits

behaviors that are consistent with early signs of ASD (Ijalba, 2016). The investigation of prenatal risk factors for ASD among Hispanic mothers are needed to understand the public health burden of ASD in border communities – including potentially unique risk factors.

Research Objectives

In 2008, the autism prevalence rate was 1 in 88 children. In 2010 and 2012, the rates increased to 1 in 68 and 1 in 69 children, respectively. The most recent 2014 data suggests an autism prevalence of 1 in 59 children (CDC, 2019). Research towards identification of ASD has improved the accuracy of diagnosis; however, further research of prevention and identification of risk factors in pregnant Hispanic women is lacking. The overall goal of this research is to identify prenatal risk that could impact the risk of ASD in Hispanic children on the Texas/Mexico border of children between the ages of 2-21 years old at time of the study. Specifically, we aimed to:

1. Identify prenatal risk factors of ASD by comparing Hispanic women who gave birth to children with ASD and Hispanic women who gave birth to typically developing children
2. Compare the prevalence of prenatal risk factors of maternal illnesses and maternal medications taken during gestation among Hispanic women living on the border to the general population
3. Investigate effects of maternal inflammation on risk of giving birth to a child with ASD

METHODS

Study Design

We completed a secondary analysis of data previously collected from a case control study of 25 cases and 25 controls. Ideally, we would have gathered data from a greater number of participants; however, funding limitations necessitated capping the recruitment of this initial pilot study.

Study Setting

The Rio Grande Valley of southern Texas is 90% Hispanic and borders Mexico, making it an ideal location to conduct this preliminary study (U.S. Census, 2017). The Hispanic Autism Research Center (HARC) sought to identify environmental exposure and prenatal risk factors in Hispanic children or their biological mothers that could cause ASD in the children (Palmer, 2014). The HARC pilot study was conducted by The University of Texas Rio Grande Valley (UTRGV).

Study Subjects

Eligibility Criteria

Inclusion criteria for all child participants included: (a) a child between the ages of 2-21 years old; (b) living with at least one biological parent; (c) has a parent who speaks English or Spanish; (d) residing in the study catchment areas of Harlingen and Brownsville, Texas, covering Hidalgo and Cameron counties, or no more than a 2-hour drive from these assessment sites (e) of Mexican American ethnicity (Palmer, 2014). Mexican American ethnicity was determined by the San Antonio Heart Study (SAHS) algorithm, a nine-item indicator which uses parental surnames, birthplace of both parents, self-declared ethnic

identity, and ethnic background of grandparents (Palmer, 2014). Inclusion for parent participants included: (a) biological parent to a child between the ages of 2-21 years old; (b) living with the participant child; (c) parent who speaks English or Spanish; (d) residing in the study catchment areas of Harlingen and Brownsville, Texas, covering Hidalgo and Cameron counties, or no more than a 2-hour drive from these assessment sites (e) of Mexican American ethnicity (Palmer, 2014).

Children in the case group were children with a clinical diagnosis of Autism Spectrum Disorder, including Autism, Asperger's, and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS) (Palmer, 2014). Adults in the case group were the biological parents of participating children in the case group. Children in the control group were typically developing children sampled from the general population. Adults in the control group were the biological parents of participating children in the control group. Exclusion criteria for cases were children or parents of children diagnosed with other neurological disabilities or are non-Mexican American. Exclusion criteria for controls were children or parents of children who do not have a clinical diagnosis of ASD or are non-Mexican American (Palmer, 2014). Due to budgetary restraints, we were unable to match controls based on age and gender to cases. For the purposes of this pilot study, 25 cases and 25 controls were recruited.

Recruitment

Multiple organizations participated in the recruitment, including an organization of community healthcare workers called *Promotoras de Salud* and The Easter Seals of the Lower Rio Grande Valley (Palmer, 2014). The connections *Promotoras de Salud* had with

school districts, faith-based entities, and other community-based organizations were useful tools in recruitment for this study because the *promotoras* had established a high level of trust and reached out to families personally through home visits and phone calls. Recruitment also relied heavily on the McAllen and Harlingen centers of The Easter Seals of the Rio Grande Valley, referrals, and advertisement (Palmer, 2014). The potential subject's parent(s) responded to advertisements or referrals from local healthcare providers (community partners), including *Promotoras de Salud* and Easter Seals. Research staff then coordinated recruitment (Palmer, 2014).

