6-2018

Epidemiological, Environmental, and Biological Risk Factors for Gastroschisis

Arti Ketan Desai

LOMA LINDA UNIVERSITY
School of Medicine
in conjunction with the
Faculty of Graduate Studies

Epidemiological, Environmental, and Biological Risk Factors for Gastroschisis

by

Arti Ketan Desai

A Dissertation submitted in partial satisfaction of
the requirements for the degree
Doctor of Philosophy in Biology

June 2018

Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

_____, Chairperson
Bryan T. Oshiro, Professor of Obstetrics and Gynecology

_____
Stephen G. Dunbar, Professor of Biology

_____
Ravi Goyal, Associate Professor of Pharmacology

_____
William K. Hayes, Professor of Biology

_____
Kerby C. Oberg, Professor of Pathology and Human Anatomy

_____
Gary M. Shaw, Professor of Pediatrics

ACKNOWLEDGEMENTS

My journey at Loma Linda University would not have been possible without the guidance, support, friendship, and faith of my family, friends who have become family, colleagues, and mentors. Starting this adventure seven years ago, I can confidently say that I have grown not only intellectually, but also personally and professionally through the various experiences and opportunities I have been able to be a part of. From brainstorming meetings to coming up with ways to conduct our project, I have been inspired to continue asking questions and think outside the box and these will be lessons I will cherish and utilize as I move forward in my career.

Thank you to my committee: Dr. Bryan Oshiro, Dr. Ravi Goyal, Dr. Stephen Dunbar, Dr. William Hayes, Dr. Kerby Oberg and Dr. Gary Shaw. I appreciate your expertise, ideas, guidance and motivation throughout this process.

Dr. Oshiro- none of this would have been possible without you and your willingness to share your passion and vision. Your dedication, commitment and positivity is unparalleled. Thank you for being my mentor and friend.

Words cannot begin to express my gratitude to my friends who keep me grounded. Shawn Plafker and Elizabeth Dameff, my time at Loma Linda would not have been what it was without you both. You have taught me so much about being a strong, confident individual and the memories, adventures and laughs we have had I will cherish forever (along with the billion or so pictures we have taken over the years). Also, the LLUSA staff and teams have been my platform to grow as a leader and the last six years working together have been some of my best memories.

The constant support from my family and their belief in me has been the best motivation. A big thank you to my parents Mita and Ketan Desai and my brother Kishan Desai for their love, patience and overwhelming support in anything and everything I pursue. Thank you so much instilling in me the value of perseverance and responsibility.

I have been immensely blessed to have the opportunity to work in a field I am extremely passionate about. I am in awe of the strength, resiliency and positivity I have encountered from the physicians, staff, and families I was able to interact with. I hope that the work and research I have been able to do contributes to and aides in making lives better.

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ABBREVIATIONS

BMI	Body Mass Index
SNP	Single Nucleotide Polymorphisms
LOS	Length of Stay
V	Vaginal Delivery
CS&L	Cesarean Section with Labor
CS	Cesarean Section
UTI	Urinary Tract Infection
P/F	Primary Fascial Closure
S	Staged Fascial Closure
RR	Risk Ratio
CI	Confidence Interval
IUGR	Intrauterine Growth Restriction
PPROM	Preterm premature membrane rupture
IUFD	Intrauterine Fetal Death
GBS	Group B streptococcus
LLUCH/LLUMC	Loma Linda University Children's Hospital/Loma Linda University Medical Center
SGA	Small for Gestational Age
GA	Gestational Age
REDCap	Research Electronic Data Capture
WHO	World Health Organization
OR	Odds Ratio

EFW	Estimated Fetal Weight
PPV	Positive Predictive Value
NPV	Negative Predictive Value
AC	Abdominal Circumference
FL	Femur Length
BPD	Biparietal Diameter
HC	Head Circumference
US	Ultrasound
ROC	Receiver Operating Characteristic
GIS	Geographic Information Systems
EPA	Environmental Protection Agency
TRI	Toxic Release Inventory
ECM	Extracellular Matrix
GFP	Green Fluorescent Protein
RNA	Ribonucleic acid
HBMEC	Human Brain Microvascular Endothelial Cell
rcf	Relative Centrifugal Force
EPA	Environmental Protection Agency
TRI	Toxic Release Inventory

ABSTRACT OF THE DISSERTATION

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by

Arti Ketan Desai

Doctor of Philosophy, Graduate Program in Biology
Loma Linda University, June 2018
Dr. Bryan T. Oshiro, Chairperson

Gastroschisis, a congenital defect of the abdominal wall, manifests as external herniation of viscera, most commonly the fetal bowel. The worldwide prevalence of gastroschisis continues to rise, and this increase can also be seen in California with an overall birth prevalence of 2-3 per 10,000 births. While the etiology and pathogenesis of gastroschisis remains unknown, previous studies indicate several risk factors including young maternal age, nulliparity, and low maternal body mass index, in addition to environmental factors and exposure, given the increase in global prevalence with a predisposition of cases to occur in clusters, and absence of a genetic link. We sought to examine etiology of gastroschisis and associated epidemiological, environmental, and biological factors by; 1) determining prevalence of gastroschisis in the Inland counties of Southern California over time; 2) assessing geospatial patterns with overlays of various environmental factors; and 3) comparing cell migration rates from biological samples in gastroschisis versus control pregnancies. We found increased maternal age and parity over time in those carrying a baby affected by gastroschisis and observed clustering of cases in the Inland Empire region. There were no significant differences in cell migration rates. Results identified changing prenatal characteristics and potential relationships with environmental hazards, which future studies will continue investigating. Continued

research can aid in improved prenatal diagnosis and better clinical outcomes for the mother and baby.

CHAPTER ONE

INTRODUCTION TO GASTROSCHISIS

Gastroschisis is a congenital birth defect of the abdominal wall resulting in an infant's intestines protruding outside the body through a perforation lateral to the umbilicus.[1] Numerous studies have shown an increased prevalence of gastroschisis throughout the world in the last few decades, and the United States and the State of California are no exceptions to this growing trend.[3, 4, 7-19] Data from The National Birth Defects Prevention Network showed the prevalence of gastroschisis in the United States during the years 1999 to 2001 as being 3.73 per 10,000 live births.[5] An updated publication reporting on data from 2004 to 2006 shows the increase in the prevalence to 4.49 per 10,000 live births.[11] Data from a population-based registry in California from 1987 to 2003 noted a birth prevalence of 2.57 per 10,000 births. During the 17- year study period in the State of California, the overall birth prevalence increased by 3.2-fold.[18] This study by Vu et al. demonstrated that the birth prevalence of gastroschisis has been gradually rising in the past two decades in California.

Furthermore, in San Bernardino and Riverside Counties of Southern California, annual rates of gastroschisis were noted as 3.2 and 6.0 per 10,000 live births and fetal deaths in the years 2005 and 2006, respectively.[19] The multitude of data throughout the years show the increased prevalence of gastroschisis on not only a worldwide scale, but at a national and state level, as well. In California, in particular the Inland Empire region of Southern California, various hypotheses have been proposed regarding the pathogenesis, etiology, and risk factors of gastroschisis. Teratogens such as organic

solvents, maternal smoking, alcohol, and illicit drugs as associated with gastroschisis.[20-24] Risk factors associated with gastroschisis include young maternal age, nulliparity, residences surrounding landfill sites, and low pregnancy body mass index (BMI).[18,25,26] Environmental factors have also been linked to increasing rates of gastroschisis, as suggested by prevalence and tendency to occur in clusters.[16,27-31] The implication of environmental factors is also supported by evidence from animal models.[32-38] While the etiology of gastroschisis remains unknown, there are several main theories of pathogenesis. First is that of teratogenic differentiation of embryonic mesenchyme with deficiency of the somatopleure. Deprived of mesenchymal support the somatopleure is reabsorbed creating the typical right periumbilical defect.[39] Others have suggested a modification of this idea, with umbilical ring mesodermal dysplasia that results in disruption [40, 41] Alternatively, the cause of gastroschisis may be a vascular developmental defect of the omphalomesenteric artery.[42] Another theory is that of premature atrophy of other abnormality of the right umbilical vein.[43, 44] A related suggestion is that gastroschisis is a consequence of failure of the yolk sac and related vitelline structures to be incorporated into the umbilical stalk.[45] Before a specific gene and/or mutation can be linked causally to this disorder, its biologic relevance must be established. The critical question is not whether cases as a group have more rare events (such as single nucleotide polymorphisms, SNP) then controls, but rather which mutation disrupting a gene is responsible for the given anomaly. Variable penetrance, epistasis, epigenetic changes and/or other gene-environment interactions can complicate this picture.

Based on previous studies and data it can be hypothesized that demographic, social, biological, obstetric, and/or environmental factors may be associated with the

prevalence of gastroschisis. Specific objectives will be analyzed to recognize risk factors and better understand the pathogenesis and mechanism of gastroschisis in order to reduce neonatal health disparities.

This study, which couple biological tissue sampling and geo-temporal and spatial mapping, is needed to move beyond speculation, and potentially lead us to a cause. Research which further advances the understanding of risk factors or causative mechanisms may ultimately result in the prevention of gastroschisis and its related morbidities. Once this is known, we can move forward with potential interventions. In addition, our characterization of patients in a prospective fashion could lead to better predictors of neonatal outcomes. By studying long term outcome data on these affected individuals, we may be better able to understand their feeding problems and nutritional requirements, and design improved treatment programs for them. Lastly, this study serves as a tool to educate, and increase awareness and knowledge in the population as a whole, about the increasing global prevalence of gastroschisis and its possible causes.

Objective and Aims

The objective of this dissertation is to examine the etiology of gastroschisis and associated epidemiological, environmental, and biological factors. The study has three main aims: 1) to determine the prevalence of gastroschisis in the Inland Empire counties of Southern California from the period January 1998 to March 2018; and 1.1) to identify associations, if any exist, between maternal and infant demographic, social, biological, and obstetric factors with the prevalence of gastroschisis; 2) to assess temporal and geographic trends to determine geographic clustering of gastroschisis cases and their

associations with environmental factors, such as waste disposal sites, water supplies, power lines, and toxic chemicals; and 3) to elucidate the etiology of gastroschisis through comparison of biological samples from mothers of babies diagnosed with gastroschisis to those of uncomplicated pregnancies.

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CHAPTER TWO

EPIDEMIOLOGICAL TRENDS OF GASTROSCHISIS

Introduction

Epidemiology plays a major role in translational research and translating scientific discoveries into clinical practice and population health impact.[1] As such, understanding and identifying risk factors and maternal characteristics are vital in elucidating etiology and pathogenesis of birth defects, such as gastroschisis.

Birth defects are characterized as structural abnormalities present at birth, yielding surgical, medical, or cosmetic importance. In the United States, birth defects account for 3% of live births annually. Birth defects are not only the leading cause of infant mortality in the United States with 1.2 deaths per 1,000 live births, but are also associated with increased morbidity, health care use, and costs. [2]

Gastroschisis is a birth defect of the abdominal wall with increased prevalence in the last few decades. Gastroschisis has increased in prevalence worldwide from approximately 0.1 per 10,000 total births in the 1970s to over 5 per 10,000 total births in the early 2000s.[3, 4] The Center for Disease Control notes an increased prevalence of gastroschisis by 30% in the United States from 3.6 per 10,000 births during 1995–2005 to 4.9 per 10,000 births during 2006–2012. Increased prevalence and limited understanding of risk factors pose concerns and an urgency to identify the causal factors contributing to this increase. [5] The overall neonatal mortality rate for gastroschisis is 5–10%, and associated morbidities, such as susceptibility to sepsis can lead to additional complications and prolonged hospitalization stays in gastroschisis patients.[6] As gastroschisis cases continue to increase, understanding the best management is essential

in improving neonatal outcomes. We sought to gain a better understanding of gastroschisis risk factors by first looking at various factors and trends associated with gastroschisis and its increased prevalence over time in the Inland Empire region of Southern California. We then looked at long term outcomes in neonates with gastroschisis to identify how these babies do over time depending on mode of delivery and type of closure at Loma Linda University Children's Hospital.

Does Time Really Tell All? Investigating the Relationship of Time on Maternal Age and Parity in Gastroschisis Cases

A retrospective chart review was performed on cases of gastroschisis (n= 257) in order to determine rates during a 14-year time period (1998-2012) at Loma Linda University Medical Center located in California's Inland Empire region. Maternal factors of age and parity were assessed to determine if longitudinal trends exist. The mean for each year was plotted for each of these variables and correlation models were generated to determine if longitudinal changes in these risk factors were present. Simple linear regression analysis was performed for maternal age and parity. **Figure 2.1** shows results depicting a line of best fit for maternal age with an R^2 value of 0.215 and a Pearson Coefficient of 0.464 with a significance of 0.082. **Figure 2.2** shows a line of best fit for maternal parity shows an R^2 value of 0.251 and a Pearson Coefficient of 0.501 with a significance of 0.057.

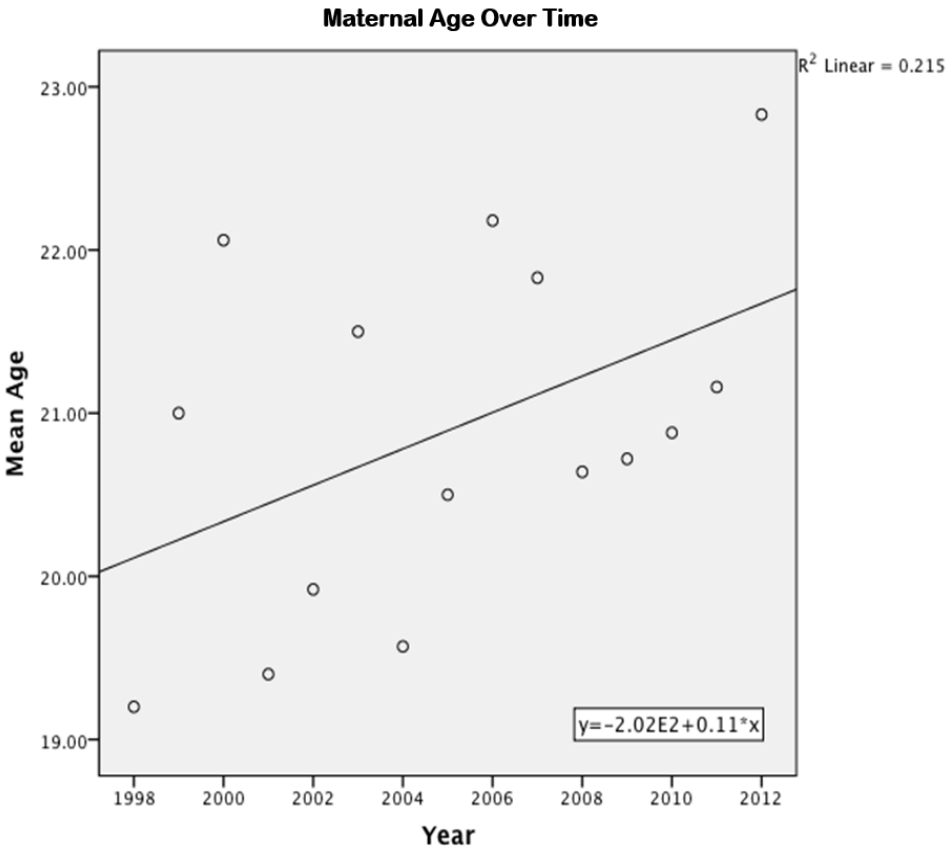

Figure 2.1. Linear regression analysis of maternal age over time.

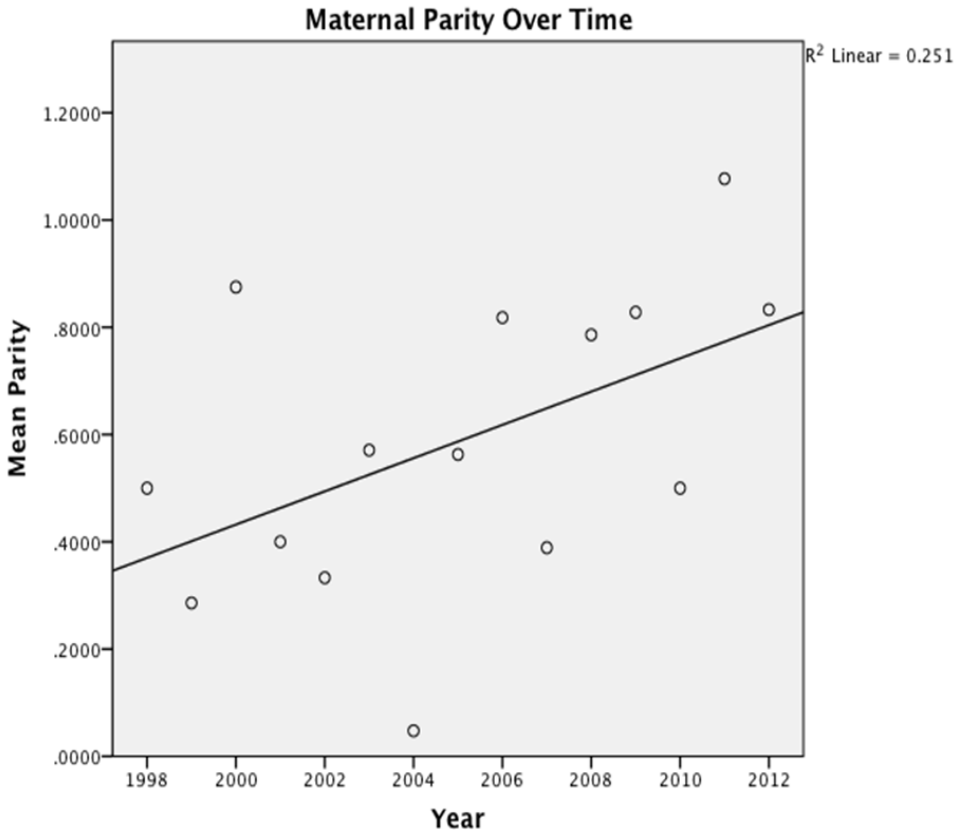

Figure 2.2. Linear regression analysis of maternal parity over time.

Cumulative maternal age and parity were higher than anticipated compared to previously published reports. Our longitudinal analysis showed an increase in maternal age and parity over time suggesting a shift in these risk factors over the past 14 years. This trend has also been noticed clinically. The results of this study allow us to recognize the changing risk factors for gastroschisis, leading to earlier prenatal diagnosis, improved clinical care, and decrease in morbidity associated with this disease.

Cesarean Sections May Increase the Risk of Sepsis in Neonates with Gastroschisis

In order to optimize management of neonates with gastroschisis we evaluated the risk of culture-proven sepsis based upon the mode of delivery. Additionally, we

investigated the effect of sepsis and mode of delivery on length of stay (LOS). A retrospective chart review was performed on records of 164 mothers that delivered babies with gastroschisis at Loma Linda University Medical Center and Lucile Packard Children's Hospital Stanford from February 1999 to December 2012. Institutional review board (IRB) approval was attained for this study at both Loma Linda University and Stanford University. Both institutions utilized Research Electronic Data Capture (REDCap), a secure, web-based research data collaborative platform as a means to collect and manage study data.[7]

Outcomes: The study's main endpoint consisted in culture proven sepsis, while the secondary endpoint was in hospital length of stay (LOS).

Main exposure: The main exposure in this research was mode of delivery defined as vaginal delivery (V), cesarean section with labor (CS&L) and cesarean section without labor (CS).

Covariates: Covariates that were considered for this study included maternal characteristics such as diagnosis of urinary tract infection, chlamydia, gonorrhea, and genital herpes, as well as delivery characteristics, such as meconium staining and preterm delivery.

Statistical analysis: Crude analyses for the association between mode of delivery and each of the covariates with sepsis diagnosis were conducted using chi-squared test. Sepsis was a common event (>10% of study population); therefore, covariates adjusted relative risk of sepsis with 95% confidence intervals was conducted using log-binomial regression. Final models were built using the purposeful model selection approach with mode of delivery, chlamydia, genital herpes and urinary tract infection (UTI) retained for

the assessment of the risk of sepsis, while mode of delivery and chlamydia were retained for the assessment of in-hospital length of stay. Model assessment did not reveal any major outlier and multicollinearity assessment using linear regression did not reveal high correlation between covariates.

Study population: Among the 164 neonates included in this research, 85 percent (51.8%) were V while 42 (25.6%) had CS&L and 37 (22.6%) had CS. Sepsis diagnosis was made for 57 (34.8%) including 12 ,26 and 19 that had been delivered by CS, CS&L, and V, respectively (Table 2.1).

Effect of mode of delivery on the risk of sepsis: Compared to V, neonates delivered by CS were 2.5 times more likely to develop sepsis during their hospital stay RR= 2.65 (1.67-4.21) while those delivered with CS&L had a 52 percent albeit not statistically significant increase in the risk of sepsis RR= 1.52 (0.83-2.79) (Table 2.2).

Effect of chlamydia on the risk of sepsis: Neonates whose mothers were diagnosed with chlamydia had RR for sepsis of 1.80 (1.08-3.00) (Table 2.2).

LOS in neonates delivered by CS&L and CS were 15 (-31 to 0.55) days and 6 (-22 to 9) days shorter, respectively, compared to V. Neonatal sepsis increased LOS by 50 (35 to 63) days compared to neonates without sepsis.