The research personnel conducted the screening process over the phone and/or in person in a private setting (Palmer, 2014). Study coordinators used a standardized script to determine eligibility, and research personnel verified that each participant met the eligibility criteria (Palmer, 2014). If the participant met initial eligibility criteria, the participant was scheduled complete the questionnaire (Palmer, 2014).

Consent Process

After the initial phone screen and if the family agrees to further participation, a research visit was scheduled at the Clinical Research Unit (CRU) (Palmer, 2014). A description of the study was provided verbally and in written form (Palmer, 2014). Any questions the participants had were answered at the time. Research coordinator or study staff guided the family through the protocol (Palmer, 2014).

All participants signed a form authorizing research to use Protected Health Information (PHI)/Private identifiable information (Palmer, 2014). PHI was collected during screening for eligibility. All participants signed a consent form and a HIPPA Waiver after

reading detailed risks, benefits, alternatives, and compensation from the research (Palmer, 2014). Study staff obtained written informed consent at the first research meeting before surveys were administered.

Adequate timing was given to parent(s) to consult with family members to obtain consent (Palmer, 2014). Research team members asked the parent(s) to repeat back what it is they were consenting to and the research team determined if they were able to understand the project well enough to consent (Palmer, 2014). After explaining the goals of the study protocol in-person to parent(s), those who could not explain in their own words the purpose of the study were deemed unable to provide consent, and their child was not enrolled (Palmer, 2014). The same process took place for Spanish speaking individuals to ensure comprehension among both languages.

Participants were compensated with $150 upon completion of the study in addition to a $50 travel allowance (Palmer, 2014). The study was at no cost to the participant.

Data Handling and Record Keeping

All surveys are stored in a locked repository at The University of Texas Rio Grande Valley (UTRGV) Harlingen Clinical Education Building, 2102 Treasure Hills Boulevard, Harlingen, TX 78550 (Palmer, 2014). The Principal Investigator (Beatriz Tapia, MD, MPH, EdD) is responsible for the repository (Palmer, 2014). Other researchers requesting information from the repository must have the approval of the PI (Palmer, 2014). Data remains on paper surveys in the repository until coded into Microsoft Excel. All electronic documents containing survey data are saved on a password-protected flash drive.

Each survey was labeled with respondent's unique study ID. The code linking ID numbers to other personal identifying information is kept in a locked file cabinet, and in electronic files that do not contain any other information and do not exist on computers containing any other information about the participants.

Data Collection and Abstraction

The thirteen-part survey was created to evaluate environmental causes and prenatal risk factors and administered in-person to participating mothers (Figure 2). Participants scheduled a time to take the survey with a member of the research team present (Palmer, 2014). Surveys were administered in-person by a member of the research team between July 2011 and August 2014. The questionnaire took approximately 2-3 hours per participant and administration was conducted in private room at the Clinical Research Unit (CRU). All survey answers were written by the mothers onto the survey, and research personnel were available for clarification of questions. Data was recorded on paper and transferred to Microsoft Excel for coding and analysis.

Abstraction of recorded survey answers from paper to Microsoft Excel included coding for multiple choice and free response answers. Coding keys for data abstraction are described in an accompanying codebook. The standard coding for close ended answers are:

1	yes
0	no
996	refuse to answer
997	don't know
998	cannot code or missing
999	not applicable

Open ended answers were transferred directly into Excel as they were written into the survey.

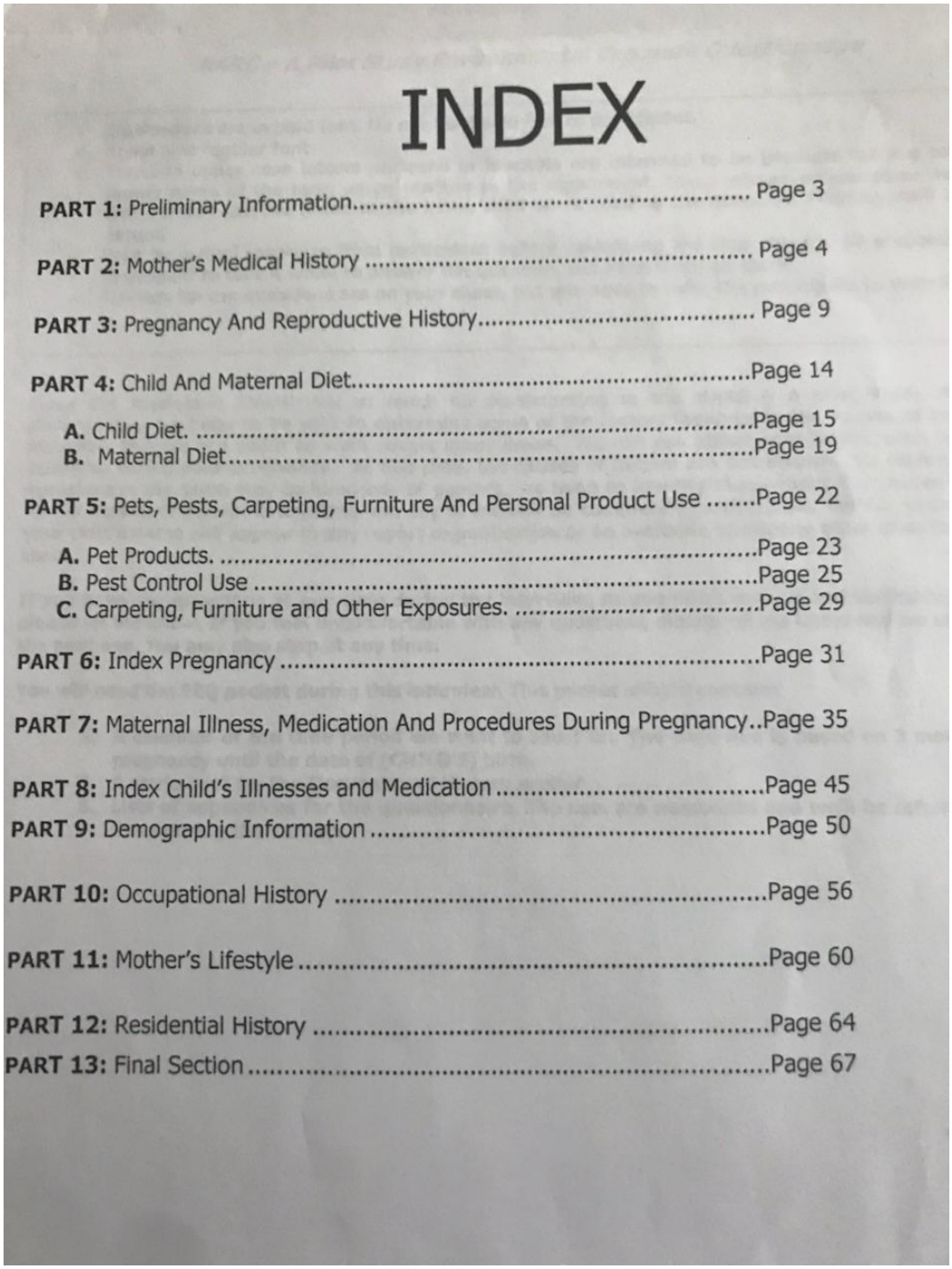
Data Analysis

Part 7 of the survey pertains to questions concerning "Maternal Illness, Medications, and Procedures during Pregnancy". Data from Part 7 was used to test prenatal risk factors. Specifically, we investigated the association between maternal illnesses and medications during pregnancy and ASD. We assessed differences in prevalence of prenatal risk factors were between case mothers and control mothers. Because of the small sample size, all statistical tests were non-parametric. All analysis was conducted using STATA 15.