Table 2.1. Population characteristics by sepsis status (Yes *vs*. No)

Characteristics	**Sepsis**			
	Yes	**No**	**Total**	**P-value***
Mode of delivery				<0.0001
C-section without labor (CS)	12 (21.1%)	25 (23.4%)	37	
C-section with labor (CS&L)	26 (45.6%)	16 (15.0%)	42	
Vaginal delivery (V)	19 (33.3%)	66 (61.7%)	85	
PPROM				0.9869
Yes	9 (15.8%)	17 (15.9%)	26	
No	48 (84.2%)	90 (84.1%)	138	
Meconium Staining				0.0945
Yes	36 (67.9%)	50 (53.6%)	86	
No	17 (32.1%)	43 (46.2%)	60	
Smoking				0.2571
Yes	4 (7.3%)	14 (13.2%)	18	
No	51 (92.7%)	92 (86.8%)	143	
Illicit Drug Use				0.7765
Yes	4 (7.1%)	9 (8.4%)	13	
No	52 (92.9%)	98 (91.6%)	150	
Alcohol				0.2531
Yes	1 (1.8%)	6 (5.6%)	7	
No	55 (98.2%)	101 (94.4%)	156	
Chlamydia				0.0157
Yes	7 (12.3%)	3 (2.8%)	10	
No	50 (87.7%)	104 (97.2%)	154	
Gonorrhea				0.6487
Yes	1 (1.8%)	1 (0.9%)	2	
No	56 (98.3%)	106 (99.1%)	162	
Genital Herpes				0.2414
Yes	2 (3.5%)	1 (0.9%)	3	
No	55 (96.5%)	106 (99.1%)	161	
UTI				0.048
Yes	3 (5.3%)	16 (15.0%)	19	
No	54 (94.7%)	91 (85.1%)	145	
Preterm Delivery				0.7917
< 37 weeks	30 (52.6%)	54 (50.5%)	84	
37 weeks +	27 (47.4%)	53 (49.5%)	80	
Length of Stay	85 days (59)	36 days (25)		<0.0001
Ethnicity				0.6762
Hispanic	41 (73.21)	75 (70.09)	116	
Non-Hispanic	15 (26.79)	32 (29.91)	47	

Some categories do not add up to 164 due to missing observations.
*Chi-square test of independence p-value.

Table 2.2. Relative risk of sepsis for mode of delivery and chlamydia

Risk factors	RR (95% CI)
Mode of delivery	
C-section without labor (CS)	2.65 (1.67-4.21)
C-section with labor (CS&L)	1.52 (0.83-2.79)
Vaginal delivery (V)	Reference
Chlamydia	
Yes	1.80 (1.08-3.00)
No	Reference

While the safest delivery method in gastroschisis cases remains controversial, previous studies have compared vaginal deliveries to cesarean deliveries, yet failed to investigate cesarean deliveries with labor and the corresponding neonatal outcomes.[8] Our study compared neonatal outcomes through sepsis rates and length of stay in patients delivered vaginally, by cesarean section with labor, and cesarean section without labor.

Neonates with gastroschisis showed a significantly increased risk of sepsis when delivered by CS&L. Neonates delivered by CS&L were over 2 times more likely to develop sepsis, which can then lead to further complications; however, their LOS was shorter compared to V. Also, neonates delivered to mothers with chlamydia showed a significantly greater risk of sepsis.

It is important to note that multiple factors, including surgeon preference, fetal responses nearing time of delivery, and defect severity all play a role in mode of delivery considerations for those babies with gastroschisis. Nonetheless, our results revealed increased sepsis in those delivered by CS&L. Fetal intolerance of labor, or alterations in the vaginal flora exposure in these neonates may have contributed to the increased sepsis rate. The increased rate of sepsis and sepsis-related complications also contributed to increased LOS.

Longitudinal, outcomes-based studies are essential in understanding additional complications related to gastroschisis and utilizing best practice approaches to ensure safety for both mother and baby.

Delayed Closure Increases the Risk of Sepsis and Length of Stay in Neonates with Gastroschisis

Neonates with gastroschisis undergo primary or delayed closure. We sought to determine whether there was any difference in neonatal sepsis based upon the closure strategy. Additionally, we investigated the effect of sepsis and type of closure on length of stay (LOS).

The records of neonates with gastroschisis managed at Loma Linda University Medical Center and Lucile Packard Children's Hospital Stanford from February 1999 to December 2012 were reviewed (n=152). The closure type was classified as: primary fascial closure (P/F) and initial silo closure with staged fascial closure (S). Primary outcome was culture proven sepsis and secondary outcome was LOS. Sepsis rates by type of closure were assessed. Crude log-binomial risk ratios (RR) and 95% confidence intervals (CI) were reported for the presence of chlamydia, gonorrhea, genital herpes, urinary tract infections, preterm premature rupture of membranes, preterm delivery, intrauterine growth restriction, meconium staining, Hispanic ethnicity, and abuse of any of the following substances: tobacco, illicit drugs, or alcohol. The effects of sepsis and type of closure on LOS were assessed using multiple linear regression. The distribution of closure strategies was as follows: P/F=44 and S=108 (Table 2.3). Sepsis incidence was 83% higher in S compared to P/F, RR=1.83 (1.02-3.30) (Table 2.4). The risk of sepsis was doubled among neonates delivered to mothers with chlamydia RR=2.07 (1.30-3.30).

Table 2.3. Population characteristics by sepsis status (Yes *vs*. No)

Characteristics	Sepsis					
	Yes	No	Total	P-value*	RR	(95% CI)
Type of Closure				0.0275		
Primary (P/F)	10 (18.2%)	34 (35.1%)	44		1	
Silo (S)	45 (81.8%)	63 (65.0%)	108		1.83	1.02 3.30
Chlamydia				0.0213		
Yes	7 (12.3%)	3 (3.1%)	10		2.07	1.30 3.30
No	48 (87.3%)	94 (96.9%)	142		1	
Gonorrhea				0.6823		
Yes	1 (1.8%)	1 (1.0%)	2		1.39	0.34 5.64
No	54 (98.2%)	96 (99.0%)	150		1	
Genital Herpes				0.2671		
Yes	2 (3.6%)	1 (1.03%)	3		1.87	0.82 4.30
No	53 (96.4%)	96 (99.0%)	149		1	
UTI				0.048		
Yes	3 (5.5%)	16 (16.5%)	19		0.40	0.14 1.17
No	52 (94.5%)	81 (83.5%)	133		1	
PPROM				0.7515		
Yes	8 (14.6%)	16 (16.5%)	24		0.91	0.49 1.67
No	47 (85.5%)	81 (83.5%)	128		1	
Preterm Delivery				0.9398		
< 37 weeks	28 (50.9%)	50 (51.6%)	78		0.98	0.65 1.50
37 weeks +	27 (49.1%)	47 (48.5%)	74		1	
IUGR				0.0308		
Yes	17 (30.9%)	47 (49.0%)	64		0.61	0.38 0.98
No	38 (69.1%)	49 (51.0%)	87		1	
Meconium Staining				0.2114		
Yes	35 (68.6%)	48 (57.8%)	83		1.34	0.83 2.17
No	16 (31.4%)	35 (42.2%)	51		1	
Substance Abuse				0.1727		
Yes	7 (12.7%)	21 (21.7%)	28		0.65	0.33 1.27
No	48 (87.3%)	76 (78.4%)	124		1	
Length of Stay	86 days (±59)	37 days (±25)		<0.0001		
Ethnicity				0.7836		
Hispanic	39 (72.22)	68 (70.10)	107		0.94	0.58 1.51
Non-Hispanic	15 (27.78)	29 (29.90)	44		1	

Some categories do not add up to 152 due to missing observations.
*Chi-square test of independence p-value.

None of the other covariates showed a significant effect on neonatal sepsis. An increase of 26 (11.89, 39.99) days was observed for neonates with S compared to P/F, while sepsis increased LOS by 45 (31.55, 58.07) days.

Table 2.4. Mean length of stay as predicted by sepsis diagnosis and Type of Closure.

Parameter	Mean LOS (95% CI)
Intercept	20.02 (7.98, 32.06)
Type of Closure	
Silo (S)	25.94 (11.89, 39.99)
Primary (P/F)	Reference
Sepsis	
Yes	44.81 (31.55, 58.07)
No	Reference

There is a significant increase in both the risk of sepsis and LOS associated with S compared to P/F, but further investigations are warranted to elucidate these effects. It is important to note that often times closure methods depend on pediatric surgeon preference and severity of defect opening and gut and/or organ protrusion. Understanding the effects various treatments play in outcomes of neonates with gastroschisis helps us understand the complexities and long term effects associated with this defect.

Outcomes of Infants Born with Gastroschisis at 12 Months of Age: A Prospective Cohort in Southern California

Given that gastroschisis is the most common birth defect of the abdominal wall, and its prevalence has risen on both global and local scales throughout the last few

decades we sought to study the long term morbidities associated with gastroschisis and to determine if there are prognostic factors that may predict adverse outcomes in affected infants.

Our objective of this study was to follow infants affected by neonatal gastroschisis prospectively to determine long-term outcomes. Specifically, our aim was to determine if there are factors at birth or during the immediate neonatal period related to poor long-term outcomes in infants with gastroschisis.

A prospective cohort study was performed on cases of gastroschisis at Loma Linda University Children's Hospital located in California's Inland Empire Region. Study participants were enrolled beginning in 2014, after this study received IRB approval. Informed consent was obtained from all study participants. Infants included in the study included all study participants who were live born and greater than 12 months of age at the time of chart review. Thirty-three infants were included, and one intrauterine fetal death (IUFD) was excluded. Infants who were enrolled in the study were excluded if they were less than 12 months of age at the time of chart review. Maternal and infant factors were assessed including: maternal pregnancy complications, maternal GBS status, gestational age at delivery, mode of delivery, weight category at delivery, type of gastroschisis, timing of closure, closure type, time to full enteral feeds, and postnatal complications. Infants were divided into the categories of good outcomes and poor outcomes based upon the number of postnatal complications. Infants with less than 3 postnatal complications were placed in the good outcome category. Infants with 3 or more postnatal complications were placed in the poor outcome category. Among the 33 infants that were included in the study, 18 infants were classified as having a good

outcome and 15 infants were classified as having a poor outcome. The groups were compared to determine if there was statistical difference between the groups. Chi squared test was used to determine if there was statistical difference between nominal data, and t-test was used to determine if there was statistical difference between numerical data. In the poor outcome group there was significantly more infants with complex gastroschisis ($p = 0.006$). Additionally, infants in the poor outcome group had significantly longer time to full feeds, with a mean of 122 days compared to 24 days in the good outcome group. The poor outcome group was noted to have more deliveries via cesarean section, although $p = 0.06$, which did not meet our cut-off for statistical significance. Although this may be due to small sample size, as it appears data are trending towards significance.

The timing of closure was not significantly different ($p = 0.17$) between infants with good and poor outcomes, and no significant difference ($p = 0.14$) was noted between weight category at time of birth or maternal GBS status ($p = 0.95$).

The strength of this study is that subjects were prospectively enrolled and followed. The limitations of this study are the relatively short duration follow-up, the small sample size, and loss of patients during follow-up visits.

Nonetheless, we noted that complex gastroschisis and delayed time to full feeds are associated with poor outcomes at greater than one year of life. Additionally, infants that are delivered via cesarean section may be at higher risk for poor long-term outcomes of the disease. Long term follow-up data will aid in identifying prognostic facts and aid in counseling for families affected by gastroschisis.

Acknowledgements

A very special thank you to The Perinatal Institute at Loma Linda University Children's Hospital for not only facilitating the space to conduct this research, but also to various staff, physicians, nurses, and administrative personnel for their support of gastroschisis research. A few people who have been instrumental in this project, especially when it was just getting started are Judy Gates, Dr. Trina Mansour, Salma Kabir, and Dr. Tara Billings. Thank you for tirelessly putting assisting with IRB paperwork, sample and data collection, always being willing to help out, especially during those beginning brainstorming days.

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SIBLING RECURRENCE RATE OF GASTROSCHISIS FROM A 12-YEAR COHORT IN SOUTHERN CALIFORNIA

By

Desai, Arti K., MPH, PhD(c)[1,3]; Billings, Tara L., DO[2,3]; Mansour, Trina, MD[3,5]; Oshiro, Bryan T., MD[4,5]

[1] Department of Earth and Biological Sciences, Basic Sciences, School of Medicine, Loma Linda University, CA

[2] Department of Pediatrics, Loma Linda University, Loma Linda, CA

[3] Perinatal Research Institute, Loma Linda University Children's Hospital, Loma Linda, CA

[4] School of Public Health, Loma Linda University, Loma Linda, CA

[5] Department of OB/GYN, School of Medicine, University of California, Riverside

This section will be submitted for publication

Abstract

Purpose: Gastroschisis is a birth defect of the abdominal wall. While the incidence of gastroschisis has increased globally in recent years, only a few familial recurrent cases have been reported. **Methods:** A cohort study was performed to determine the sibling recurrence rate of gastroschisis at our tertiary care medical center located in Southern California. 186 neonates with gastroschisis were delivered from 2003-2015 to 183 mothers. Among these mothers, 55 were multiparous and therefore included in calculating the rate of gastroschisis occurring in siblings. Primiparous women were excluded from this study. **Results:** Of 55 multiparous women included in the study, 3 women had pregnancies affected by recurrent gastroschisis. This represents a 5.45% sibling recurrence rate of gastroschisis in our study cohort. **Conclusions:** Literature has failed to demonstrate hereditary factors in gastroschisis pathogenesis. However, the recurrence rate of familial gastroschisis suggests that women with a history of a prior neonate with gastroschisis may be at a higher risk than previously noted. Therefore, families with a history of gastroschisis may benefit from pre-conceptional counseling to discuss the higher risk of gastroschisis in a future pregnancy. Expectant mothers who have had a pregnancy complicated by gastroschisis should undergo early ultrasound and counseling to ensure the best management of care for mother and neonate.

Introduction

Gastroschisis is a birth defect of the abdominal wall where an infant's intestines protrude outside of the body usually through an opening to the right of the umbilical cord. [1, 2] Studies show an increased prevalence of gastroschisis on both global and local scales

throughout the last few decades. [3-9] The prevalence of gastroschisis is usually noted as 0.5- 1 cases per 10,000 live births, yet in England the incidence of gastroschisis doubled in about a decade span (1987-1995) to 1.35 per 10,000 births and in Southwestern England the incidence has been as high as 4.4 per 10,000 births. [6] Mexico noted an increased prevalence from 2.09 per 10,000 live births in 2000 to 6.85 per 10,000 in 2014.[8] In the United States, the state of California and its Inland Empire counties are no exception to this trend.[9-18] In one of the largest studies conducted in the United States, researchers found the prevalence of gastroschisis nearly doubled from 1995 to 2005, increasing from 2.3 per 10,000 live births in 1995 to 4.4 per 10,000 live births in 2005. [16] Additionally, a 17-year study (1987 to 2003) utilizing data from the California Birth Defects Monitoring Program concluded that the overall birth prevalence of gastroschisis increased by 3.2-fold and continues to increase in California. [17] Specifically, in San Bernardino and Riverside Counties of Southern California annual rates of gastroschisis were noted as 3.2 and 6.0 per 10,000 live births in the years 2005 and 2006, respectively.[18]

While the prevalence of gastroschisis has increased globally in recent years, its etiology and pathogenesis remain unknown. [1-3, 19, 20] Many studies have considered genetic and environmental factors. For instance, demographics, such as young maternal age, low gravidity and parity, low socioeconomic status, and maternal smoking have been proposed, yet no strong associations have been identified and proven true. [2, 14, 16-22]

Interestingly, a few familial recurrent cases are reported. Specifically, a familial recurrence risk of 2.4% was calculated by Kohl et al, from population-based registries where 10 familial cases were noted amongst 412 gastroschisis births. [19] Additionally, a

study by Torfs and Curry noted a familial recurrence risk of 4.7% where 6 out of 127 families had more than one relative affected by gastroschisis and a sibling recurrence risk of 3.5%. [21]

Given that the incidence of gastroschisis has increased globally in recent years, and identification of various risk factors have not yet led to concrete etiology, understanding familial recurrence risk is a step towards preventing poor outcomes. With familial recurrence rates of gastroschisis more frequent than previously noted, expectant mothers who have had a pregnancy complicated by gastroschisis or have had a family member with gastroschisis should undergo early ultrasound and counseling to ensure the best management of care for mother and neonate.

Material and Methods

A cohort study was performed to determine the sibling recurrence rate of gastroschisis at Loma Linda University Children's Hospital, a tertiary care medical center located in Southern California. We identified the delivery of 186 neonates with gastroschisis from 2003-2015 to 183 mothers. Among these mothers, 55 were multiparous, and therefore included in calculating the rate of gastroschisis occurring in siblings, while the remaining primiparous women were excluded from this study.

Results

Of the 55 multiparous women included in the study, three women had pregnancies affected by recurrent gastroschisis, representing a 5.45% sibling recurrence rate of gastroschisis in our study cohort. Further investigation showed one patient was a

19-year-old G3P0212 who delivered a female neonate affected by gastroschisis in 11/2009, and subsequently delivered a male neonate affected by gastroschisis in 12/2010. A second patient was a 21-year-old G3P1202 who delivered a female neonate in 1/2006 affected by gastroschisis, a female neonate not affected by gastroschisis in 8/2006 which resulted in neonatal death, and subsequently delivered a female neonate in 9/2009 affected by gastroschisis. The third patient was a 21-year-old G2P2002 who delivered a female neonate in 2/2011 affected by gastroschisis, and subsequently delivered a female neonate in 10/2015 affected by gastroschisis. (Table 2.5).

Table 2.5. Sibling recurrences of gastroschisis reported from 2003- 2015 at Loma Linda University Children's Hospital

Case Number	Maternal Age	Gestational History	Neonate Sex	Neonatal Birth Year
1	19/21	G3P0212	Female/Male	2009/2010
2	21/24	G3P1202	Female/Female	2006/2009
3	21/25	G2P1001	Female/Female	2011/2015

Discussion

While literature has failed to demonstrate hereditary factors in gastroschisis pathogenesis, the recurrence rate of familial gastroschisis suggests that women with a history of a prior neonate with gastroschisis or with a family history of gastroschisis may be at higher risk than previously noted. A 5.45% recurrence rate was noted during our cohort study, corroborating recurrence trends previously noted.

These observations are met with a few limitations. While our 13-year tracking yielded a robust sample size, these patients are limited to Southern California's Inland Empire region and its surrounding referral area. Next, in collecting family history, often times it was noted that medical records only provided brief medical histories and/or a patient was unaware of their extended family history. Also, unidentified paternal identity and unknown paternal family history provides an incomplete neonatal pedigree. Further studies should try to obtain more comprehensive patient and family histories, and even longitudinally track families with a pregnancy affected by gastroschisis to see the outcome of subsequent pregnancies. In conclusion, families with a history of gastroschisis may benefit from pre-conceptual counseling to discuss the higher risk of gastroschisis in a future pregnancy. Expectant mothers who have had their own previous pregnancy or that of a family member complicated by gastroschisis should undergo early ultrasound and counseling to ensure the best management of care for mother and neonate.

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PERIPARTUM AND NEONATAL OUTCOMES OF SMALL-FOR-GESTATIONAL-AGE INFANTS WITH GASTROSCHISIS

By

Anna I. Girsen[1], Samantha Do[1], Alexis S. Davis[3,6], Susan R. Hintz[2,6], **Arti K. Desai**[4], Trina Mansour[4], T. Allen Merritt[5], Bryan T. Oshiro[4], Yasser Y. El-Sayed[1,6] and Yair J. Blumenfeld[1,6]

[1]Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, CA, USA

[2]Division of Neonatal and Developmental Medicine, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA

[3]Pediatrix Medical Group, San Jose, CA, USA

[4]Department of Obstetrics and Gynecology, Loma Linda University School of Medicine, Loma Linda, CA, USA

[5]Division of Neonatology, Department of Pediatrics, Loma Linda University School of Medicine, Loma Linda, CA, USA

[6]The Fetal and Pregnancy Health Program, Lucile Packard Children's Hospital Stanford, Palo Alto, CA, USA

This paper has been published in Prenatal Diagnosis 2015, 35, 477–482.

Abstract

Objectives Neonates with gastroschisis are often small for gestational age (SGA) based on population nomograms. Our objective was to evaluate the effect of SGA on perinatal and neonatal outcomes in cases of gastroschisis. **Methods** This is a retrospective study of neonates with prenatally diagnosed gastroschisis from two academic centers between 2008 and 13. Perinatal and neonatal outcomes of neonates with SGA at birth were compared with appropriate-for-gestational-age (AGA) neonates. The primary composite outcome was defined as any of the following: neonatal sepsis, short bowel syndrome at discharge, prolonged mechanical ventilation (upper quartile for the cohort), bowel atresia or death. **Results** We identified 112 cases of gastroschisis, 25 of whom (22%) were SGA at birth. There were no differences in adverse peripartum outcomes between SGA and AGA infants. No difference was found in the primary composite neonatal outcome (52% vs 36%, p = 0.21), but SGA infants were more likely to have prolonged mechanical ventilation (44% vs 22%, p = 0.04) and prolonged length of stay (LOS) (52% vs 22%, p = 0.007). After adjusting for GA at delivery, SGA remained associated with prolonged LOS (OR = 4.3, CI: 1.6–11.8). **Conclusion** Among infants with gastroschisis, SGA at birth is associated with a fourfold increase in odds for prolonged LOS, independent of GA.