Human Subjects, Animal Subjects, or Safety Considerations

This study is a secondary analysis of data that has already been collected. This study involves human subjects and is therefore subject to the Committee for Protection of Human Subjects. Participants have the right to refuse to participate in any aspect of the study or to withdraw from the study at any time. All survey data was deidentified, so the risk to humans physically/mentally is minimal. This study has been approved by the IRB.

Figure 2: *Index of survey provided to participant mothers concerning environmental and prenatal risk factors for ASD in the child*



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RESULTS

Characteristics of Study Cohort

Demographics of known risk factors include maternal age at conception of index child, and sex, birth weight, and gestational age of index child (Table 1). Average age of conception of index child in case mothers was 29.01 with standard deviation of 6.56 and overall range of 17.6 to 43.9 years. Average age of conception of index child in control mothers was 27.36 with standard deviation of 6.71 and overall range of 16.9 to 38.9 years. Males constituted 96% of case children and 72% of control children. Average birth weight of case children was 109.75 ounces with standard deviation of 20.81 and overall range 70 to 152 ounces, whereas average birthweight in control children was 115.8 ounces with standard deviation 13.93 and overall range of 92 to 136 ounces. Average gestational age was 37.44 weeks for case children, standard deviation 2.04 and overall range 32 to 40 weeks, and 39.3 weeks for control children, standard deviation 1.12 and overall range 37 to 42 weeks. Due to limited funding, cases and controls were not matched.

There were no meaningful differences between the age at conception or birth weight of cases and controls. A Wilcoxon rank sum was executed for age at conception, birth weight, and gestational age because the data was nonparametric. Wilcoxon rank sum indicated no difference in the median ages of conception between case and control mothers, 28.89 and 25.74 years, respectively ($p = 0.432$). There was no meaningful differences between the medians of birthweight between cases and controls, 107 and 121 ounces, respectively ($p = 0.2002$).

The proportion of male cases was larger than male controls (two-tailed p: 0.0206; one-tailed p: 0.0103), which is consistent with research reflecting autism rates affecting males 4 times higher than females (Baio, Wiggins, & Christensen, 2018). Wilcoxon rank sum indicated a difference in gestational age medians of cases and controls, 38 and 40 weeks, respectively (p = 0.0004).

Table 1: *Descriptive statistics of known risk factors for mothers and index children*

Variables	Cases (n=25), sd	Controls (n=25), sd	P-value
Mother			
Age at Conception (years)	29.01, 6.56	27.36, 6.71	0.432
Index Child			
Sex (percentage, male)	96, -	72, -	0.0206
Birth Weight (ounces)	109.75, 20.81	115.8, 13.93	0.2002
Gestational Age (weeks)	37.28, 2.04	39.3, 1.12	0.0004

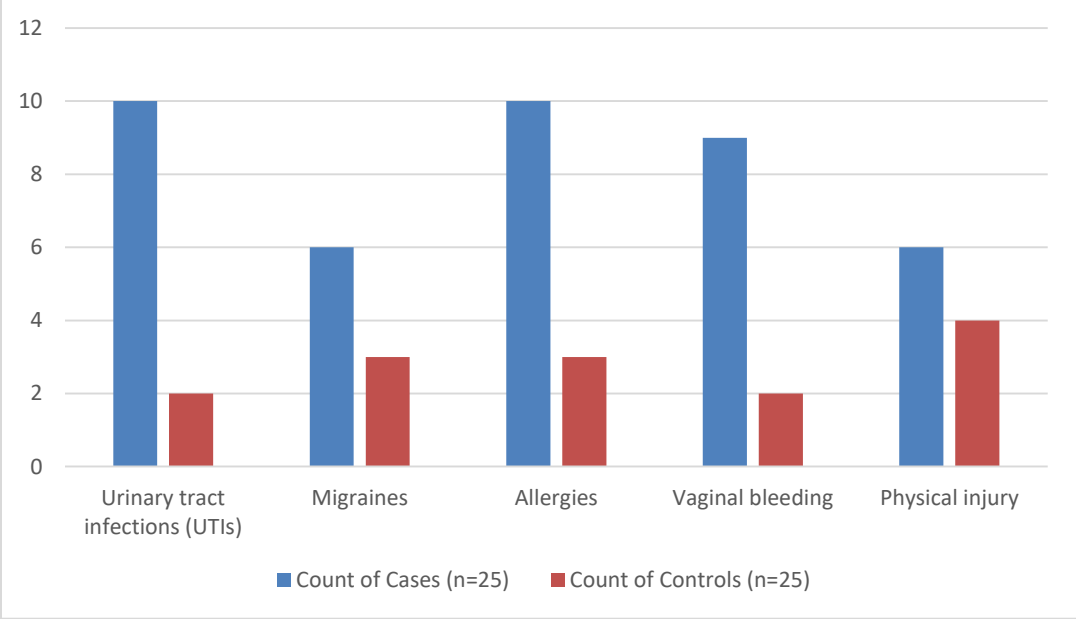
Prevalence of Risk Factors

Two separate categories of prenatal risk factors were analyzed: maternal illness during pregnancy and medications taken during pregnancy. The maternal illnesses surveyed were urinary tract infections (UTIs), migraines, allergies, vaginal bleeding, and physical injury. Mothers reported whether they experienced any of the illness at any point during index pregnancy (Table 2) (Figure 2). All illnesses were reported more often in cases than controls.

Table 2: *Differences in prevalence (counts) of reported maternal illnesses during gestation of index pregnancy*

Maternal Illnesses	Count of Cases (n=25)	Count of Controls (n=25)	Fisher's exact
Urinary tract infections (UTIs)	10	2	0.018
Migraines	6	3	0.463
Allergies	10	3	0.051
Vaginal bleeding	9	2	0.037
Physical injury	6	4	0.496

Figure 3: *Reported maternal illnesses during gestation of index pregnancy*

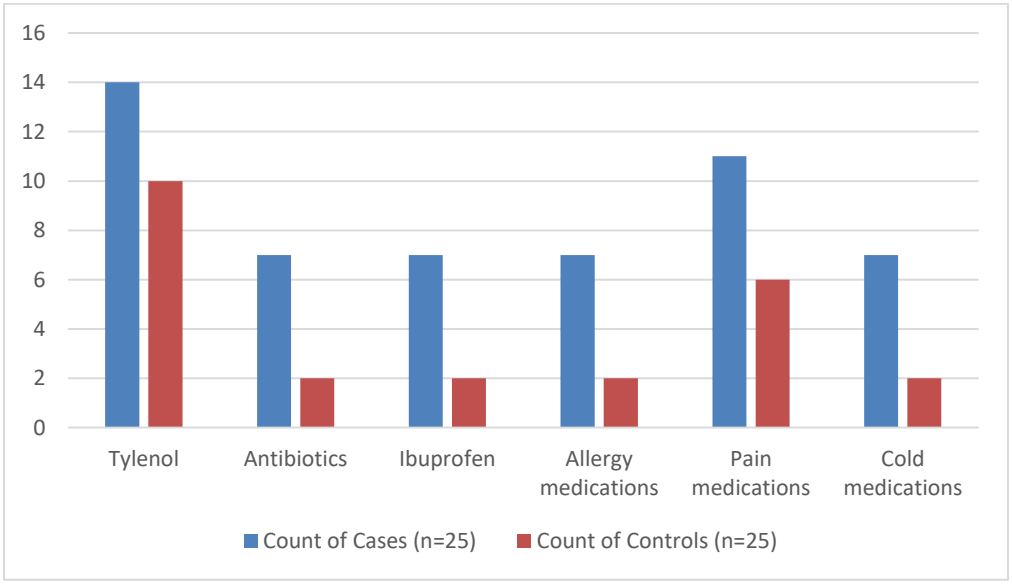


Mothers also reported whether they took any of the following medications or medications for ailments at least once during their index pregnancy: Tylenol, antibiotics of any kind, ibuprofen, allergy medication, pain medication, and cold medication (Table 3). All medications were reported more often in cases than controls.