Introduction

Gastroschisis is a severe paraumbilical defect of the fetal abdominal wall that occurs in approximately one to five cases per 10 000 live births.[1] Fetal gastroschisis is commonly diagnosed in utero by routine ultrasound that identifies the defect with high

sensitivity and specificity starting as early as the first trimester.[2,3] Although the overall neonatal mortality among gastroschisis cases is low,[4] pregnancies with gastroschisis are at increased risk for severe peripartum complications including meconium staining, intrauterine growth restriction (IUGR) and stillbirth, as well as neonatal gastrointestinal morbidities including bowel dysfunction, bowel atresia, bowel necrosis and short-bowel syndrome.[5]

Prior studies have identified the association between gastroschisis and prenatally suspected IUGR and small for gestational age (SGA) at delivery.[6,7] It has been reported that pregnancies complicated by IUGR are more likely to result in increased neonatal morbidity, including increased surgical complications, longer hospital stay, delay in establishment of full enteral feeds and impaired long-term growth.[8–11] However, because of the underestimation of the fetal abdominal circumference by prenatal ultrasound using most estimated fetal weight formulas,[12] the false positive rates for suspected IUGR diagnosis may be high and a misdiagnosis may lead to unnecessary iatrogenic preterm delivery and related morbidities. Moreover, prenatal prediction of SGA is erroneous even in non-gastroschisis cases.[13,14] Therefore, understanding the association between 'true SGA' at birth and perinatal and neonatal outcomes in cases of gastroschisis is warranted. Our aim was to evaluate the association between SGA and perinatal and neonatal outcomes in cases of prenatally diagnosed gastroschisis.

Materials and Methods

Study population This was a retrospective cohort study of all infants with prenatally diagnosed gastroschisis whose mothers received prenatal care at Loma Linda

University Medical Center and Lucile Packard Children's Hospital at Stanford between 2008 and 2013. Both institutions are tertiary care referral centers in California with maternal–fetal medicine, prenatal ultrasound, level IV neonatal intensive care units and pediatric surgical expertise in managing gastroschisis.

Gastroschisis cases were identified from separate institutional databases in which pregnancies with fetal anomalies are prospectively entered. Only cases with information on SGA diagnosis were included in the analysis. In both institutions, pregnancies complicated by fetal gastroschisis are managed in outpatient high-risk pregnancy clinics, with serial ultrasound surveillance and antenatal non-stress testing. Indications for delivery prior to 37 completed weeks gestation are severe maternal medical or obstetric complications, or non-reassuring fetal status including suspected IUGR or abnormal antenatal testing. In the absence of associated fetal or maternal morbidity, delivery for gastroschisis cases is recommended between 36 and 37 completed weeks in both institutions in order to avoid early term stillbirth, the precise timing of which is left to the discretion of the primary care provider. A trial of labor is preferred over cesarean delivery in the absence of obstetric contraindications.

Study data from both institutions were collected and managed using Research Electronic Data Capture (REDCap), [15] a secure, Web-based application designed to support data capture for research studies. Institutional review board approval from both Loma Linda University and Stanford University was obtained prior to initiation of the study.

Study definitions Detailed perinatal, intrapartum and neonatal variables were collected from electronic medical records. In addition, ultrasound reports and stored ultrasound

images were reviewed by trained research nurses and physicians. A prenatal diagnosis of suspected IUGR was made based on ultrasound estimation of fetal weight less than 10% by Hadlock formula, [16] with or without the presence of oligohydramnios and/or umbilical artery Doppler abnormalities. Doppler abnormalities in the ductus venosus, umbilical vein or middle cerebral artery are not routinely performed in prenatally diagnosed cases of fetal gastroschisis in either center, [17] and umbilical artery Doppler assessment is only performed in cases of suspected IUGR. Maternal pre-pregnancy body mass index (BMI) was calculated as (BMI=weight in kilograms/height^2 in meters) using height and documented weight at pre-pregnancy.

Small for gestational age was defined by a birth weight less than 10th percentile at delivery using the Fenton growth charts for infants <38 weeks GA[18] and the WHO growth charts for infants ≥38 weeks GA.[19] Neonatal prolonged length of stay (LOS) was defined a priori as the upper quartile (>75th percentile) of LOS (in days) for the entire cohort. Prolonged mechanical ventilation was defined similarly a priori as the upper quartile of the length of mechanical ventilation for the cohort. Neonatal sepsis or infection diagnosis was based on a positive blood culture during initial neonatal hospitalization.

The primary outcome of the study was a composite neonatal outcome, defined as any of the following: any culture-positive sepsis or infection, short bowel syndrome at discharge (defined clinically by the neonatologist and/or surgeon as recorded in medical record), prolonged mechanical ventilation, bowel atresia (as documented in the medical record by the neonatal or surgical teams) or death prior to discharge. Secondary outcomes

were the individual components of the primary outcome (listed earlier), prolonged hospital LOS and prolonged mechanical ventilation requirement.

Statistical analysis Statistical analysis was performed using R application (version 2.15.0, R Development Core Team, 2011, Vienna, Austria). Unadjusted analyses were performed using chi-squared test or Fisher's test for categorical variables and Student's t-test or Wilcoxon test for continuous variables. Sensitivity and specificity analyses were performed in subgroups of pregnancies with prenatal ultrasonography performed within 14 and 7 days prior to delivery, respectively. Multivariable logistic regression models were constructed to determine the independent association of SGA with neonatal outcomes while adjusting for GA. Results of the model were expressed as odds ratios (OR) and 95% confidence intervals (CI). The level of significance was set at $p<0.05$.

Results

A total of 112 cases of prenatally diagnosed gastroschisis were identified and included in the analysis, of which 25 neonates (22%) were diagnosed with SGA at delivery. Of the 25 SGA neonates, 17 (68%) had a birth weight <10th percentile and 8 (32%) had birth weight <3rd percentile. There were no cases of antepartum or peripartum stillbirth, and all cases had both perinatal and neonatal data available for analysis. No significant differences were seen in maternal age, race/ethnicity, payer status, study center, gravidity or parity, pre-pregnancy BMI or smoking between the SGA and appropriate-for-gestational-age (AGA) groups (Table 2.6).

Neonates that were SGA had similar peripartum complication rates when compared with AGA neonates. There were no differences in meconium staining at

delivery, gestational age at delivery, oligohydramnios rated, preterm premature membrane rupture (PPROM) rates, preterm delivery less than 37 weeks or cesarean delivery between the two groups (Table 2.7). Prenatal IUGR diagnosis was suspected in 76% of the SGA neonates compared with 41% in the AGA cohort ($p = 0.005$) but the incidence of abnormal umbilical artery Doppler flow was not different between the groups (8% vs 0% in AGA, $p = 0.07$) (Table 2.7).We analyzed cases that underwent an ultrasound exam within 14 days of delivery ($n = 65$) and found the sensitivity and specificity of suspected IUGR diagnosis to be 100% and 42%, respectively. When limiting the analysis to those undergoing an ultrasound exam within 7 days of delivery ($n = 33$), the sensitivity and specificity were 100% and 35%.

Small-for-gestational-age neonates had a significantly smaller mean birth weight at delivery compared with AGA neonates (2051 ± 268 g vs 2639 ± 425 g, $p<0.0001$), although no differences in the gestational age at delivery or 5 min Apgar scores were seen (Table 2.8). No significant difference was seen in the unadjusted rate of the primary composite outcome between the SGA and AGA groups (52% vs 36%, $p = 0.21$). Although no significant difference was found in absolute hospital LOS or length of mechanical ventilation, the SGA neonates were more likely to have prolonged hospital stay, which was ≥53 days (52% vs 22%, $p<0.01$) and prolonged mechanical ventilation (≥11 days; 44% vs 22%, $p = 0.04$) compared with AGA neonates. There were no differences in the type of closure performed, with 32% achieving primary closure in the SGA group and 37% in the AGA group, $p = 0.84$. There was also no difference in the incidences of bowel atresia (0% vs 7%, $p = 0.43$) or neonatal sepsis (32% vs 20%, $p =$

0.30) between the SGA and AGA groups (Table 2.8). One neonatal death occurred in the AGA group at the age of 46 days.

A multivariable logistic regression analyses were then performed in order to assess the association between neonatal outcomes and SGA, adjusting for gestational age at delivery (Table 2.9). The odds for prolonged LOS were significantly increased in SGA neonates compared with AGA neonates (adjusted OR: 4.3, 95% CI: 1.3–15.3). In addition, the odds of prolonged mechanical ventilation were higher in SGA neonates (OR = 3.0, CI: 1.1–8.1) compared with AGA neonates. No significant association was found between SGA and primary composite outcome, culture proven sepsis/infection or preterm delivery at <37 weeks gestation (Table 2.9).

Table 2.6. Maternal demographics in cases of small-for-gestational-age neonates (SGA) compared with appropriately grown (AGA) neonates with gastroschisis

	SGA = 25	AGA = 87	*p*-value[a]
Maternal age (years)	20.9 (4.2)	21.6 (4.1)	0.50
Race/ethnicity			0.10
Non-Hispanic White	2 (8%)	21 (24%)	
Hispanic	14 (56%)	32 (37%)	
Other	7 (28%)	31 (36%)	
Missing	2 (8%)	3 (3%)	
Payer status			0.23
Public	19 (76%)	67 (77%)	
Private	5 (20%)	7 (8%)	
Self-pay	0 (0%)	3 (3%)	
Unknown/missing	1 (4%)	10 (12%)	
Academic center			0.46
Loma Linda University	14 (56%)	58 (67%)	
Stanford University	11 (44%)	29 (33%)	
Gravida	2 (1–3)	2 (1–2)	0.65
Parity			0.99
Nulliparous	16 (64%)	58 (67%)	
Multiparous	9 (36%)	29 (33%)	
Pre-pregnancy BMI (kg/m^2)	21.4 (16.6–30.0)	23.0 (16.6–40.6)	0.20
Smoking during pregnancy	4 (16%)	13 (15%)	0.88
Unknown/missing	1 (4%)	2 (2%)	

Data are presented as mean (SD), median (interquartile range, IQR) or *n* (%).
BMI, body mass index.
[a]Chi-squared test or Fisher's exact test for categorical variables and *t*-test or Wilcoxon test for continuous variables.

Table 2.7. Perinatal outcomes of small-for-gestational-age (SGA) neonates compared with appropriately grown (AGA) neonates with gastroschisis

	SGA = 25	AGA = 87	*p*-value[a]
Suspected IUGR by prenatal ultrasound	19 (76%)	36 (41%)	<0.01
Time from last ultrasound exam to delivery (days)	10 (6–21)	12 (4–19)	0.85
Oligohydramnios	1 (4%)	13 (15%)	0.26
Abnormal umbilical artery Doppler assessment	2 (8%)	0 (0%)	0.07
Prenatal bowel dilation by ultrasound	14 (56%)	55 (63%)	0.64
Missing data on prenatal bowel assessment	11 (44%)	32 (37%)	
PPROM	5 (20%)	15 (17%)	0.98
Preterm delivery <37 weeks	14 (56%)	46 (53%)	0.96
Meconium staining	12 (48%)	43 (49%)	0.82
Gestational age at delivery (weeks)	36.5 (1.3)	36.6 (1.4)	0.72
Type of delivery			0.36
Spontaneous or augmented labor	6 (24%)	31 (36%)	
Induced labor	16 (64%)	43 (49%)	
Scheduled cesarean	3 (12%)	11 (13%)	
Missing	0 (0%)	2 (2%)	

Data are presented as *n* (%), median (IQR) or mean (SD).
IUGR, intrauterine growth restriction; PPROM, preterm premature rupture of membrane.
[a]Chi-square test or Fisher's exact test for categorical variables, *t*-test or Wilcoxon test for continuous variables.

Table 2.8. Neonatal outcomes of small-for-gestational-age (SGA) neonates compared with appropriate-for-gestational-age (AGA) neonates with gastroschisis

	SGA = 25	AGA = 87	*p*-value[a]
Birth weight (g)	2051 (268)	2639 (425)	<0.001
Apgar score <7 at 5 min	1 (4%)	7 (8%)	0.68
Defect closure			0.84
Primary closure	8 (32%)	32 (37%)	
Delayed closure	17 (68%)	55 (63%)	
Primary composite outcome[b]	13 (52%)	31 (36%)	0.21
Days on ventilator	9 (4–12)	5 (2–10)	0.06
Bowel atresia	0 (0%)	6 (6.9%)	0.43
Neonatal death	0 (0%)	1 (1%)	0.50
Culture-proven sepsis or infection	8 (32%)	17 (20%)	0.30
Hospital LOS (days)	53 (25–70)	35 (26–49)	0.25
Prolonged hospital LOS[c]	13 (52%)	19 (22%)	<0.01
Prolonged mechanical ventilation[d]	11 (44%)	19 (22%)	0.04
Missing	2 (8%)	5 (6%)	

Data are presented as *n* (%) or median (IQR).
LOS, length of stay.
[a]Chi-squared test or Fisher's exact test for categorical variables and Wilcoxon test for continuous variables.
[b]Variables included culture-proven sepsis or infection, neonatal short bowel syndrome, prolonged stay on ventilator, bowel atresia or neonatal death.
[c]Defined as upper quartile (>75th percentile) of LOS (>53 days).
[d]Defined as upper quartile (>11 days).

Table 2.9. Association between neonatal outcomes and SGA among infants with gastroschisis

	SGA OR (95% CI)[a]	AGA
Primary composite outcome	1.9 (0.8–4.9)	Reference
Prolonged mechanical ventilation	3.0 (1.1–8.1)	Reference
Culture-proven sepsis or infection	1.9 (0.7–5.2)	Reference
Prolonged LOS	4.3 (1.6–11.8)	Reference
Preterm delivery at <37 weeks	0.4 (0.04–2.9)	Reference

[a]Models adjusted for gestational age.
AGA, appropriate-for-gestational age; SGA, small-for-gestational-age; LOS, length of stay; OR, odds ratio; CI, confidence interval.

Discussion

The prevalence of fetal gastroschisis is increasing. In a recent study of over 13 million live births from 15 states, 4713 of whom were complicated by fetal gastroschisis, a consistent increase in the prevalence of gastroschisis was noted from 2.32 per 10000 births in 1995 to 4.42 per 10 000 in 2005.[1] This increase is particularly worrisome given the association between gastroschisis and severe peripartum and neonatal morbidity and mortality. In this study, we evaluated the effect of SGA on perinatal and neonatal outcomes in cases of prenatally diagnosed gastroschisis using a cohort from two tertiary care centers in California and found that prolonged length of stay was significantly associated with SGA compared with AGA, independent of gestational age.

The association between gastroschisis and SGA at delivery is well established, with most studies describing an approximate 20% rate of SGA[20] while much higher rates, up to 40–60%, have also been described in gastroschisis.[21,6,22] Reasons for differences in these rates are unclear and may be related to different definitions of SGA (<3% vs <10%), different maternal baseline demographics and potential differences in exposure to

environmental toxins, some of which have been linked with the development of gastroschisis.[23] Our cohort comprised mostly young nulliparous women with relatively low pre-pregnancy BMI, and the maternal smoking rate of 15% in our cohort is slightly higher than that generally seen in California.[24]

The current study provides important and robust data regarding the association between SGA at birth and both perinatal and neonatal outcomes not previously described. In our cohort, we did not find a higher rate of adverse perinatal outcomes including meconium staining, PPROM, preterm delivery or cesarean delivery when comparing SGA neonates with their AGA counterparts. Although SGA is by large postulated to be placentally mediated, in this study the lack of difference in perinatal outcomes between those with and without growth restriction suggests that some of the fetuses with suboptimal growth may have been constitutionally small rather than pathologically grown due to abnormal placentation.[25] Also, mothers of SGA neonates had similar pre-pregnancy BMI, smoking status and parity when compared with mothers of AGA neonates. Unfortunately, placental pathological assessment was not routinely performed in our cohort, and further studies are necessary to characterize possible pathological mechanisms leading suboptimal growth in gastroschisis cases.

From a neonatal perspective, SGA neonates did experience a longer hospital length of stay and prolonged mechanical ventilation when compared with those who were AGA, but we did not find a similar association with other neonatal morbidities, including the composite adverse neonatal outcome. In a recent study of 191 gastroschisis cases from the University of California database, SGA was not found to be associated with a composite neonatal outcome including death, bowel complications requiring reoperation,

gastrostomy and necrotizing enterocolitis.[20] In another cohort of 66 gastroschisis cases from Texas, evidence of growth restriction, defined as birth weight less than 3%, was not found to be associated with longer length of stay or longer total parenteral nutrition,[21] despite the fact that SGA neonates in that cohort were more likely to require delayed closure. Reasons for the prolonged hospitalization in our SGA cohort remain unclear, as there were no differences in gestational age, type of closure, sepsis or bowel atresia between the groups. However, the finding of significantly increased rate of prolonged mechanical ventilation in the SGA group may suggest associated clinical variables and morbidities. Of note, sepsis was more common among SGA infants compared with AGA infants, although the difference did not reach statistical significance likely due to relatively small patient numbers.

The association between gastroschisis and SGA is well known to prenatal sonographers, resulting in possible bias and leading to a lower specificity and higher false positive rates of prenatal suspected IUGR diagnosis.[26, 27] In our cohort, SGA was suspected prenatally more frequently in SGA neonates compared with non-SGA neonates, and the ultrasound sensitivity and specificity of SGA were 100% and 42% within 14 days of delivery and 100% and 35% within 7 days of delivery. The low specificity may have resulted from our definition of IUGR, which was an estimated fetal weight <10% for gestational age with or without umbilical artery Doppler and amniotic fluid abnormalities. Of note, there were no differences in either abnormal umbilical artery Doppler findings or oligohydramnios between the study groups, but we were likely underpowered to study those outcomes. Others have noted a similar low specificity for prenatal IUGR diagnosis in gastroschisis cases, with ultrasound predicting IUGR in 43%

of cases in one study but SGA present in only 23%.[26] A study by Ajayi *et al.* also found that only 50% of fetuses with abdominal circumference<2.5th percentile were SGA at birth.[27] In contrast, the reported sensitivity for SGA prediction in non-gastroschisis cases ranges from approximately 64% to 85%, with a specificity of 63–94%.[13,14] An ultrasound diagnosis of suspected IUGR may be inaccurate for SGA determination in cases of fetal gastroschisis because the abdominal circumference is often smaller than expected due to a large amount of bowel being located outside of the abdominal cavity.[28,29] In addition, it has been shown in a non-gastroschisis population that the SGA versus AGA comparison used does not properly reflect the percentage of body fat among these infants.[30] Given that the finding of IUGR may prompt iatrogenic preterm delivery in pregnancies with gastroschisis, it is important to develop more reliable methods of predicting SGA.

Our study is not without limitations. First, our approach was a retrospective review along with its inherent biases. We included all cases of prenatally diagnosed gastroschisis that received both prenatal and postnatal care in our centers during the study period, and it is possible that ascertainment bias exist; specifically, some prenatal stillbirth cases may have occurred before an initial referral to our centers could occur, and their data are therefore not included in the analysis. In accordance with this limitation, we had no cases of prenatal stillbirth in either group in either institution while the stillbirth rate described in other cohorts ranges from 1% to 3%.[10,11] Also, our centers do not employ a common standardized algorithm for the prenatal and postnatal management of gastroschisis, and therefore, approach to care may have been individualized. Unfortunately, the use of umbilical artery Doppler was not standardized in either institution, thus limiting the interpretation of this data. Sample size limitations prevented

an analysis of the effects of individual provider factors on perinatal and neonatal outcomes and we were possibly underpowered to see certain rare adverse neonatal outcomes. Finally, SGA at delivery may not indicate pathological growth, but rather constitutional growth in some cases.[25] Unfortunately, we were unable to analyze additional factors associated with pathological growth that might have confounded the results including certain prenatal Doppler abnormalities or postnatal assessments, such as the ponderal index, because those were not universally collected in our centers.

Strengths of the analysis include the relative size of the cohort and the inclusion of detailed prenatal, perinatal and neonatal data from two large referral centers, thereby making our results more generalizable. By focusing on 'true SGA' at birth, and not suspected IUGR based on prenatal ultrasound, we were able to analyze the effect of likely pathological growth on both perinatal and neonatal outcomes. Our finding of an independent association of SGA and prolonged neonatal LOS may help providers counsel their patients and warrants further study as to possible underlying etiologies.

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EFFECT OF ANTEPARTUM MECONIUM STAINING ON PERINATAL AND NEONATAL OUTCOMES AMONG PREGNANCIES WITH GASTROSCHISIS

By

Anna I. Girsen[1], Matthew B. Wallenstein[2], Alexis S. Davis[3,4], Susan R. Hintz[2,4], **Arti K. Desai**[5], Trina Mansour[5], T. Allen Merritt[6], Maurice L. Druzin[1,4], Bryan T. Oshiro[5], and Yair J. Blumenfeld[1,4]

[1]Department of Obstetrics & Gynecology, Stanford University School of Medicine, Stanford, CA, USA

[2]Division of Neonatal and Developmental Medicine, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA

[3]Pediatrix Medical Group, San Jose, CA, USA

[4]The Fetal and Pregnancy Health Program, Lucile Packard Children's Hospital Stanford, Palo Alto, CA, USA

[5]Department of Obstetrics and Gynecology, Loma Linda University School of Medicine, Loma Linda, CA, USA

[6]Division of Neonatology, Department of Pediatrics, Loma Linda University School of Medicine, Loma Linda, CA, USA

This paper has been published in The Journal of Maternal-Fetal & Neonatal Medicine. 2016 Aug 2;29(15):2499-503.

Abstract

Objective: To investigate the association between meconium staining and perinatal and neonatal outcomes in pregnancies with gastroschisis. Methods: Retrospective analysis of infants with prenatally diagnosed gastroschisis born in two academic medical centers between 2008 and 2013. Neonatal outcomes of deliveries with and without meconium staining were compared. Primary outcome was defined as any of the following: neonatal sepsis, prolonged mechanical ventilation, bowel atresia or death. Secondary outcomes were preterm delivery, preterm-premature rupture of membranes (PPROM) and prolonged hospital length of stay. Results: One hundred and eight infants with gastroschisis were included of which 56 (52%) had meconium staining at delivery. Infants with meconium staining had a lower gestational age at delivery (36.3 (±1.4) versus 37.0 (±1.2) weeks, $p = 0.007$), and a higher rate of PPROM (25% versus 8%, $p = 0.03$) than infants without meconium. Meconium staining was not significantly associated with the primary composite outcome or with any of its components. After adjustments, meconium staining remained significantly associated with preterm delivery at <36 weeks [odds ratio OR = 4.0, 95% confidence intervals (CI): 1.5–11.4] and PPROM (OR = 3.8, 95% CI: 1.2–14.5). Conclusions: Among infants with gastroschisis, meconium staining was associated with prematurity and PPROM. No significant increase in other adverse neonatal outcomes was seen among infants with meconium staining, suggesting a limited prognostic value of this finding.

Introduction

Gastroschisis is a congenital abdominal wall defect that occurs in approximately

1–5 cases per 10,000 live births [1]. Gastroschisis is commonly diagnosed in utero by routine ultrasound that can identify the defect as early as the first trimester [2, 3]. Although the overall outcome for infants with gastroschisis is favorable [4], they remain at increased risk for multiple peripartum and neonatal complications, including intrauterine growth restriction (IUGR), stillbirth, bowel dysfunction, bowel atresia, bowel necrosis and short-bowel syndrome [5,6]. Meconium staining at delivery has long been known to be associated with gastroschisis, although the exact implications of meconium staining remain unclear. There is a wide variation in the documented incidence of meconium staining in gastroschisis cases, with prior studies demonstrating rates between 25% and 83% [6–8]. Prior studies in animal models have suggested that meconium in amniotic fluid is related to intestinal damage in infants with gastroschisis [9–11]. In addition, a recent cohort study found an increased risk of umbilical ‘‘peel’’ and edema among infants with meconium staining [12]. Although prior studies have examined the association between meconium staining and neonatal bowel outcomes, reports detailing the adverse effects, if any, between meconium and intrapartum and other neonatal outcomes are limited. Our objective in this retrospective cohort study was to evaluate the association between meconium stained amniotic fluid and perinatal and neonatal outcomes among pregnancies with gastroschisis. Because meconium has been described to be associated with inflammation, we hypothesized that meconium staining would be related to increased adverse antepartum and neonatal outcomes, particularly preterm birth and neonatal respiratory effects.

Materials and Methods

Study Population

We performed a retrospective study of all infants with prenatally diagnosed gastroschisis whose mothers received prenatal care at Loma Linda University Medical Center and Lucile Packard Children's Hospital Stanford between October 2008 and 2013. Both institutions are tertiary care referral centers in California with expertise in managing gastroschisis, including maternal-fetal medicine, pediatric surgery, prenatal ultrasound and level IV neonatal intensive care.

In both institutions, pregnancies complicated by fetal gastroschisis are managed in outpatient high-risk pregnancy clinics, with serial ultrasound surveillance and antenatal nonstress testing. A delivery prior to 37 completed weeks gestation is indicated in cases with severe maternal medical or obstetric complications, or non-reassuring fetal status including suspected IUGR or abnormal antenatal testing. In the absence of fetal or maternal morbidity, delivery for gastroschisis cases was generally undertaken at 36–37 weeks (Stanford) or by 38 weeks (Loma Linda) during the study period in order to avoid early term stillbirth, the precise timing of which was left to be decided by the primary care provider. In both institutions, a trial of labor is preferred over cesarean delivery in the absence of obstetric contraindications.

The study data from both institutions were collected and managed using Research Electronic Data Capture (REDCap) [13], a secure, web-based application designed to support data capture for research studies. A total of 113 gastroschisis cases were identified from separate institutional databases in which pregnancies with fetal anomalies are prospectively entered. Cases with missing information on meconium staining (n=5)

were excluded from the analysis. Study approval was obtained from institutional review boards at Loma Linda University and Stanford University.

Study Definitions

Detailed perinatal, intrapartum and neonatal variables were collected from electronic medical records. Meconium staining of the amniotic fluid was defined clinically as the presence of meconium at any point during labor and delivery (both thin and thick meconium) by the obstetrical team and documented in the medical record. Neonatal prolonged length of stay (LOS) was defined *a priori* as the upper quartile (≥ 75th percentile) of LOS (in days) for the entire cohort. Prolonged mechanical ventilation was defined similarly *a priori* as the upper quartile of the total length of mechanical ventilation (in days) for the cohort. Diagnosis of neonatal sepsis was based on a positive blood culture during initial neonatal hospitalization. Maternal prepregnancy body mass index (BMI) was calculated as (BMI = weight in kilograms/height^2 in meters) using height and documented prepregnancy weight. Small for- gestational-age (SGA) was defined by a birth weight less than 10th percentile at delivery using the Fenton growth charts for infants <38 weeks GA [14] and the WHO growth charts for infants ≥38 weeks GA [15].

The primary outcome of the study was a composite neonatal outcome, defined as any of the following: culture positive sepsis or presumed infection (as determined clinically by the treating neonatologist), prolonged mechanical ventilation, bowel atresia (as documented in the medical record by the neonatal or surgical teams) or death prior to discharge. Secondary outcomes were the individual components of the primary outcome,

preterm delivery at <36 weeks gestation, preterm-premature rupture of membranes (PPROM) and prolonged hospital LOS. A preterm gestational age cut-off of 36 weeks was chosen because many pregnancies complicated by gastroschisis are iatrogenically delivered between 36 and 37 weeks due to concerns about near-term and early term *in utero* demise.

Statistical Analysis

Statistical analysis was performed using R application (version 2.15.0, R Development Core Team, 2011, Vienna, Austria). Unadjusted analyses were performed using chi-square test or Fisher's exact test for categorical variables, and Student's *t*-test or Wilcoxon rank-sum test for continuous variables, as appropriate. Multivariable logistic regression models were constructed to determine the independent association of meconium staining with neonatal outcomes while adjusting for gestational age and study center. Results of the model were expressed as odds ratios (OR) and 95% confidence intervals (CI). The level of significance was set at $p < 0.05$.

Results

A total of 108 cases with prenatally diagnosed gastroschisis were included in the analysis of which 56 (52%) had meconium staining at delivery. Forty-one patients with meconium staining (73%) delivered at Loma Linda University and 15 (27%) at Stanford University ($p = 0.04$). No cases of antepartum or peripartum stillbirths were recorded in this study. Maternal demographics are shown in Table 2.10. No significant differences were seen in maternal age, race/ethnicity, payer status, gravidity or parity, prepregnancy

BMI or smoking between the groups with and without meconium staining (Table 2.10). Meconium staining at delivery was associated with preterm birth before 37 weeks gestation [37 (66%) versus 20 (39%), $p = 0.007$], preterm birth before 36 weeks gestation [20 (36%) versus 9 (17%), $p = 0.01$] and PPROM [14 (25%) versus 4 (8%), $p = 0.03$]. Mean gestational age at birth was lower in the meconium group [36.3 weeks (±1.4) versus 37.0 weeks (±1.2) ($p = 0.007$)] (Table 2.11). No significant difference was found in the incidence of oligohydramnios, abnormal umbilical artery Doppler or in the mode of delivery at term or at <36 weeks gestation (spontaneous preterm birth 14% versus 10% in no meconium; induced labor 11% versus 4% in no meconium, $p = 0.79$) between the groups (Table 2.11). No significant difference was found in the rate of preterm delivery at <36 weeks in regards to the study center [24 (33%) in Loma Linda versus 8 (20%) in Stanford, $p = 0.75$] and the mode of delivery <36 weeks was similar between the study centers [spontaneous, n =9 (12%) in Loma Linda versus n =4 (10%) in Stanford; induced n =4 (5%) versus n =4 (10%) in Stanford; scheduled C-section n =7 (10%) versus 0% in Stanford]. Indications for labor induction at <36 weeks were similar between the two centers [Loma Linda: non-reassuring fetal testing (n =1); PPROM (n =3) and other (n =1) versus non-reassuring fetal testing (n =2), PPROM (n =2) and other (n =1) in Stanford]. The indications for scheduled C-section in Loma Linda were non-reassuring fetal status (n =5) and other (n =3). In addition, no significant difference was found in the rate of meconium staining at deliveries <36 weeks between the study centers [n =16 (22%) in Loma Linda versus n =4 (10%) in Stanford, p =0.21]. No significant difference was seen in the unadjusted rate of primary composite outcome between the meconium staining and no meconium staining groups [26 (46%) versus 18 (35%), p =0.29]. In unadjusted

analyses, neonates with gastroschisis and meconium staining required longer duration of mechanical ventilation [median 9 days (1–31) versus 3 days (1–58), p =0.002], and were more likely to receive delayed abdominal closure (75% versus 56%, p =0.04) compared to those without meconium (Table 2.12). No significant difference was noted in neonatal birth weight or incidence of SGA between the two groups (Table 2.12). Similar rates of bowel atresia, neonatal sepsis/infection, prolonged hospital LOS and prolonged mechanical ventilation were also noted for the groups with meconium and without meconium staining (Table 2.12). After adjusting for gestational age and study center in multivariable models, meconium staining was not related to primary composite outcome or any of its individual components (Table 2.13). After adjusting for study center, both preterm delivery <36 weeks (OR =3.3, 95% CI: 1.2–10.3) and PPROM (OR =4.0, 95% CI: 1.5–11.4) remained significantly related to meconium staining, respectively (Table 2.13).

Table 2.10. Maternal demographics in pregnancies with gastroschisis and meconium staining compared to no meconium staining.

	Meconium staining $N=56$	No meconium staining $N=52$	p values*
Maternal age (years)	21.1 (3.6)	21.7 (4.5)	0.44
Race/ethnicity			0.08
Non-Hispanic White	13 (23%)	9 (17%)	
Hispanic	19 (34%)	27 (52%)	
Other	23 (41%)	12 (23%)	
Unknown	1 (2%)	4 (8%)	
Payer status			0.29
Public	42 (75%)	41 (79%)	
Private	5 (9%)	7 (14%)	
Self-pay	2 (4%)	0 (0%)	
Unknown	7 (13%)	4 (8%)	
Study center			0.04
Loma Linda	41 (73%)	27 (52%)	
Stanford	15 (27%)	25 (48%)	
Gravida	2 (1 - 2)	2 (1 - 2)	0.93
Parity			0.45
Nulliparous	35 (63%)	37 (71%)	
Multiparous	21 (37%)	15 (29%)	
Prepregnancy BMI (kg/m^2)	28.4 (26.1–29.9)	27.8 (25.4–31.2)	0.73
Smoking during pregnancy	10 (18%)	6 (12%)	0.49

Data are presented as mean (SD), median (IQR) or n (%). BMI, body mass index.

*Chi-square test or Fisher's exact test for categorical variables, t-test or Wilcoxon test for continuous variables.

Table 2.11. Perinatal findings and outcomes of gastroschisis pregnancies with meconium staining compared to no meconium staining.

	Meconium staining *N*=56	No meconium staining *N*=52	*p* values*
Oligohydramnios	9 (16%)	4 (8%)	0.30
Abnormal umbilical artery Doppler assessment	1 (2%)	1 (2%)	1.00
Prenatal bowel dilation by ultrasound	37 (66%)	29 (56%)	0.59
Gestational age at delivery (weeks)	36.3 (1.4)	37.0 (1.2)	0.007
PPROM	14 (25%)	4 (8%)	0.03
Preterm delivery <37 weeks	37 (66%)	20 (39%)	0.007
Preterm delivery <36 weeks	20 (36%)	9 (17%)	0.01
Mode of delivery <36 weeks			0.79
Spontaneous	8 (14%)	5 (10%)	
Induced labor	6 (11%)	2 (4%)	
Scheduled CS	5 (9%)	2 (4%)	
Indication for labor induction <36 weeks†			0.89
Pathological fetal status	2 (4%)	1 (2%)	
PPROM	4 (7%)	1 (2%)	
Other	1 (2%)	1 (2%)	
Indication for CS <36 weeks†			0.84
Pathological fetal status	4 (7%)	1 (2%)	
Other	1 (2%)	2 (4%)	
Mode of all deliveries			0.28
Spontaneous	22 (39%)	14 (27%)	
Induced labor	27 (48%)	33 (64%)	
Scheduled CS	8 (12%)	5 (10%)	
Missing	2 (4%)	0 (0%)	

Data are presented as n (%), or mean (SD). PPROM, preterm-premature-rupture-of-membrane; CS, cesarean delivery.

*Chi-square test or Fisher's exact test for categorical variables, *t*-test for continuous variables.

†All indications that applied were reported.

Table 2.12. Neonatal outcomes in pregnancies with gastroschisis and meconium staining at delivery compared to no meconium staining.

	Meconium staining $N = 56$	No meconium staining $N = 52$	p values*
Birth weight (g)	2500 (453)	2528 (496)	0.76
SGA	12 (21%)	13 (25%)	0.87
Apgar score <7 at 5 min	4 (7%)	4 (8%)	1.00
Defect closure			0.04
Primary closure	14 (25%)	23 (44%)	
Delayed closure	42 (75%)	29 (56%)	
Primary composite outcome†	26 (46%)	18 (35%)	0.29
Days on ventilator	9 (1–31)	3 (1–58)	0.002
Bowel atresia	3 (5%)	2 (4%)	1.00
Neonatal death	0 (0%)	1 (2%)	0.97
Culture proven sepsis or infection	14 (25%)	12 (23%)	0.99
Hospital LOS (days)	36 (17–238)	34 (4–174)	0.29
Prolonged hospital LOS‡	17 (30%)	14 (27%)	0.86
Prolonged mechanical ventilation§	21 (38%)	10 (19%)	0.08

Data are presented as mean (SD), *n* (%) or median (range). SGA, small-for-gestational-age; LOS, length of stay.

*Chi-square test or Fisher's exact test for categorical variables, Wilcoxon test for continuous variables.

†Variables included: culture proven sepsis or culture proven infection, neonatal short bowel syndrome, prolonged stay on ventilator, bowel atresia or neonatal death.

‡Defined as upper quartile (>75th percentile) of LOS (>53 days).

§Defined as upper quartile (>11 days).

Table 2.13. Association between neonatal outcomes and meconium staining among infants with gastroschisis.

	Meconium staining‡ OR (95% CI)
Primary composite outcome	1.3 (0.6–3.0)*
Prolonged mechanical ventilation	1.9 (0.8–5.1)*
Culture proven sepsis or infection	0.8 (0.3–2.2)*
Prolonged LOS	1.0 (0.4–2.6)*
Preterm delivery <36 weeks	4.0 (1.5–11.4)†
PPROM	2.6 (0.7– 0.6)*

LOS, length of stay; PPROM, preterm-premature-rupture of membranes.

*Adjusted for gestational age at delivery and study center.

†Adjusted for study center.

‡No meconium staining as a reference.

Discussion

In this study, no significant difference was seen in the rate of bowel atresia, neonatal sepsis/infection, prolonged LOS or prolonged mechanical ventilation. The current study adds to the existing literature, as meconium staining in infants with gastroschisis was associated with preterm birth at <36 weeks and PPROM.

In the non-gastroschisis population, up to 20% of live births are complicated by meconium staining [16, 17]. Among term pregnancies without gastroschisis, meconium staining has shown to have limited predictive value for poor neonatal outcomes, with some studies showing an increased rate of adverse outcomes in neonates with meconium staining [16] and others demonstrating no significant association with perinatal asphyxia or neonatal neurologic outcome [18–20]. Among infants with gastroschisis, meconium rates of up to 25–83% have been reported [6–8]. In the current study, 52% of the infants with gastroschisis had meconium staining at delivery, which is consistent with that of the recent study from the University of California database [6]. The variation in the rates of

meconium staining among prior studies may reflect differences in definition of meconium staining and differences in baseline meconium staining between institutions. Unfortunately, baseline rates of meconium were not available in either center, as those are not routinely tracked for clinical or quality assurance purposes. Even in this study, a difference in the rate of meconium staining was found between the two centers (27% versus 73%), although both are consistent with reported rates in the literature.

Studies analyzing the association between meconium staining and adverse neonatal outcomes have been previously reported. In a study of 191 infants with gastroschisis by Overcash et al., no significant relationship between meconium staining and a composite adverse outcome including neonatal death, bowel complications requiring re-operation, gastrostomy or necrotizing enterocolitis was found [6], a finding which is consistent with our results. Another study demonstrated an association between meconium staining and abnormal cardiotocography and/or SGA [7], which was not seen in our study. Although neonates with gastroschisis and meconium staining demonstrated significantly increased days on mechanical ventilation in unadjusted analysis, there was no significant difference in prolonged mechanical ventilation in unadjusted or multivariable analyses. The difference in delayed abdominal closure between groups may have been driven by the study center, as the decision is not standardized across both institutions and the decision on primary versus delayed closure is based on the discretion of the treating surgeon.

In this study, meconium staining among infants with gastroschisis was found to be associated with prematurity before 36 weeks' gestation as well as with PPROM. Studies in non-gastroschisis populations have demonstrated that meconium is rarely

passed before 34 weeks' gestation and appears more often as gestational age increases [21–23]. Meconium staining is found in approximately 4% of preterm pregnancies in non-gastroschisis population [24, 25], whereas in our study, 65% of gastroschisis infants born at <37 weeks' gestation demonstrated meconium staining. Underlying etiologies for the presence of meconium in preterm gastroschisis cases are unclear, especially since the exact timing when the meconium occurred (antepartum versus intrapartum) in our population is unknown. Although, meconium staining was significantly related to study center in this study, no significant difference was found in the rates of preterm pregnancies between the two study centers, nor did the meconium staining and study center significantly correlate in a subgroup of deliveries at <36 weeks gestation. One possibility could be that meconium stained amniotic fluid enhanced the bowel inflammation prior to delivery, thus, leading to spontaneous preterm delivery and preterm rupture of membranes. Prior studies in fertilized chick eggs have documented increased gastrointestinal inflammation related to meconium stained amniotic fluid [9, 10]. Another possibility could be that PPROM itself irritates the exposed bowel leading to meconium staining.

Our study is not without limitations. First, our study design is limited by the inherent biases of a retrospective review. Although we included all cases of prenatally diagnosed gastroschisis that received prenatal, intrapartum and postnatal care in our centers during the study period, it is possible that some prenatal stillbirth cases may have occurred before an initial referral to our centers, and therefore their data are not included in the analysis. Another limitation is that the meconium staining in this study was defined subjectively by the providers at the time of delivery and was not based on standardized,

prospectively applied criteria. Moreover, meconium staining was not sub-classified as ‘‘thick’’ versus ‘‘thin’’ meconium, and it is unclear whether one is more prevalent in cases of gastroschisis or more commonly associated with the adverse outcomes we analyzed. It is also uncertain whether the meconium was present prior to the onset of labor or secondary due to stress during labor, even among cases with PPROM. Our centers do not utilize a common standardized algorithm for the prenatal, intrapartum and postnatal management of gastroschisis, and therefore approach to care was individualized. Lastly, because of the limited sample size, we were underpowered to detect certain rare neonatal outcomes in this study, including many of the individual variables included in the composite primary outcome. That being said, strengths of our study include the relatively large size of the cohort compared to previously published studies [7,8,12] and the inclusion of prenatal, perinatal and neonatal data from two tertiary referral centers, thereby making our results more generalizable. By comparing the perinatal and neonatal outcomes of gastroschisis infants with and without meconium staining at delivery, this study brings new important findings to this rather understudied question.

In conclusion, peripartum meconium staining in pregnancies complicated by gastroschisis may be associated with preterm delivery and PPROM. However, the lack of a strong association between meconium staining and severe adverse neonatal outcomes suggests a poor prognostic value to this occurrence. Future studies are needed to establish possible mechanism underlying the finding of meconium staining in premature deliveries of infants with gastroschisis.

Declaration of Interest

The authors report no declarations of interest.

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UTILITY OF THIRD TRIMESTER SONOGRAPHIC MEASUREMENTS FOR PREDICTING SGA IN CASES OF FETAL GASTROSCHISIS

By

YJ Blumenfeld[1,2], S Do[1], AI Girsen[1], AS Davis[2,3], SR Hintz[2,3], **AK Desai**[4], T Mansour[4], TA Merritt[5], BT Oshiro[4], YY El-Sayed[1,2,] AA Shamshirsaz[6] and HC Lee[3]

[1]Department of Obstetrics & Gynecology, Stanford University School of Medicine, Stanford, CA, USA

[2]The Fetal and Pregnancy Health Program, Lucile Packard Children's Hospital Stanford, Palo Alto, CA, USA

[3]Department of Pediatrics, Division of Neonatal and Developmental Medicine, Stanford University School of Medicine, Stanford, CA, USA

[4]Department of Obstetrics and Gynecology, Loma Linda University School of Medicine, Loma Linda, CA, USA

[5]Department of Pediatrics, Division of Neonatology, Loma Linda University School of Medicine, Loma Linda, CA, USA

[6]Department of Obstetrics & Gynecology, Baylor College of Medicine, Texas Children's Hospital, Houston, TX, USA.

This paper has been published in Journal of Perinatology (2017) 37, 498–501.

Abstract

OBJECTIVE: To assess the accuracy of different sonographic estimated fetal weight (EFW) cutoffs, and combinations of EFW and biometric measurements for predicting small for gestational age (SGA) in fetal gastroschisis. STUDY DESIGN: Gastroschisis cases from two centers were included. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated for different EFW cutoffs, as well as EFW and biometric measurement combinations. RESULTS: Seventy gastroschisis cases were analyzed. An EFW <10% had 94% sensitivity, 43% specificity, 33% PPV and 96% NPV for SGA at delivery. Using an EFW cutoff of <5% improved the specificity to 63% and PPV to 41%, but decreased the sensitivity to 88%. Combining an abdominal circumference (AC) or femur length (FL) z-score less than − 2 with the total EFW improved the specificity and PPV but decreased the sensitivity. CONCLUSION: A combination of a small AC or FL along with EFW increases the specificity and PPV, but decreases the sensitivity of predicting SGA.