Table 3: *Differences in prevalence (counts) of reported maternal medications taken during gestation of index pregnancy*

Maternal Medications	Count of Cases (n=25)	Count of Controls (n=25)	Fisher's exact
Tylenol	14	10	0.396
Antibiotics	7	2	0.138
Ibuprofen	7	2	0.138
Allergy medications	7	2	0.138
Pain medications	11	6	0.232
Cold medications	7	2	0.138

Figure 4: *Reported maternal medications taken during gestation of index pregnancy*



Measures of Association concerning Prevalence

Odds ratios were calculated conducted to measure the association between the individual maternal illnesses and ASD (Table 4) and maternal medications and ASD (Table 5).

Table 4: *Odds ratios of reported maternal illnesses during gestation of index pregnancy*

Maternal illnesses reported during gestation	Odds ratio	95% Confidence Interval	p-value
Urinary tract infections (UTIs)	7.667	[1.470, 39.987]	0.008
Migraines	2.316	[.509, 10.543]	0.270
Allergies	4.889	[1.150, 20.790]	0.024
Vaginal bleeding	6.469	[1.230, 34.011]	0.017
Injury	1.750	[.426, 7.190]	0.435

Table 5: *Odds ratios of reported maternal medications taken during gestation of index pregnancy*

Maternal medications reported taken during gestation	Odds ratio	95% Confidence Interval	p-value
Tylenol	1.909	[.620, 5.876]	0.258
Antibiotics	4.472	[.827, 24.193]	0.066
Ibuprofen	4.472	[.827, 24.193]	0.066
Allergy medications	4.472	[.827, 24.193]	0.066
Pain medications	2.488	[.741, 8.350]	0.136
Cold medications	4.472	[.827, 24.193]	0.066

DISCUSSION

The authors of this study acknowledge that the small sample size (n = 25) reduces the power of the study, and thereby the ability to detect meaningful differences between the two groups. However, a comprehensive view of statistical significance, point estimates, and confidence intervals can provide meaningful implications of the results.

While all illnesses were reported more often in cases than controls, meaningful differences in counts were calculated through Chi-squared analysis of vaginal bleeding and UTIs (Table 2). This is corroborated by confidence intervals indicating a positive association between vaginal bleedings, UTIs, and ASD in the child (Table 4). The confidence intervals are extremely wide, indicating a lack of precision in the point estimate and low statistical quality. However, the high upper limits of the confidence intervals and positive association of the exposure to the outcome could provide clinical or practical implications that UTIs and vaginal bleeding are potential prenatal risk factors to further investigate. These implications can also be applied to the other illnesses that were not evaluated to be statistically significant by p-values or confidence intervals. Point estimates of all maternal illnesses were positive, indicating a general positive association between the prenatal risk factors and the outcome of ASD.

Urinary tract infections (UTIs)

The odds of ASD in the index child are 7.667 times greater when born to mothers who experienced UTIs during gestation of the child than those who did not (CI: [1.470, 37.987], p = 0.008) (Table 4). Previous case-control studies investigating the risk between maternal infections during pregnancy and risk of ASD found a mild, but significant

association between maternal bacterial infections in the second trimester and ASD. Overall, women with bacterial infections diagnosed during hospital admissions were at increased risk of delivering a child with ASD (ORadj: 1.58; 95% CI: 1.06-2.37). Genitourinary infections were one of the most commonly diagnosed infections during pregnancy, with UTIs being among the most common of the genitourinary type. Bacterial infections during pregnancy in the second and third trimesters were also associated with a moderately increased ASD risk. UTIs were the most common bacterial infections in the third trimester (Zerbo, et al., 2016).

Vaginal Bleeding

The odds of ASD in the index child are 6.469 times greater when born to mothers who experienced vaginal bleeding during gestation of the child than those who did not (CI: [1.230, 34.012], p = 0.017) (Table 4). Increased prevalence of vaginal bleeding in this study is consistent with past peer-reviewed studies of prenatal factors, which found that 16.7% of case mothers experienced vaginal bleeding. This was significantly higher than the national rate of gestational vaginal bleeding of 6.6% (p = 0.001) (Brimacombe, Ming, & Lamendola, 2007). In this study, 36% of case mothers experienced vaginal bleeding and the most recent rate for the state of Texas is 20.8% (PRAMS, 2011).