Introduction

Gastroschisis is a severe paraumbilical abdominal wall defect that occurs in approximately one to five cases per 10 000 live births.[1] Fetal gastroschisis is commonly diagnosed in utero by routine ultrasound starting as early as the first trimester. Approximately 15 to 30% of fetal gastroschisis cases are born small for gestational age (SGA) less than 10% for gestational age.[2–7] Although the etiology of SGA among gastroschisis cases remains unclear, it may be an intrinsic part of gastroschisis physiology with some investigators suggesting involvement of the vascular endothelial growth

factor–nitric oxide synthase 3 pathway.[3,8] SGA is associated with adverse neonatal outcomes including prolonged neonatal intensive care unit length of stay, and surgical complications including perioperative infections and delayed closure of the abdominal wall defect.[2,4,9] Therefore, accurately predicting SGA prenatally is important for patient counseling and delivery planning.

The incidence of suspected intrauterine growth restriction (IUGR) less than 10% by prenatal ultrasound in gastroschisis cases is found in 50 to 75% of pregnancies, higher than the incidence of SGA at birth.[2,10] Intrauterine growth restriction often begins in the second trimester, and is driven largely by the fact that the herniated viscera lead to decreased abdominal circumference (AC), which is one of the major component of different estimated fetal weight ultrasound formulas.[4,11–14] Numerous studies, including a recent study by our group, have assessed the predictive utility of the total estimated fetal weight (EFW) in predicting SGA at birth in gastroschisis cases.[2,4,10,11] Most have shown that prenatal ultrasound generally underestimates the actual birth weight, especially when the common Hadlock formula is used, resulting in high false-positive rates.[4,10–12] Given the high sensitivity but more modest specificity of prenatal ultrasound, improving the accuracy of SGA prediction in these cases is warranted. The aim of our study was to assess the accuracy of different EFW cutoffs, and combinations of EFW and biometric measurements for predicting SGA in gastroschisis cases.

Materials and Methods

Study Population

This was a retrospective study of all infants with prenatally diagnosed

gastroschisis whose mothers received prenatal care at Loma Linda University Medical Center and Lucile Packard Children's Hospital Stanford between 2008 and 2013. Both institutions are tertiary care referral centers in California with maternal–fetal medicine, prenatal ultrasound, level IV neonatal intensive care units and pediatric surgical expertise in the management of gastroschisis.

Gastroschisis cases were identified from separate institutional databases in which pregnancies with fetal anomalies are prospectively entered. Only cases with information on SGA diagnosis were included in the current analysis. Pregnancies complicated by fetal gastroschisis are managed in outpatient high-risk pregnancy clinics in both centers, with serial ultrasound surveillance and antenatal non-stress testing. Indications for iatrogenic preterm delivery include severe maternal medical or obstetric complications, or non-reassuring fetal status including suspected IUGR or abnormal antenatal testing. In the absence of associated fetal or maternal morbidity, delivery for gastroschisis cases is typically recommended between 36 0/7 and 37 6/7 weeks in both institutions in order to avoid term stillbirth, although the precise timing of which is left to the discretion of the primary care provider. A trial of labor is preferred over cesarean delivery in the absence of obstetric contraindications.

Study data from both institutions were collected and managed using Research Electronic Data Capture (REDCap), a secure, web-based application designed to support data capture for research studies. Institutional review board approvals from both Loma Linda University and Stanford University were obtained prior to initiation of the study.

Study Definitions

Detailed perinatal, intrapartum and neonatal variables were collected from electronic medical records. In addition, ultrasound reports and stored ultrasound images were reviewed by trained research nurses and physicians. The prenatal EFW was assessed using a Hadlock formula incorporating the biparietal diameter (BPD), head circumference (HC), AC and femur length (FL) (Log10 (weight) = 1.3596 − 0.00386 × AC × FL +0.0064 × HC+0.00061 × BPD × AC+0.0424 × AC+0.174 × FL).[14] The EFW percentile for a given gestational age was then estimated using a Hadlock EFW percentile calculator.[12, 13] Only cases with ultrasound assessment 2 weeks prior to delivery were included in the analysis. Doppler studies of the ductus venosus, umbilical vein or middle cerebral artery are not routinely performed in prenatally diagnosed cases of fetal gastroschisis in either center, and umbilical artery Doppler assessment is only performed in cases of suspected IUGR (EFW<10%). Maternal pre-pregnancy body mass index was calculated as (body mass index = weight in kilograms/height^2 in meters) using height and documented weight at pre-pregnancy. SGA was defined by a birth weight less than 10th percentile at delivery using gender-specific Fenton growth charts for infants.[15]

Statistical Analysis

To account for differences in the gestational age at the last scan between the patients, Z-scores were calculated for the different biometric measurements. Z-scores (assessment of the standard deviation from the expected mean for gestational age) for individual sonographic parameters were calculated based on published formulas incorporating the gestational age at the time of the ultrasound exam.[12] Statistical analysis was performed using Stata/SE 14.1 (Stata Corp. College Station, TX, USA). Unadjusted

analyses were performed using $\chi 2$ test for categorical variables, and Student's t-test for continuous variables. Receiver operating characteristic curves, sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) were calculated for the total EFW and individual biometric parameters. EFW less than the 5th and 10th percentiles were considered as cutoffs for estimating diagnostic parameters. The level of significance was set at $P < 0.05$. Area under the curve was estimated using logistic regression with individual or combination of biometric parameters as predictor variables.

Results

Of 178 total gastroschisis cases managed in our centers during the time period, we excluded 108 cases that did not have an ultrasound performed within 2 weeks of delivery, yielding a total of 70 cases for analysis. Of those, 16 infants (23%) were determined to be SGA at birth. When comparing baseline demographic data between the SGA and appropriate for gestational age (AGA) groups, there was no difference in mean maternal age (21.2 vs 21.6 years, P=0.78), the gestational age of the last ultrasound exam (35.9 vs 35.2 weeks, P = 0.14), days between the last ultrasound exam and delivery (6.0 vs 5.8, P = 0.91), or the gestational age of delivery (36.8 vs 36.0 weeks, P = 0.12) between those with and without SGA. Women with and without SGA neonates had similar pre-pregnancy body mass index (22.5 vs 27.4, P = 0.30) (Table 2.14).

The mean EFW and individual biometric parameters were compared between those with and without SGA at delivery (Table 2.15). There was a statistically significant difference between the mean AC in the SGA group when compared with the AGA group (27.0 vs 28.9 cm, P = 0.020). All of the other parameters, including the mean EFW were

found to be similar between the groups. When considering EFW percentile, SGA neonates had significantly lower EFW percentile (3.8 percentile) compared with AGA neonates (16.5 percentile, P = 0.021). Gestational age-specific Z-scores for the individual sonographic parameters were compared between SGA and AGA neonates (Table 2.16). There was a statistically significant difference between the z-score of the AC (−3.4 vs − 1.6, P <0.0001) and FL (−2.3 vs − 1.6, P = 0.013) between the groups. The other parameters, HC and BPD, were similar between groups. Prediction of SGA was assessed using receiver operating characteristic analysis along with the sensitivity, specificity, PPV and NPV of the total EFW and individual parameters using z-scores less than − 2, which is consistent with less than 5% for gestational age (Table 2.17 and Figure 2.3). An EFW less than 10% had 94% sensitivity, 43% specificity, 33% PPV and 96% NPV for SGA at delivery. Thirty-four cases had a prenatal sonographic EFW less than 5% for gestational age, 14 of whom were born SGA. Using an EFW cutoff of < 5% improved the specificity to 63% and PPV to 41% but decreased the sensitivity to 88%. The only individual parameter with similar area under the curve was an AC z-score less than − 2.

In order to study the predictive utility of biometric measurements, we then analyzed different combinations of EFW cutoffs and parameters with a z-score less than − 2. Combining an EFW less than 5% and AC z-score less than − 2 (requiring both to be true to predict SGA) increased the specificity from 61–63 to 72%, with a decrease in sensitivity from 88 to 81% when compared with the EFW less than 5% alone. The combination of EFW less than 5% and AC z-score less than − 2 also yielded a higher PPV (46%) and similar NPV (93%) compared with the EFW alone. Adding an FL z score less than − 2 to the EFW less than 5% (requiring both to be true to predict SGA)

increased specificity to 77%, but dramatically decreased the sensitivity to 56% and NPV to 85% when compared with the EFW less than 5% alone. A combination of EFW less than 5%+AC z-score less than − 2+FL z-score less than − 2 (requiring all three to predict SGA) increased specificity to 91%, but decreased the sensitivity to 25% and the NPV to 80% when compared with the total EFW less than 5% alone.

Table 2.14. Maternal demographic and obstetric factors in SGA and AGA gastroschisis cases.

	SGA (n = 16)	*AGA (n = 54)*	P-*value*
Maternal age (years)	21.2 (4.2)	21.6 (4.2)	0.78
Race			0.45
White	44%	46%	
Asian	6%	4%	
American Indian/Alaska Native	6%	0	
Other	25%	30%	
Unknown	19%	20%	
Ethnicity—Hispanic	88%	70%	0.17
Nulliparity	75%	68%	0.59
BMI (pre-pregnancy)*	22.5 (3.9)	23.0 (3.4)	0.57
Maternal weight gain (kg)*	27.8 (10.2)	35.7 (15.4)	0.057
Smoking*	19%	11%	0.44
GA of last US exam (weeks)	35.9 (1.4)	35.2 (1.8)	0.14
Days between last US exam and delivery	6.0 (4.3)	5.8 (5.1)	0.91
GA at delivery (weeks)	36.8 (1.3)	36.0 (1.8)	0.12

Abbreviations: AGA, appropriate for gestational age; BMI, body mass index; GA, gestational age; SGA, small for gestational age; US, ultrasound. Means (standard deviation) or column %'s are shown. *Missing variables—BMI $n = 3$, smoking $n = 1$, maternal weight gain $n = 1$.

Table 2.15. Total estimated fetal weight (EFW), head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), femur length (FL) at the last ultrasound before delivery.

	SGA (n = 16)	*AGA (n = 54)*	P-*value*
EFW at last US, g	1940 (376)	2178 (453)	0.059
HC, cm	30.5 (1.1)	30.6 (1.7)	0.80
BPD, cm	8.4 (0.5)	8.4 (0.6)	0.94
AC, cm	27.0 (2.3)	28.9 (2.8)	0.020
FL, cm[a]	6.3 (0.4)	6.4 (0.4)	0.48

Abbreviation: AGA, appropriate for gestational age. Means (s.d.) are shown.
[a]Femur length was missing for one AGA patient.

Table 2.16. Mean *z*-scores for individual sonographic biometric parameters and risk of small for gestinational age (SGA).

	SGA (n = 19)	*AGA (n = 49)*	P-*value*	*Odds ratio for SGA status*[a]	*95% CI*
HC	− 2.2	− 1.6	0.11	0.70	0.44, 1.09
BPD	− 1.5	− 1.0	0.15	0.71	0.45, 1.13
AC	− 3.4	− 1.6	< 0.0001	0.46	0.29, 0.72
FL	− 2.3	− 1.6	0.013	0.50	0.28, 0.89

Abbreviations: AC, abdominal circumference; AGA, appropriate for gestational age; BPD, biparietal diameter;FL, femur length; HC, head circumference. [a]Odds ratios with 95% CI estimated per increase in *z*-score of 1. Femur length not known for one AGA patient.

Table 2.17. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the total estimated fetal weight (EFW) and individual sonographic parameters and combination of parameters for prediction of small for gestational age.

	Sensitivity	*Specificity*	*PPV*	*NPV*
EFW $<$ 5%	88%	63%	41%	94%
EFW $<$ 10%	94%	43%	33%	96%
HC *z*-score $\leqslant -2$	63%	67%	36%	86%
BPD *z*-score $\leqslant -2$	38%	80%	35%	81%
AC *z*-score $\leqslant -2$	88%	61%	40%	94%
FL *z*-score $\leqslant -2$	56%	70%	36%	84%
EFW $<$ 5%+AC *z*-score $\leqslant -2$	81%	72%	46%	93%
EFW $<$ 5%+FL *z*-score $\leqslant -2$	56%	77%	43%	85%
EFW $<$ 5%+AC *z*-score $\leqslant -2$+FL *z*-score $\leqslant -2$	25%	91%	44%	80%
EFW $<$ 10%+AC *z*-score $\leqslant -2$	88%	63%	41%	94%
EFW $<$ 10%+FL *z*-score $\leqslant -2$	56%	70%	36%	84%
EFW $<$ 10%+AC *z*-score $\leqslant -2$+FL *z*-score $\leqslant -2$	31%	83%	36%	80%

Abbreviations: AC, abdominal circumference; BPD, biparietal diameter; FL, femur length; HC, head circumference; US, ultrasound. EFW at last US considered as percentile; other parameters based on z-score according to gestational age.

	AUC	95% CI	
EFW at last US (percentile)	0.832	0.716	0.949
HC	0.660	0.517	0.802
BPD	0.632	0.481	0.783
AC	0.833	0.706	0.960
FL	0.697	0.548	0.846
EFW + AC	0.833	0.706	0.960
EFW + FL	0.798	0.670	0.926
EFW + AC + FL	0.835	0.706	0.964

US=ultrasound; HC=head circumference; BPD=biparietal diameter; AC=abdominal circumference; FL=femur length

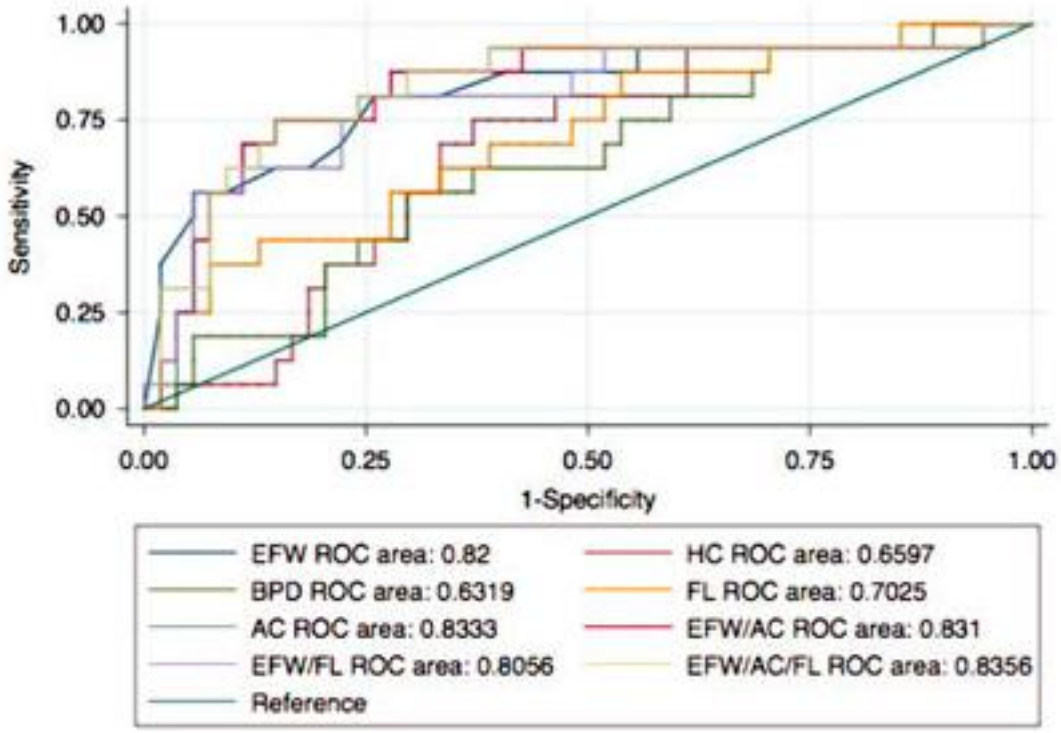

Figure 2.3. Prediction of SGA at delivery among fetuses with gastroschisis using the third trimester estimated fetal weight (EFW) and biometric measurements (AC, FL, BPD, HS). AC, abdominal circumference; BPD, biparietal diameter; FL, femur length; HC, head circumference; ROC, receiver operating characteristic; US, ultrasound.

Discussion

In this multi-institutional cohort of gastroschisis cases we found differences in sonographic AC and FL z-scores within 2 weeks of delivery between SGA and AGA

infants. However, the addition of a very short AC or FL with a z-score less than − 2 to the overall EFW increased the specificity and PPV of prenatal ultrasound, but decreased the sensitivity compared with the EFW alone. Our findings have several important contributions to the existing literature and potential clinical implications. First, our data showing a smaller FL in addition to the expected smaller AC support the theory that prenatal IUGR may be an intrinsic part of gastroschisis physiology. In their study of 70 gastroschisis cases, Centofanti *et al.*[16] found that fetal measurements of HC, AC and FL were all smaller during the second half of the pregnancy in fetuses with gastroschisis compared with normal controls. The etiology for SGA in gastroschisis cases deserves further research, but the mechanism is likely intrinsic to the fetus rather than placental since prior studies found similar rates of oligohydramnios and umbilical artery Doppler flow abnormalities between SGA and AGA cases.[2] In their study of 42 gastroschisis cases undergoing long-term follow-up (median age 9), Harris *et al.*[17] described significant catch up growth between birth and follow-up for the majority of children; however, those with complex gastroschisis (bowel complications such as atresia and volvulus) at birth had a significantly lower median body mass index and weight z-scores at follow-up.

Second, our data provide clinicians comprehensive data about the predictive utility of different EFW cutoffs, and combinations of EFW and small biometric measurements in predicting SGA at delivery. Multiple studies have shown the relatively poor accuracy of prenatal ultrasound in predicting SGA irrespective of EFW formulas.[6, 11] In their study of 53 gastroschisis cases, Nicholas *et al.*[11] compared the Honarvar, Siemer and Hadlock formulas for EFW. While none of the three formulas met the criteria for

ideal formula (low systematic error and high precision) the authors found the Hadlock formula to have the best bias and precision combination. In a similar study of 62 gastroschisis cases with an ultrasound performed within 2 weeks of delivery, Chaudhury *et al*.[6] compared the accuracy of five different EFW formulas. They found similar accuracy rates using the Hadlock formula (89% sensitivity and 68 to 70% specificity) but higher specificity (up to 86%) and positive predictive value (67%) when using either the Shepard or Siemer formulas. In our study, we showed increased specificity and PPV with using an EFW cutoff less than 5% compared with o10% although at a slightly decreased sensitivity. We also showed that adding an AC or FL z-score less than −2 to the overall EFW irrespective of cutoff using a Hadlock formula may improve the specificity and positive predictive rate as well but at a cost of decreasing the sensitivity. It is unclear if a similar effect can be seen by using the Shepard or Siemer formulas.

Whether improving SGA prediction in the late preterm period will improve prenatal management and neonatal outcomes warrants further investigation. In a recently published study analyzing prenatal, intrapartum and neonatal differences between SGA and AGA gastroschisis cases, we found no difference in preterm premature membrane rupture rates, preterm delivery rates, meconium staining, or mode of delivery between SGA and AGA cases.[2] That being said, it is plausible that prenatal providers suspecting IUGR at later preterm gestational ages may iatrogenically induce gastroschisis pregnancies prematurely in order to avoid stillbirth. Several studies have correlated earlier gestational age at delivery with adverse neonatal outcomes among gastroschisis cases, but prospective implementation of our findings is warranted to assess whether a

later gestational age of delivery can be achieved by optimizing the accuracy of prenatal ultrasound.[18–20]

Our study is not without limitations. This was a retrospective study utilizing existing records, and the protocol for ultrasound surveillance was not standardized across both institutions. Thus, it is possible that selection bias exists in our data set since providers concerned about IUGR may have been more likely to perform an ultrasound at late preterm gestational ages. That being said the rate of SGA seen in our cohort is consistent with other published cohorts, and at worst this bias may have affected the PPV and NPV, but not the sensitivity and specificity. Moreover, while other studies have suggested that the Hadlock formula may not be the ideal formula for IUGR determination in gastroschisis cases, given its wide prevalence in many prenatal diagnostic centers and prior gastroschisis studies we specifically targeted this formula.[4, 6, 10, 16] Additional studies would be needed to study our approach using additional EFW formulas. Finally, we assessed different methods of predicting SGA at delivery and not necessarily additional neonatal morbidity. The predictive utility of sonographic assessments for adverse neonatal outcomes will be the topic of future analyses.

Despite these limitations, it is important to note the strengths of our study. First, given the relatively low incidence of gastroschisis (1 in 2000 to 1 in 3000 pregnancies) we provided data from a robust cohort analyzing both the accuracy of the total EFW and individual ultrasound parameters within 2 weeks of delivery. In fact, the average days from ultrasound to delivery in our cohort was less than 7 days. Second, we used a commonly used gender specific neonatal weight nomogram (the Fenton curve) to diagnose SGA, and used calculations provided by Hadlock and colleagues to determine

the z-scores of individual biometric parameters. This allowed us to present the accuracy of individual parameters, the total EFW, and a combination of the EFW and individual parameters. Finally, combining data from two separate institutions makes our findings generalizable to other sites using the Hadlock formula to determine the EFW.

In conclusion, adding third trimester AC or FL with a z-score less than − 2 to the total EFW improves the specificity and PPV for suspected SGA in gastroschisis cases, but lowers the overall sensitivity. Our data can assist providers suspecting IUGR in the third trimester in pregnancies complicated by fetal gastroschisis.

Conflict of Interest

The authors declare no conflict of interest.

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CHAPTER THREE

ENVIRONMENTAL TRENDS OF GASTROSCHISIS

Abstract

Increased prevalence of gastroschisis has been linked to environmental factors and teratogens, as well as the presence of clustering of cases in North Carolina, Texas, the state of Washington and California. Geographic Information Systems (GIS) was utilized to identify not only where gastroschisis cases are referred from to Loma Linda University Medical Center, but also what is occurring in those surrounding areas. We mapped out known cases, overlayed environmental factors such as farmlands, superfund, and toxic release inventory sites, then used spatial statistics to quantify the presence of hot spots. Hot spot analysis and Gettis-Ord statistics showed areas of high gastroschisis case density, or cluster occurrence by noting a high z-score of 5.89 and low p-value of 0.00, indicating that it is statistically significantly unlikely that the observed spatial pattern reflects a random pattern. This study demonstrates the importance of geoinformation technology and the usefulness of the spatial scan statistics in exploratory etiologic research.

Introduction

Gastroschisis is a congenital birth defect of the abdominal wall, resulting in external herniation of intestines and potentially other abdominal organs.[1] Given its increase in prevalence worldwide, examining geographic distribution of birth defects, such as gastroschisis, can aid in exploratory etiologic research.[2] By identifying clusters of defects, environmental risk factors may help in the understanding of underlying factors

contributing to gastroschisis etiology. [3] In California, a slightly elevated risk of gastroschisis was reported[4] and potential clustering of cases was considered, given the increased prevalence of cases particularly in the Inland Empire region of Southern California when compared to the entire State of California. The highest incidence appearing to be concentrated in the agricultural and inland areas, with San Bernardino and Riverside County having approximately twice the incidence of the coastal regions. During the 17 year study period in the State of California, the overall birth prevalence increased by 3.2-fold.[5] This study by Vu et al. demonstrated that the birth prevalence of gastroschisis has been gradually rising in the past two decades in California. Additionally, in San Bernardino and Riverside Counties of Southern California, annual rates of gastroschisis were noted as 3.2 and 6.0 per 10,000 live births and fetal deaths in the years 2005 and 2006, respectively.[6] Various environmental risk factors, including potential teratogens such as organic solvents and residence surrounding landfill sites, have been linked to the increasing rates of gastroschisis due to increased prevalence and tendency to occur in clusters. [5, 7-11] The implication of environmental factors is also supported by evidence from animal models.[12-18]

Geographic Information Systems (GIS) was utilized to analyze geographic trends of gastroschisis to determine whether geographic clustering of gastroschisis cases is present, and whether any associations with environmental factors, such as waste disposal sites, water supply, power lines, and toxic chemicals, exist.

Materials and Methods

Institution review board approval was granted to conduct a retrospective chart

review at Loma Linda University Medical Center on gastroschisis cases (n= 257) in order to determine rates during a 14 year time period (1998- 2012) in the Inland Empire Region of Southern California.

Data Management/Analysis

Data Sorting

To ensure patient anonymity and to protect privacy, data for maternal residence was sorted by geo-codes.

Geographic Information Systems (GIS) Analysis and Methodology

Evidence of geographic clustering was evaluated by the distribution of individual gastroschisis cases using zip codes (nominal) of maternal residence. Potential temporal clustering of gastroschisis was sought by tabulating the frequency of zip codes during the 1998-2012 time frame. GIS software packages, including ArcMap and ClusterSeer2, and statistical methods (Gettis-Ord Statistics) was used to determine and display the associations between spatial-environmental and spatial-temporal factors and the incidence of gastroschisis. A GIS visual map displayed results of the analysis. Additionally, the approximate date of conception and the mother's residence just prior to, at the time of, and/or immediately after conception was recorded to facilitate GIS analysis of the incidence of gastroschisis in the study area. It is believed that the teratogen needs to be present before the time of the anterior abdominal wall development in order to create this defect. It is anticipated that exposure to teratogens prior to the period of gut formation produces metabolites which are responsible for the gastroschisis anomaly. The mother's location(s) were geocoded and aggregated to a larger geographical area using

"Centrus" Desktop. Emission site locations were also geocoded at the street level. The rationale for this investigation was to identify any associations between gastroschisis and environmental toxins from archived United States Environmental Protection Agency (EPA) voluntary sites, superfund sites, current industries, and agencies reporting permitted emission releases, as well as proximities to agricultural spraying zones and freeway corridors (air, soil, and water).

Spatial Analysis

Part 1: Map out which areas cases are coming from to get a visual representation of geographic distribution of gastroschisis cases coming to Loma Linda University Health.

Part 2: Testing for spatial clusters and association of gastroschisis cases and a possible environmental association. Farmlands, superfund sites and Toxics Release Inventory (TRI) sites as deemed by the EPA were used as overlays to note any environmental associations.

Part 3: Hotspot analysis was conducted to show areas of density (high or low) and likeliness of clustering patterns. Spatial statistical tests (Gettis-Ord Statistics) was run to determine the statistical probability of the observed spatial pattern occurring as a statistically random or non-random pattern.

Results

Spatial analysis shows possible geographic clustering surrounding major transportation routes, suggesting environmental or chemical contaminants contributing to

increased gastroschisis cases. Additionally, visual inspection reflects a greater density of cases surrounding the high desert (Victorville), Palm Springs, Highland, and Jurupa Valley areas (Figure 3.1). The Loma Linda University Medical Center (LLUMC) gastroschisis coded cases are overlayed with noted farmlands in Figure 3.2 depicting the proximity of gastroschisis incidence to known agricultural/ farmlands. EPA- deemed superfund and TRI sites were then added an added overlay (Figure 3.3) showing that superfund site placement does seem to visually indicate a relationship of association with gastroschisis case incidence. Upon visual inspection, hot spot analysis and Gettis-Ord statistics show areas of high density or cluster occurrence. A high z-score of 5.89 and low p-value of <0.0001 indicates that it is statistically significantly unlikely that the observed spatial pattern reflects a random pattern (Figure 3.4).

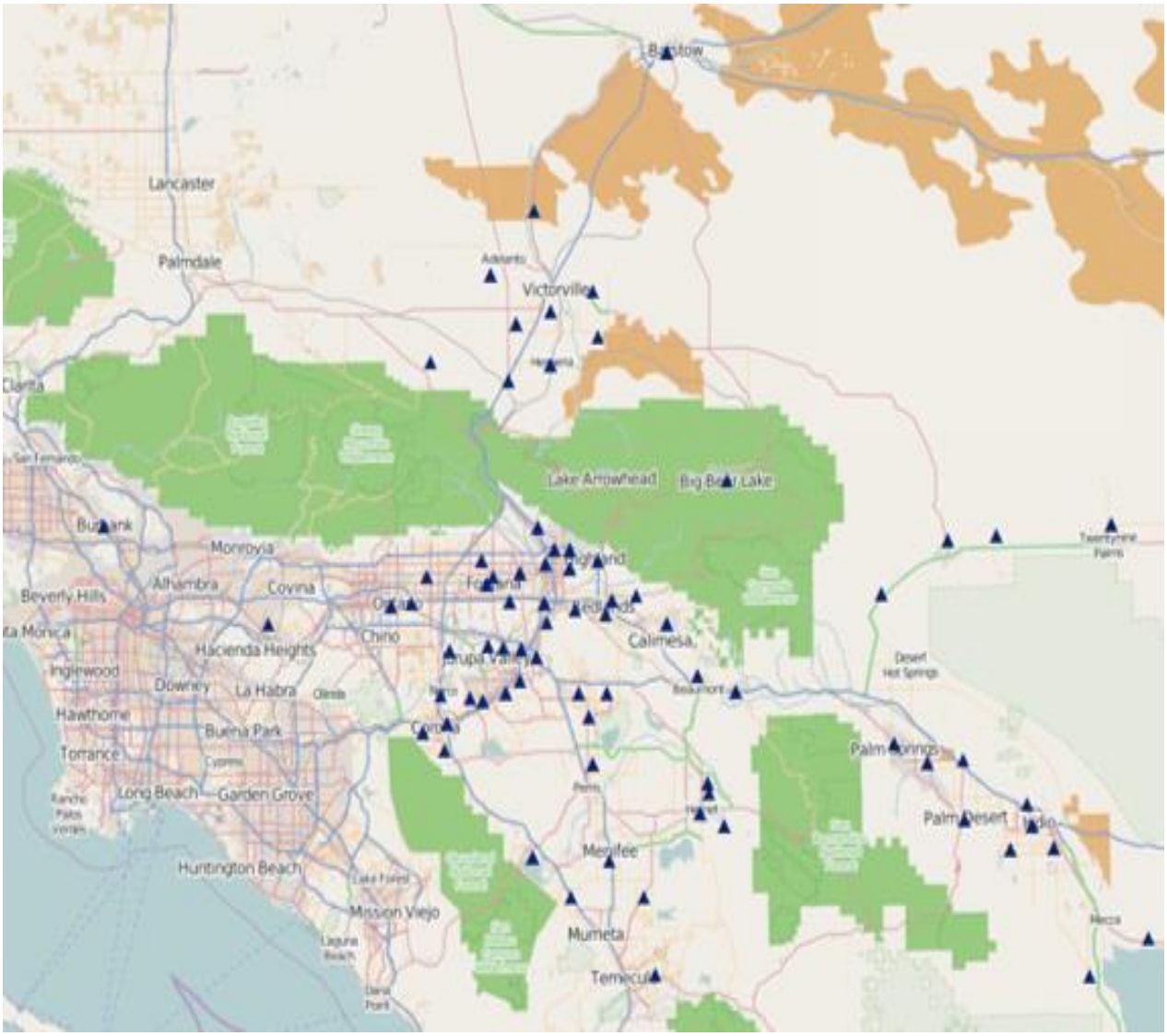


Figure 3.1. Geographic Information Systems map of cases of neonatal gastroschisis graphed for in the Inland Empire region of Southern California.

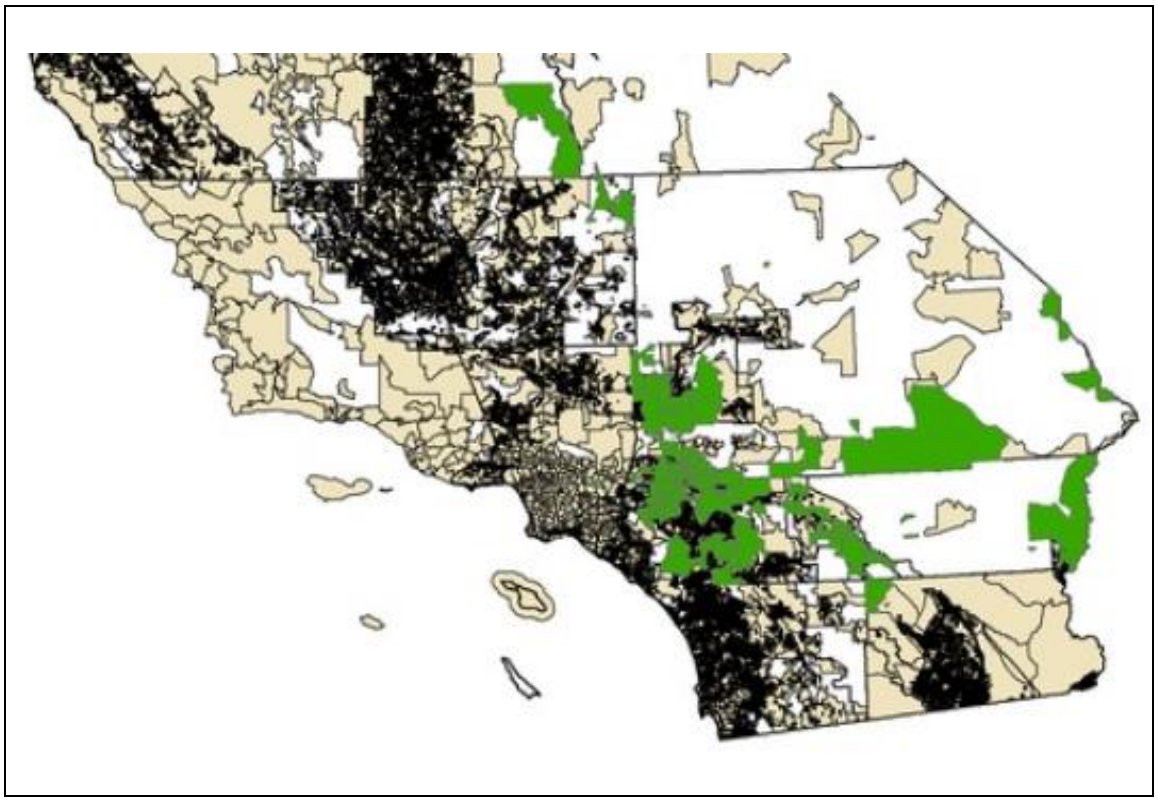
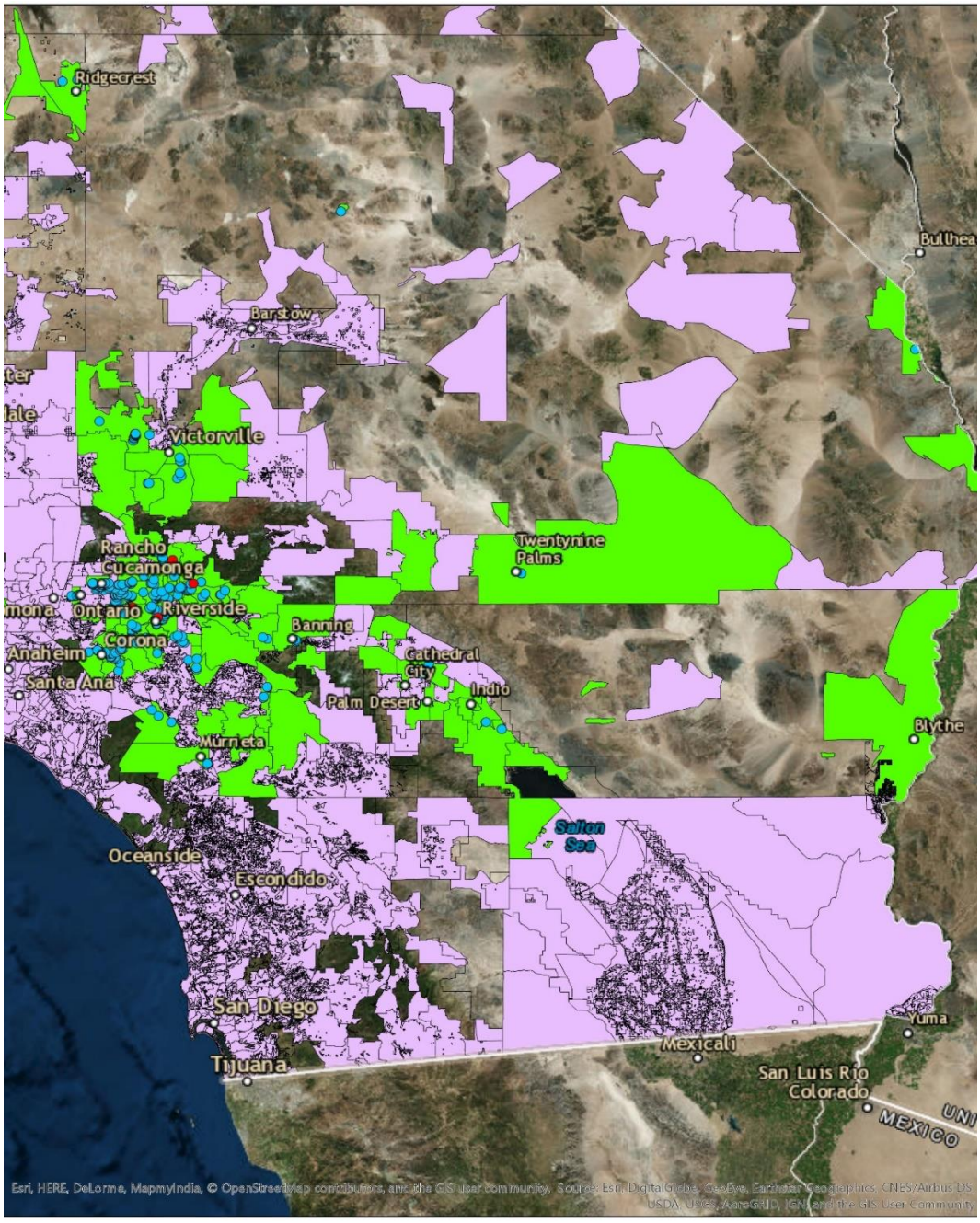


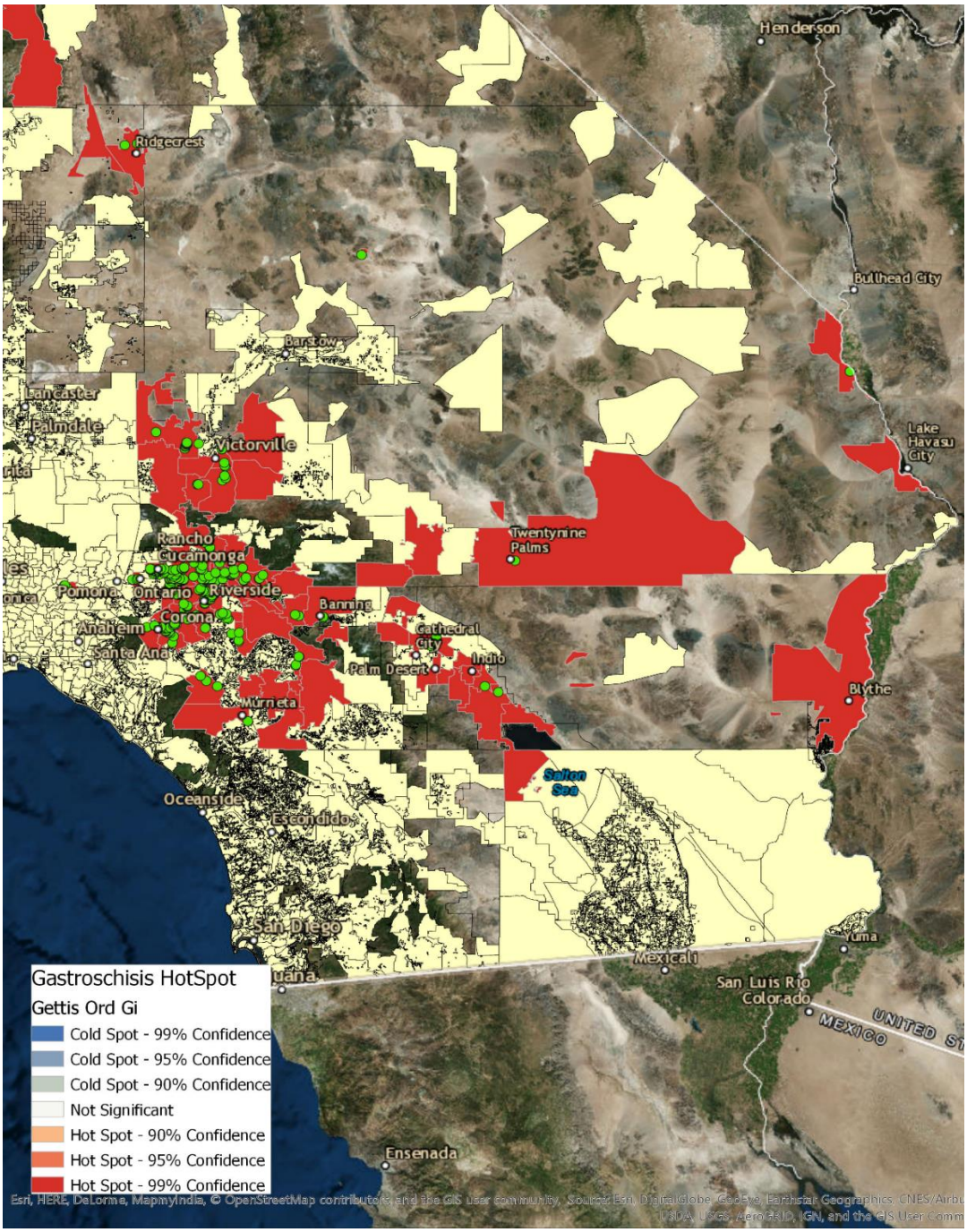
Figure 3.2. A map displaying areas where gastroschisis patients presented at Loma Linda University Medical Center (LLUMC) (green region) overlayed with noted farmlands (black region). *It is important to note that this map only represents patients seen at LLUMC and further research should consider Los Angeles, Orange, Central Valley and San Diego counties.



Key

Purple — Known zip codes
Green — Gastroschisis case in zip code area
Black — Farmlands
Teal dots: Superfund sites
Red dots: TRI sites

Figure 3.3. Gastroschisis GIS Mapping- Cases at LLUCH by Zip Code Overlayed with Farmlands, Superfund, and Toxics Release Inventory (TRI) Sites. Data sources: LLUCH and TOXMap from EPA.gov (Updated May 2017)



Gettis-Ord Statistics (General G Summary)

Observed General G:	0.000021
Expected General G:	0.000014
Variance:	0.000000
z-score:	5.891194
p-value:	0.000000

Figure 3.4. Hotspot map of gastroschisis cases at LLUCH by Zip Code showing areas of cold spot (low density) to hot spot (high density) occurrence of clustering.

Discussion

We sought to utilize GIS and statistical analyses to assess the relationship between the built environment and gastroschisis in the Inland Empire Region of Southern California. Investigating the geographic distribution of this birth defect can be useful in exploratory research. Elucidating prenatal predictors of gastroschisis is key to improving diagnosis. While spatial analysis shows possible geographic clustering surrounding major transportation routes, suggesting environmental or chemical contaminants contributing to increased gastroschisis cases, it is vital to note the limited sample size and usage of cases only admitted at Loma Linda University Medical Center. These data give a snapshot into gastroschisis cases in this catchment area, yet fail to consider other potential cases referred to surrounding facilities. This limitation can be overcome by accessing San Bernardino County birth records, or birth certificate data from the California Department of Public Health Vital Records.