Allergies

The odds of ASD in the index child are 4.889 times greater when born to mothers who experienced allergies during gestation of the child than those who did not (CI: [1.150, 20.790], p = 0.024). Ongoing research has attempted to link allergic conditions with autoimmune disorders because they are both hypersensitivity responses (Sayed, Christy, Quirion, & Brown, 2008). One study found elevated ASD risk for maternal allergy diagnoses

during the second trimester of pregnancy (ORadj: 2.5, 95% CI: 1.2 – 5.2) (Croen, Grether, & Yoshida, 2005).

Maternal medications

Statistically, no maternal medications demonstrated significant difference in prevalence counts or point estimates. However, all point estimates were positive and the upper limit of the confidence intervals were high (ranging from 8.35 to 24.193, Table 5). Prior studies strongly suggest a relationship between medication mechanism and autism development, specifically Tylenol. While acetaminophen (Tylenol) is typically used to reduce inflammation, studies show that even low doses of the drug can induce immune system activation and oxidative stress responses associated with autism development (Parker, et al., 2017).

An additional evaluation elicited from this study is that maternal illnesses and maternal medications were more frequently reported across all variables in case mothers. While this may be a result of recall bias, it may also indicate behavioral patterns of frequent illness or medication consumption in mothers who will give birth to children with autism.

Limitations

One major limitation of this pilot study is its small sample size. Because this is a pilot study, the researchers only had enough funding for 25 cases and 25 controls. Subsequently, the small sample size may not provide enough statistical power to detect significant results in variables measured. In future research, the power could be improved by adjusting the type I error level to reflect the sample size and variability of the data. Another way to improve power is increasing the sample size with increased funding and greater recruiting.

Another consequence of limited funding and small sample size is that confounders could not be addressed. The author is aware there are many variables that may ask as confounders to autism risk, including but not limited to: infant sex, infant birth weight, and maternal age at conception (Goines, et al., 2011). Frequency matching for these variables was not economically feasible, and the sample size was too small for logistic regression.

This study only measured occurrence of prenatal risk factors and did not delve deeper into timing of the infection. As critical as timing is in fetal development, it would be prudent to accurately identify the timing of the symptoms as way of identifying which point in the gestational pathway maternal inflammation is most potent.

Lastly, all data was self-reported by the mothers retroactively and is subject to recall bias. Survey responses were not cross-referenced with medical records due to lack of funding, so all data is reliant on maternal recall.

Strengths

The results of our analysis of data collected in this preliminary pilot study are consistent with the idea that maternal inflammation increases risk for autism in the child. Additionally, we found meaningful differences in prevalence and odds of vaginal bleeding, UTIs, and allergies during gestation. These trends have also been studied and supported in numerous past peer-reviewed studies of larger scale (Brimacombe, Ming, & Lamendola, 2007) (Zerbo, et al., 2016) (Croen, Grether, & Yoshida, 2005).

Additionally, this study used a very specific sample of Hispanic women and children who live on the Texas/Mexico border. While this group has typically lower diagnosis rates of ASD, the data of this small study on a specific group of individuals is consistent with larger

studies concerning all races and ethnicities. The finding that prenatal risk factors can similarly influence Hispanic women and their children as they do other women and children strengthens the idea that the Hispanic children are underdiagnosed and there is not a protective genetic component in Hispanics.

CONCLUSION

The current pilot study and past peer-reviewed literature indicate a strong association between prenatal risk factors and autism risk in the child. This association is supported by research of maternal inflammation and its role in fetal neurodevelopment. As a result, three prenatal risk factors were found to have association with ASD should be studied further for their role in autism risk: maternal vaginal bleeding, UTIs, and allergies. Early identification and treatment of these risk factors during gestation may play a role in reducing ASD risk.

This research has the potential to significantly impact the health and quality of life of Hispanic women and children living on the Texas/Mexico border. This population is medically underserved, and as a result autism is underdiagnosed. Identification and appropriate treatment of the prenatal risk factors should be taught to local clinicians and community health workers. The public health community can use this data to create awareness and educate Hispanic women on risk factors to be mindful of while they are pregnant.

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