In trying to identify what is causing the clustering of gastroschisis cases, various factors were considered. Riverside, San Bernardino, and Ontario, CA being listed as number three on the list of "The 10 Most Air-Polluted Cities in the U.S." by the World Health Organization (WHO) in 2011 may indicate environmental associations. In addition to traffic and pollution from major transportation routes and agriculture areas in the Inland Empire, socioeconomic status was also taken into account.

This study demonstrates the usefulness of spatial scan statistics in exploratory etiologic research and can potentially lead to earlier prenatal diagnoses, improved clinical care, decreased morbidity, and potential to reduce health disparities through the use of geoinformation technology.

Further study should be directed at specific geographic data and determining spatio-temporal associations between gastroschisis case clusters and environmental toxins from archived EPA superfund and TRI sites. Additionally, using county wide data may also increase the sample size and offer a more accurate representation of gastroschisis occurrences within California's Inland Empire region.

Acknowledgements

Thank you to Dustin Baumbach for sharing his expertise and knowledge in all things GIS. Your patience, ability to explain, and teach various GIS concepts, tools, and trends not only created visual representation of where gastroschisis cases are occurring, but also added a new understanding of how multiple factors play into considerations for gastroschisis development in the Southern California region. Thank you!

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CHAPTER FOUR

BIOLOGICAL CONSIDERATIONS OF GASTROSCHISIS

Abstract

Objective: Gastroschisis is the most common birth defect of the abdominal wall. It manifests as a defect in the anterior abdominal wall located most commonly to the right of the umbilicus with evisceration of abdominal viscera. The etiology is unknown and various theories have been proposed. Environmental teratogens have been implicated as possible causative factors in some epidemiologic studies. We sought to determine if maternal or cord blood serum or amniotic fluid taken from gastroschisis pregnancies had an impact on cell migration.

Methods: Maternal blood, cord blood, and amniotic fluid were obtained from pregnant women and their newborns with gastroschisis and controls at delivery and were stored at -80^0 Celsius. Endothelial cells were grown to create a confluent monolayer then incubated with maternal blood, cord blood, and amniotic fluid. The samples were matched for gestational age and mode of delivery. *In vitro scratch* assay was utilized. The Invitrogen EVOS FL Auto Imaging System is used to capture images of cellular movement time.

Results: Cell migration rates were not significantly different between cells incubated with third trimester maternal blood, cord blood, and amniotic fluid (p-value= 0.66 for 3rd trimester blood, p-value = 0.45 for cord blood, and p- value= 0.47 for amniotic fluid).

Conclusion: There was no difference in cell migration rates between control and gastroschisis pregnancies.

Introduction

Multiple hypotheses have been proposed for gastroschisis pathogenesis including a disruption of the blood supply to the developing abdominal wall, failure of mesoderm to form in the body wall, abnormal involution of the right umbilical vein, and abnormalities in ventral wall folding.[1]

Maternal risk factors that confer susceptibility to gastroschisis include genetic polymorphisms relating to angiogenesis, further supporting a vascular hypothesis. [2] Torfs et al.'s study of 57 gastroschisis cases and 506 non- malformed controls noted risks associated with polymorphisms of 32 genes representing enzymes involved in angiogenesis, blood vessel integrity, inflammation, wound repair, and dermal or epidermal strength. [3] Therefore, environmental factors, if present in pregnancies affected by gastroschisis, may impact cell growth.

In vitro assays allow for the identification of direct effects on endothelial cell function in addition to analysis of isolated processes that contribute to angiogenesis and variables such as matrix components in isolation. The in vitro scratch assay, a method to study cell migration in vitro, is based on the observation that upon creation of a new artificial gap (''scratch'') on a confluent cell monolayer, the cells on the edge of the newly created gap will move toward (or away from) the opening to close the ''scratch'' until new cell–cell contacts are established again. Steps involved include creation of a ''scratch'' on monolayer cells, capture of images at the beginning and regular intervals during cell migration to close the scratch, and comparison of the images to determine the rate of cell migration.[4] A major advantage of this method is that it mimics migration of

cells in vivo which aides in understanding the behavior and patterns of cell migration. This understanding may play an important role in the pathogenesis of gastroschisis. [5]

Materials and Methods

Sample Collection

Maternal blood during the third trimester of pregnancy, and amniotic fluid and cord blood at time of delivery were collected in 10 ml heparinized tubes. Blood was centrifuged (2000 rcf) at 4 degrees centigrade for 5 minutes to separate the plasma from the whole blood. The plasma is then aliquoted and placed in a negative 80 degree C freezer. Samples were collected from mothers carrying a fetus diagnosed prenatally with gastroschisis. Controls were matched for gestational age at delivery to within ±1 week.

Scratch Assay

Cell culture dishes were coated with the proper ECM substrate then incubated overnight without shaking. The unbound ECM substrate was then removed and washed once with Cells Systems media. Dishes were refilled with 3-5 ml of media prior to plating the cells grown from Human Brain Microvascular Endothelial Cells. Subconfluent cells were resuspended in a tissue culture dish with cells washed twice with Cells Systems media, adding versene containing 0.05% trypsin then mixing cells with the medium containing serum. The solution was gently pipetted, and the dish was rocked to disperse the cells equally. An aliquot from the cell suspension was taken and the cell counts was determined using a hemocytometer. Cells were plated onto a prepared 96 well confluent cell monolayer plate.

The cell monolayer was scraped in a straight line to create a 7 mm "scratch" using a 20 ml pipet tip to create an artificial gap, or scratch on a confluent cell monolayer plate, mimicking the gastroschisis opening as a "wound" (Image 4.1). The debris was then removed, and the edge of the scratch will be made uniform by washing the cells once with 1 ml of the growth medium then replaced with control, uncomplicated, or gastroschisis pregnancy serum in the wells. Wells were photographed at 2 hour intervals from 0hr – 8hr, then 24 hrs later using The Invitrogen EVOS FL Auto Imaging System. Once all the images were taken, they were analyzed for rate of scratch closure using Ilastik[6]

Statistical Analysis

A repeated measures ANOVA was performed to compare the area of cell migration in normal and gastroschisis samples from third trimester blood, amniotic fluid and umbilical cord blood at 0hr, 2hr, 4hr, 6hr, 8hr and 24hrs. We excluded the control group as well as results from hour 8 and 24 from analysis due to biological insignificance.

Third trimester maternal blood: Extreme outliers [E11, F11] were excluded from the analysis. Hemolysis was noted in 8 samples [Subject A6, B6, C6, D6, E6, F6, G6, and F6] and were excluded from analysis.

Umbilical cord blood: Extreme outliers [E11, F11] were excluded from the analysis.

Amniotic fluid: Extreme outliers [A1 and H1] were excluded from the analysis.

Results

Third trimester maternal blood: Normality assumption was met (Wilks lambda p-value=0.14). The condition of sphericity (Mauchly's $p<0.001$) was violated therefore the Greenhouse-Geisser Epsilon correction 0.38 suggests reporting multivariate results. A mixed model approach using the unstructured covariance model best represented the data (AICC unstructured vs Autoregressive heterogeneous $p<0.01$). There was no significant difference between normal and gastroschisis ($p=0.66$) throughout time in the study.

Umbilical cord blood: Normality assumption was met (Wilks lambda p-value=0.47). The condition of sphericity (Mauchly's $p<0.001$) was violated therefore the Greenhouse-Geisser Epsilon correction 0.62 suggests reporting multivariate results. A mixed model approach using the unstructured covariance model best represented the data (testing unstructured vs Autoregressive heterogeneous demonstrated that the arH model is indefensible $p<0.01$).

There was no significant difference between normal and gastroschisis ($p=0.45$) throughout time in the study. Exploration of Tukey's post hoc multiple comparison test indicated that the average area of the two groups were not significantly different at various times but did show that there was significant change within the group over time.

Amniotic fluid: Normality assumption was met (Wilks lambda p-value=0.49). The condition of sphericity (Mauchly's $p<0.001$) was violated therefore the Greenhouse-Geisser Epsilon correction 0.81 suggests reporting within subject effect results. A mixed model approach using the unstructured covariance model best represented the data (testing unstructured vs Autoregressive heterogeneous demonstrated that the arH model is indefensible $p<0.01$).

There was no significant difference between normal and gastroschisis (p=0.47) throughout time in the study. Exploration of Tukey's post hoc multiple comparison test indicated that gastroschisis and normal groups started at hour 0 significantly different (p=0.03), and at two other times as well; hour 4 (p=0.005), hour 6 (p=0.002).

Third trimester maternal blood, cord blood, and amniotic fluid groups over time are not significantly different in their cell migration rates (p-value= 0.66 for 3rd trimester blood, p-value = 0.45 for cord blood, and p- value= 0.47 for amniotic fluid). Table 4.1, 4.2, and 4.3 show repeated measures ANOVAs between 3rd trimester maternal blood, cord blood, and amniotic fluid of normal and gastroschisis plates throughout four time periods, respectively, with corresponding figures showing the average area of wound healing over a 24 hour time period. Additionally, Image 4.2, 4.3, and 4.4 show serum plated from third trimester maternal blood, cord blood, and amniotic fluid, respectively, at 0hr, 2hr, 6hr, 8hr, and 24hr intervals. The scratch closure can be noted, indicating the presence of wound healing in all three sample types. Regardless of the lack of inhibition in cell migration rates this was a novel approach to understanding gastroschisis development

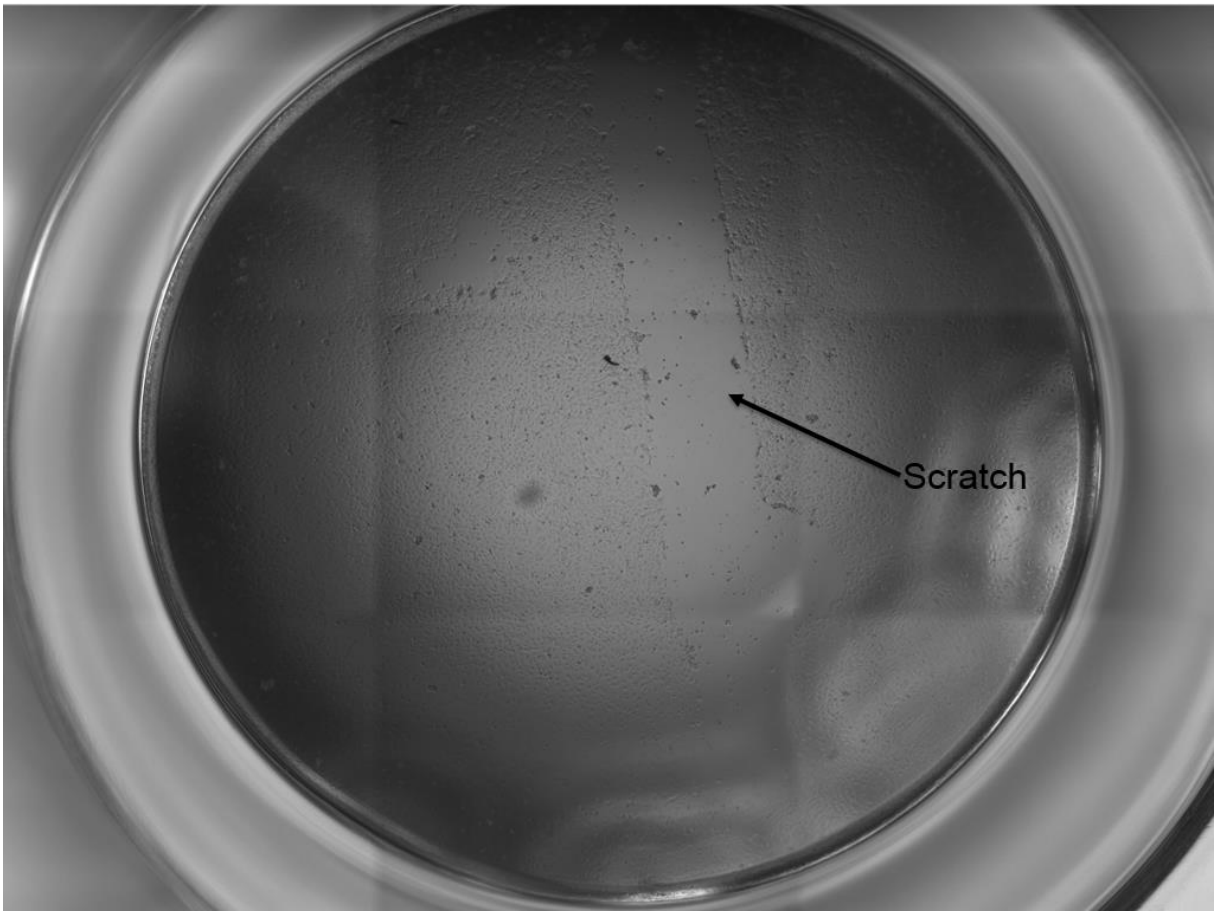

Image 4.1. The cell monolayer scraped in a straight line to create a "scratch", mimicking a wound

Table 4.1. Repeated measures ANOVA between 3rd trimester maternal blood of normal and gastroschisis plates throughout four time periods.

Within-subject factor	Between-subject factor								
	Normal (n=32)			Gastroschisis (n=46)			P-value		
		95% Confidence Interval			95% Confidence Interval				
Time	Estimated Marginal Mean	Lower Bound	Upper Bound	Estimated Marginal Mean	Lower Bound	Upper Bound	Group	Time	Group x time
0hrs	246059.6	186686.6	305432.6	210968.3	175914.2	246022.4	0.72*	0.34*	0.66*
2hrs	191621.2	150464.6	232777.9	210396.7	176544.2	244249.1			
4hrs	196932.6	155470.7	238394.4	211350.5	166547.8	256153.1			
6hrs	196473.6	142767.8	250179.4	204784.8	163160.3	246409.4			

Note: *Normality assumption was met using natural log transformation.

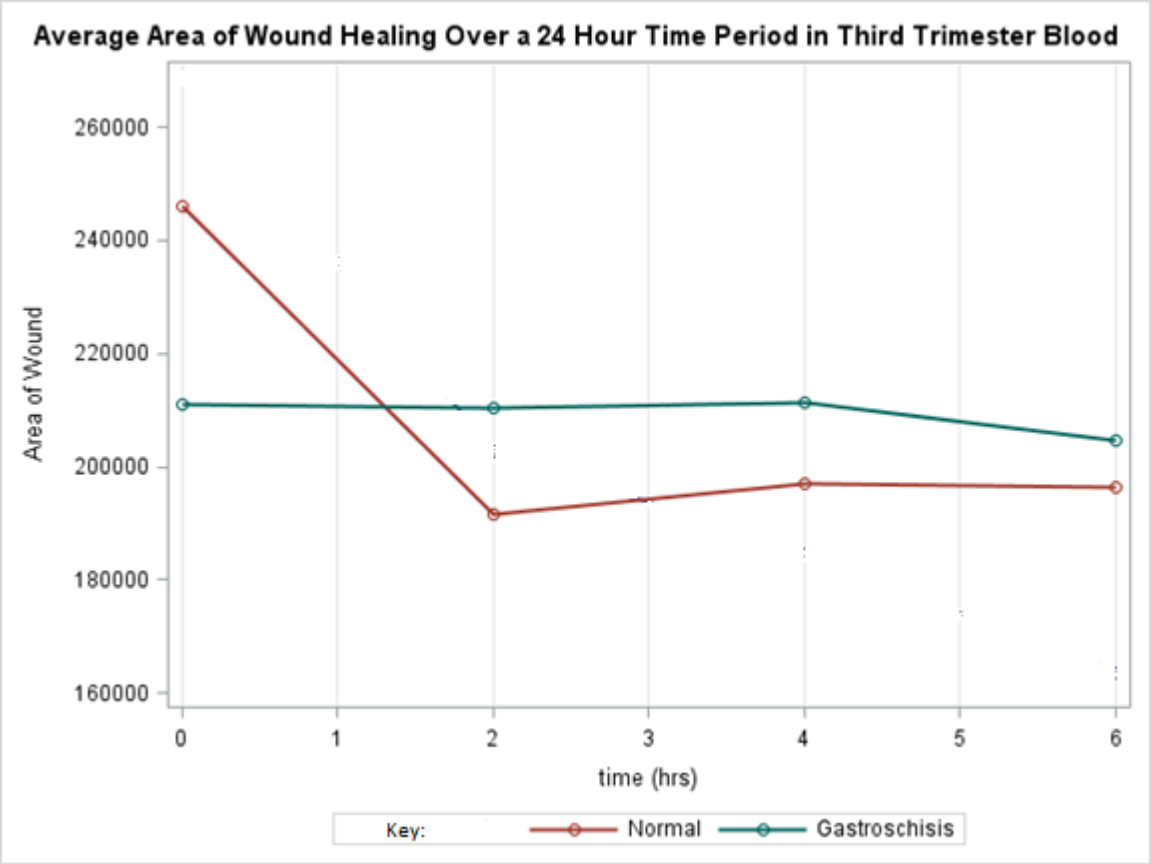

Figure 4.1. Average area of wound healing over a 24 hour time period in third trimester blood

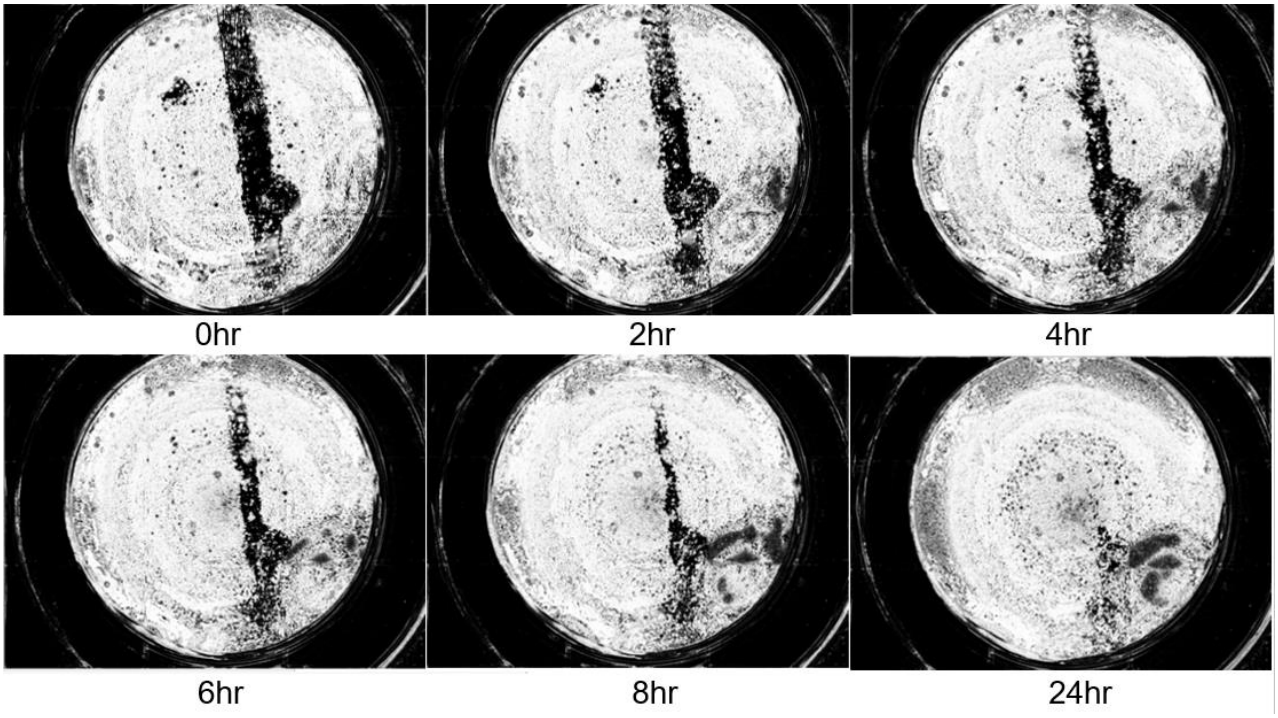

Image 4.2. Third trimester blood control sample plated in scratch well at 0hr, 2hr, 6hr, and 24hr. Scratch closure can be noted indicating wound healing.

Table 4.2. Repeated measures ANOVA between cord blood of normal and gastroschisis plates throughout four time periods.

Within-subject factor	Between-subject factor								
	Normal (n=40)			Gastroschisis (n=47)			P-value		
		95% Confidence Interval			95% Confidence Interval				
Time	Estimated Marginal Mean	Lower Bound	Upper Bound	Estimated Marginal Mean	Lower Bound	Upper Bound	Group	Time	Group x time
0hrs	235933.6	191350.7	280516.4	269925.1	227542.8	312307.5			
2hrs	226799.3	155817.1	297781.6	258561.5	208055.1	309068.0			
4hrs	235766.3	163242.9	308289.7	229684.8	186060.6	273308.9	**0.43***	**<0.0001***	**0.45***
6hrs	204669.2	130761.8	278576.5	202462.6	153961.3	250963.9			

Note: *Normality assumption was met using natural log transformation.

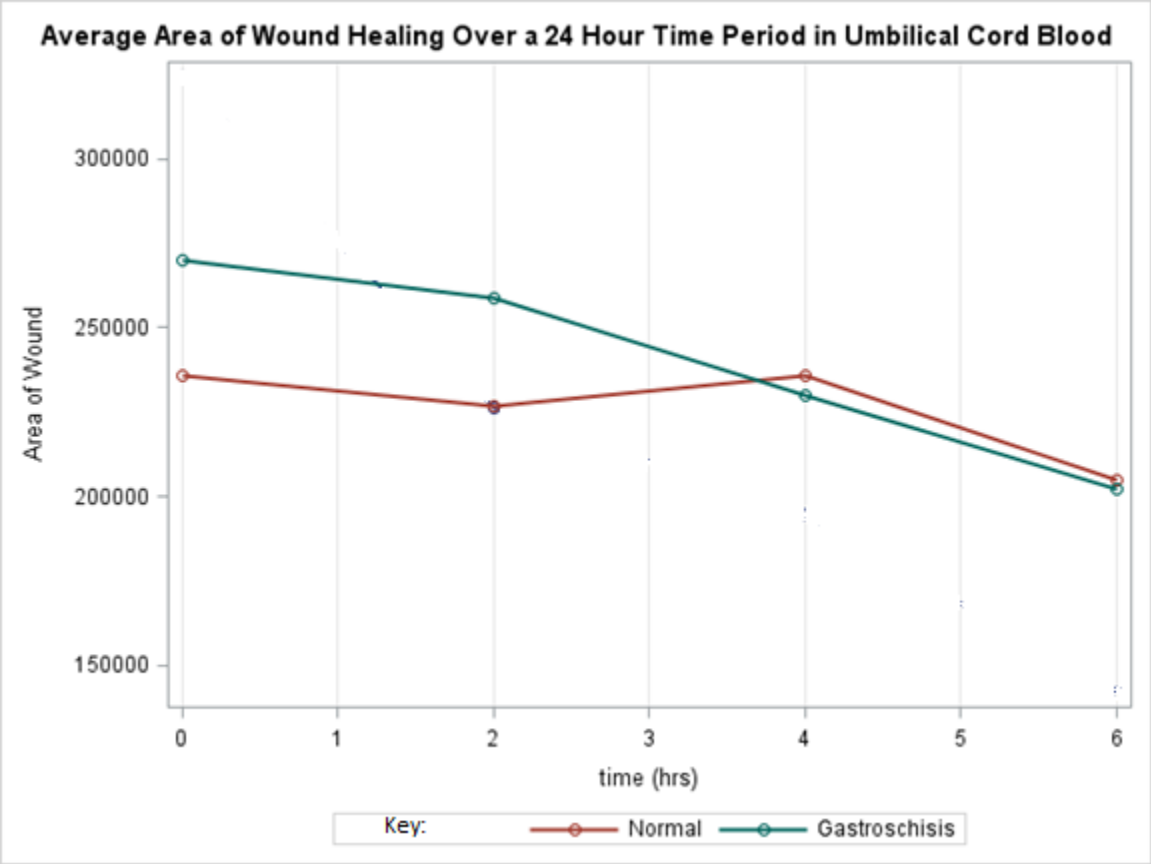

Figure 4.2. Average area of wound healing over a 24 hour time period in cord blood.

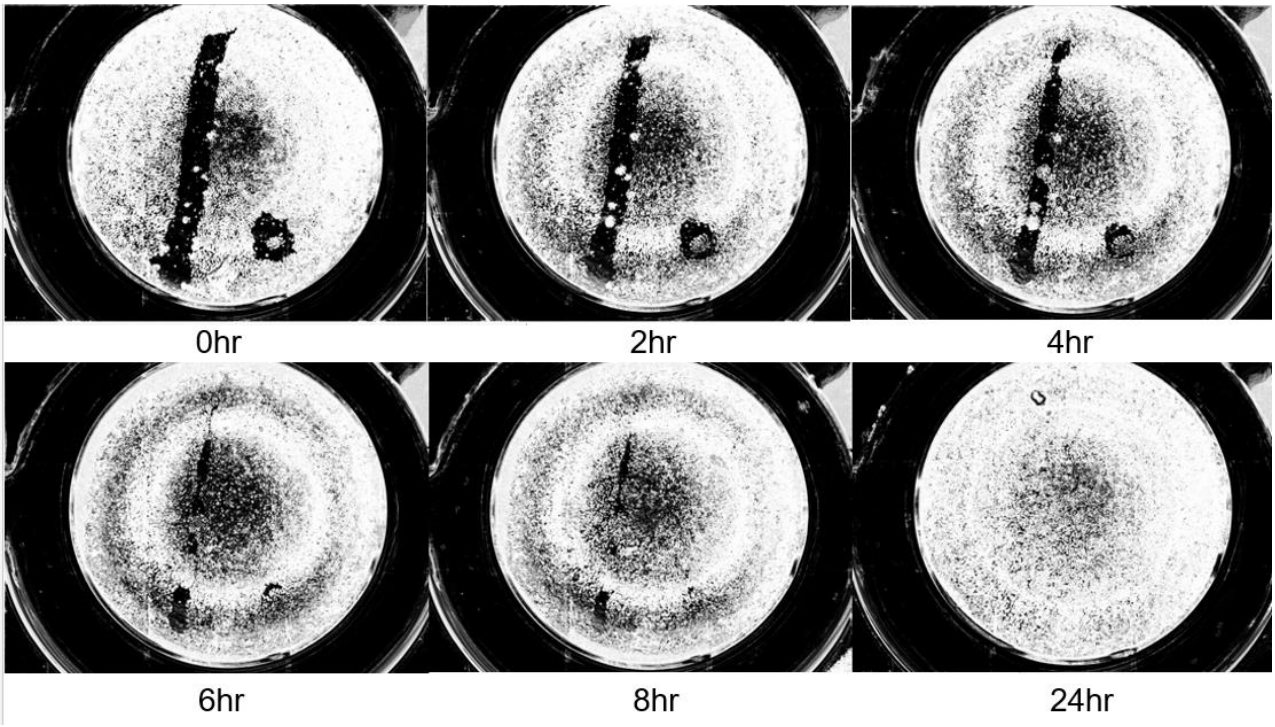

Image 4.3. Cord blood from an uncomplicated pregnancy plated in scratch well at 0hr, 2hr, 6hr, and 24hr. Scratch closure can be noted indicating wound healing.

Table 4.3. Repeated measures ANOVA between amniotic fluid of normal and gastroschisis plates throughout four time periods.

	Between-subject factor								
Within-subject factor	Normal (n=40)			Gastroschisis (n=48)			P-value		
		95% Confidence Interval			95% Confidence Interval				
Time	Estimated Marginal Mean	Lower Bound	Upper Bound	Estimated Marginal Mean	Lower Bound	Upper Bound	Group	Time	Group x time
0hrs	123792.6	87223.87	160361.2	236771.7	154552.7	318990.8			
2hrs	273524.6	174877.6	372171.5	393165.6	292844.4	493486.7			
4hrs	278804.9	176308.4	381301.4	429205.3	332306.8	526103.7	**0.001***	**<0.0001***	**0.47***
6hrs	284598.3	206040.6	363156.0	503946.9	398643.6	609250.2			

Note: *Normality assumption was met using natural log transformation.

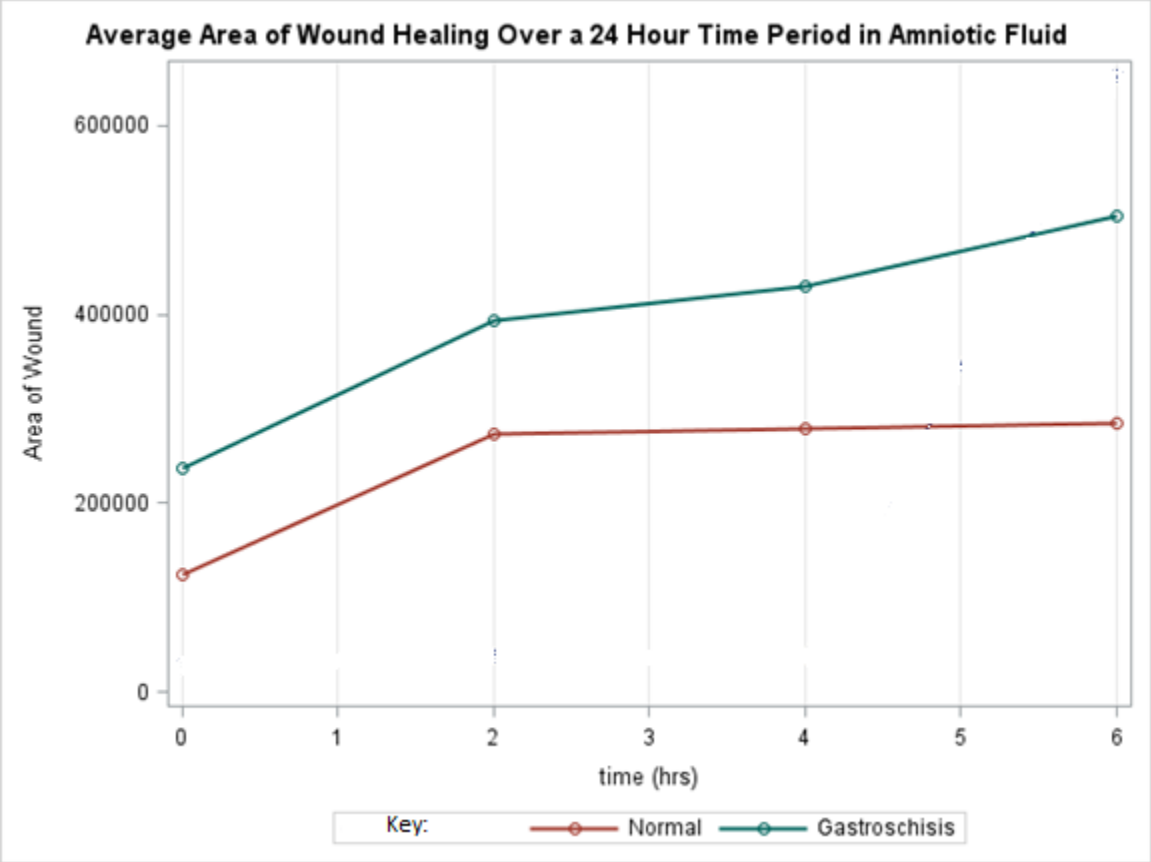

Figure 4.3. Average area of wound healing over a 24 hour time period in amniotic fluid.

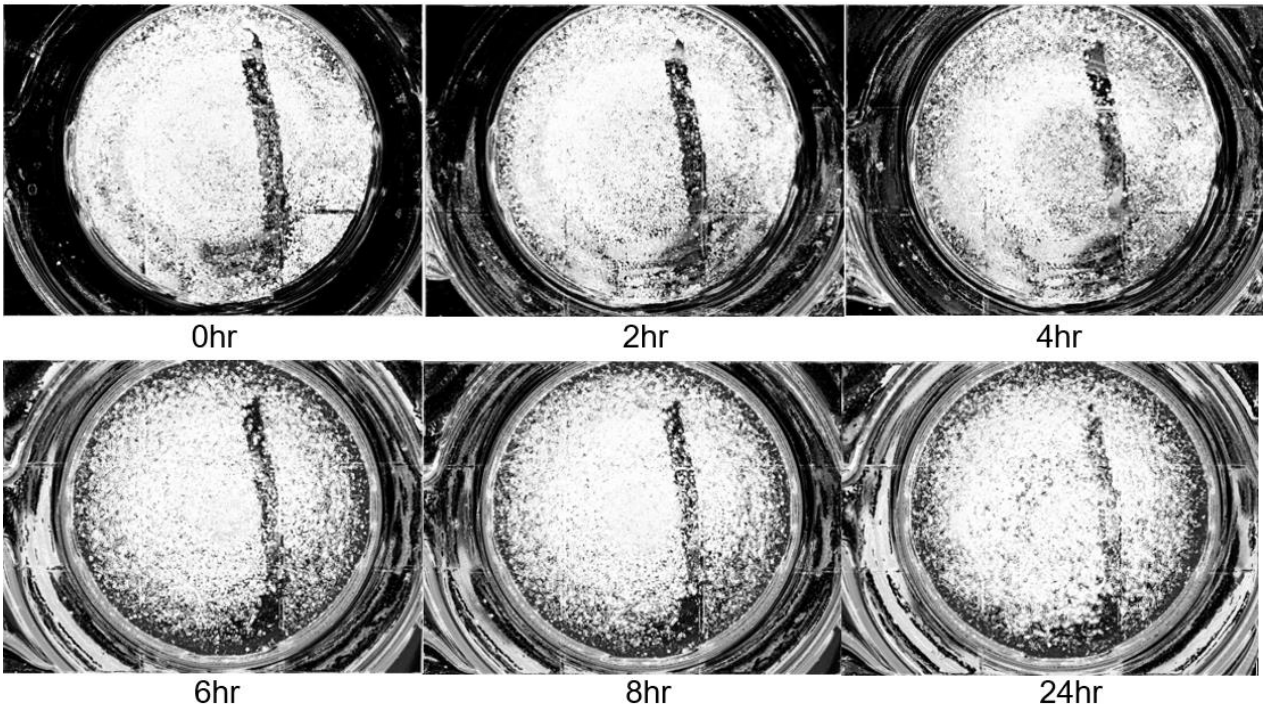

Image 4.4. Amniotic fluid from a gastroschisis pregnancy plated in scratch well at 0hr, 2hr, 6hr, and 24hr. Scratch closure can be noted indicating wound healing.

Discussion

In vitro scratch assays showed migration of cells in vivo to aid in visualizing behavior and patterns of cell migration in control and gastroschisis pregnancies. A scratch created in the well mimicked the gastroschisis "wound" and closure rates were compared. There were no significant differences found in cell migration rates in third trimester maternal blood, cord blood, and amniotic fluid samples between control and gastroschisis pregnancies over time. While no or slower rate of cell migration in gastroschisis samples were seen, the lack of a significant difference in closure in control and gastroschisis samples indicate that cell migration is not affected by factors that may be present in late pregnancy in amniotic fluid or in the maternal or fetal circulation. This does not eliminate the possibility that incubation taken from samples early in the

pregnancy would have yielded different results. Future work will consider collecting biological samples in early stages of pregnancy, or at time of gastroschisis development to yield different results as gastroschisis development occurs in the sixth to tenth week of fetal development.

There is need for further understanding the pathogenesis and risk factors associated with gastroschisis. Numerous studies have noted factors, such as age, body mass index, race, and exposure to toxins (tobacco, alcohol, illicit drugs, and pesticides). Still, there has yet to be substantial evidence and correlation in gastroschisis development. Despite many hypotheses the pathogenesis of gastroschisis remains unknown.

The exploratory experimental design of this study was aimed to aid in understanding gastroschisis formation on a cellular level. The *in vitro scratch* assay approach is a method novel to studies of gastroschisis, yet its ability to capture cell migration is key in our investigation of determining cell-cell interactions during cell migration. Given that it has been suggested that the defect occurs early in development, at the time when, in the area of the defect, there is a shift in vascularization [7] or that there may be an interruption of the blood flow in the vascular plexus that will form the right vitelline arteries [8], we hypothesized that either process might involve apoptosis, angiogenesis, cell migration, or cell adhesion. Additional considerations included comparing cell migration rates from samples collected at an earlier gestational age to account for disruptions occurring during early development. This may also be connected with several environmental factors adversely influencing the process, as well.

Tissue formation during embryonic development, wound healing and immune responses all require the coordinated movement of cells in particular directions to specific locations. Errors during this process have serious consequences, including vascular disease, tumor formation and metastasis. An understanding of the mechanism by which cells migrate may lead to the development of novel therapeutic strategies for controlling cell migration, proliferation, or apoptosis, which may contribute to gastroschisis pathogenesis.

Various biological samples from control and gastroschisis pregnancies were examined for differences in cell migration rates. While no significant difference in cell migration rates were noted, this adds to our understanding of gastroschisis from a cellular framework during embryonic developmental processes. However, further research is required to elucidate the mechanisms involved with the development of gastroschisis.

Acknowledgments

This portion of the project was able to go from concept to reality due to the time, interest and assistance of Dr. Ravi Goyal and his lab. From the lab space, equipment and supplies, Dr. Goyal graciously provided the support, mentorship, and guidance to conduct this cell migration work. He challenged my reasoning and discussed various methods that could be utilized in further understanding of gastroschisis pathogenesis. Additionally, Dipali Goyal shared her expertise and knowledge by teaching me correct lab techniques to complete this project. I also need to acknowledge Dr. Sheel Shah for making me a more inquisitive researcher, patiently answering my endless amount of questions, and for not only taking an interest in my work, but also always being willing to help. You added

new depth to my project by introducing me to various image analysis tools and machine learning. Thank you! Furthermore, Mericarmen Peralta's assistance with statistical analysis and data consulting was key in understanding the significance and implications of this data set. Lastly, Dr. Bryan T. Oshiro, without your vision and passion this project would just be an unusual coincidence of gastroschisis cases, rather than an opportunity to study and work towards further understanding.

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CHAPTER FIVE

CONCLUSIONS

Our study was unique it that we moved from retrospective chart reviews to prospective follow-ups, resulting in longitudinal analysis of 1- year follow-up. We noticed a change in maternal demographics. Mothers carrying a baby affected with gastroschisis were previously noted as being young in age, nulliparous, and with low maternal body mass index (BMI). However, we saw a trend approaching significance of an increase in maternal age and parity in mothers of gastroschisis babies over time, while BMI was not significantly different. Still, clinically an increased BMI was seen. This information aids in clinical practice by increasing awareness of changing maternal demographics. Additionally, utilizing data from two institutions we were able to achieve a larger sample size to elucidate trends related to LOS and sepsis, depending on mode of delivery and type of closure. We saw that cesarean section with labor (CS&L) had a 2.5 times higher chance of sepsis verses vaginal deliveries, and silo closure yielded an 83% increase in sepsis versus primary closures. As the study at Loma Linda University Children's Hospital moved prospective we observed familial recurrences, which was interesting given that gastroschisis is not known to have genetic links. A 5.45% sibling recurrence rate was noted where a mother had a baby with gastroschisis and a subsequent child also affected by gastroschisis. Given this observation, mothers with a previous pregnancy affected by gastroschisis should undergo preconception counseling and early prenatal care to ensure the best outcomes for her and her baby. Finally, babies with complex gastroschisis and delayed times to full feeds were associated with poorer outcomes at year one of life. Given the nature of human studies, we are often limited by

losing patients at follow- up because they can move, change addresses, and phone numbers without informing the hospital. Furthermore, charts contain missing values for certain variables we were studying, thus excluding the patient from our study. Understanding and utilizing data to elucidate epidemiological trends can play a great role in further understanding gastroschisis and its changing characteristics, as well as in potentially identifying the best plan of care. Results from this study may aid in clinical practice and add to knowledge of gastroschisis demographics, risk factors, and outcomes.

Loma Linda University Children's Hospital (LLUCH) has a unique opportunity to further study gastroschisis etiology given its high clustering of cases in the Inland Empire region. We noticed that cases at Loma Linda University Medical Center (LLUMC) came predominately from the high desert, Victorville area, in addition to Palm Springs, Highland, and Jurupa Valley. Furthermore, spatial statistics revealed that, based on the cases we utilized, it is statistically significantly unlikely that the observed spatial cluster pattern reflects a random pattern. It is important to note that we were limited to cases solely for Loma Linda University Medical Center and therefore are not able to account for gastroschisis birth are surrounding centers.

We compared cell migration patterns from blood and amniotic fluid samples from mothers carrying babies with gastroschisis versus control mothers carrying healthy babies with an uncomplicated pregnancy to understand gastroschisis at a biological level. Cell migration rates did not significantly differ among the gastroschisis and control pregnancies in third trimester maternal blood, cord blood, or amniotic fluid group over time. Collecting biological samples in early stages of pregnancy may yield different

results as gastroschisis development occurs in the sixth to tenth week of fetal development.

Future Work

Additional samples, such as maternal hair, were also collected and may be analyzed for environmental, chemical, and drug toxicology. Hair analysis has several distinct advantages and unique qualities providing it with particular promise in a number of regards to biomonitoring.[1] Successful analysis can assist in the diagnosis, prognosis, and treatment of disease and morbidity, and assess exposure to toxins and polluted environments which the body has been exposed to. Substances can remain and be analyzed for a longer period of time in hair than traditional blood and urine samples. Blood or urine samples are also collected and will be analyzed for metabolites and proteomics. Cord blood samples will be submitted to Metabolon[2] for metabolic pathway, metabolite and biomarker identification. Other studies include the addition of tissue sampling of discarded tissue from infant around defect site. For the study group this will be from the gastroschisis ring defect at the time of surgical repair of the baby. For the control group the sampling will occur during abdominal wall/scare revisions. To ensure neonatal safety, the sampling will only occur if the Pediatric Surgeon determines it appropriate. This will not require reconsent as tissue sampling will be done prospectively as new subjects are consented. A sample of discarded tissue will be obtained by the pediatric surgeon at the time of closure or repair. If there is no tissue to discard, the sample will not be obtained. No injuries to infants or mothers are anticipated. Discomfort to baby is no different than what baby is already exposed to during surgical procedure.

Anesthesia will be in place and post-operative healing is the same with or without the tissue sampling. The addition of the tissue biospecimen help elucidate the pathophysiology of gastroschisis. We will compare the vascular morphology in skin and rectus muscle samples, measure vascular structure (light microscopy), the relative expression levels of several vascular and other cellular proteins (collagen, actin, tubulin, by use of advanced epifluorescence and/or confocal microscopy and/or Western immunoblot). We also will quantify capillary density with these techniques.

This biological aspect of the study, coupled with additional geo-temporal and geo-spatial mapping is needed to move beyond speculation and potentially lead us to a cause. Research which further advances the understanding of risk factors or causative mechanisms may ultimately result in the prevention of gastroschisis and its related morbidities. Once this is known, we can move forward with potential interventions with the potential to reduce neonatal health disparities. In addition, our characterization of patients in a prospective fashion could lead to better predictors of neonatal outcomes. We hope that by studying long term outcome data on these affected individuals, that we will be better able to understand their feeding problems and nutritional requirements and design an improved treatment program for them. Lastly, this study serves as a tool to educate, and increase awareness and knowledge in the population as a whole about gastroschisis and its increasing prevalence throughout the world.

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