UNIVERSITY OF WITWATERSRAND

FACULTY OF HEALTH SCIENCES

SCHOOL OF PUBLIC HEALTH

FACTORS ASSOCIATED WITH NECROTIZING ENTEROCOLITIS IN A PUBLIC HOSPITAL IN JOHANNESBURG, SOUTH AFRICA, 2013 – 2018, A CROSS SECTIONAL STUDY.

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A research report submitted to the Faculty of Health Sciences, University of The Witwatersrand, in partial fulfillment of the requirement for the degree of Masters of Science in Epidemiology (Epidemiology & Biostatistics)

October, 2020.

DECLARATION

I, AJAYI Oluseyi Mojisola declare that this research report is my own work. It is being submitted for the award of a Master of Science degree in Epidemiology in the field of Epidemiology and Biostatistics to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination of degree at this or any other University.

Date: 1st Day of April, 2020 Signature: O.M. AJAYI

To God, my strength, my sustainer and my provider.

ABSTRACT

Background: Necrotizing Enterocolitis (NEC) is a devastating gastrointestinal disease that usually affects newborns. NEC is an important cause of neonatal[1] deaths in the neonatal intensive care unit. This condition is more predominant in babies with a smaller gestational age. The purpose of this study was to determine the factors associated with necrotizing enterocolitis among the very low birth weight (VLBW; weight <1,500g) neonates in Charlotte Maxeke Johannesburg Academic hospital.

Methods: Data was extracted from the VLBW standard database used for registering all VLBW neonates who are born and/or admitted within the first 28 days (neonatal period) of life. The study included all VLBW neonates born or admitted to the neonatal unit at (CMJAH) between 1st January, 2013 and 31st December, 2018. Data analysis was conducted using statistical software for data management and analysis (STATA 15.0). A diagnosis of NEC was established based on the clinical and radiological evidence of stage II or III, with respect to the Bell‟s criteria. Logistic regression analysis was performed to determine the significant risk factors associated with NEC.

Results: The majority of the sample population was males (53%).Two hundred and forty-six (10.58%) infants developed NEC, out of which 83 (33.74%) neonates died. Over the six year period, there was a 7.4% Annual Percent Change (APC) and the overall prevalence of NEC almost doubled between 2016 (8.06%) and 2018 (15.24%). The prevalence of NEC was highest in 2018 (15.24).

Results from the bivariate analysis showed that conventional ventilation (OR 7.962, 95% CI 5.944 – 10.665; P < 0.0001), neonates that were given oxygen on day 36 (OR 2.799, 95% CI 2.005 - 3.907; P < 0.0001), increasing maternal age (OR 1.026, 95% CI 1.003 - 1.051; P < 0.03), patent ductus arteriosus (OR 2.119, 95% CI 1.511 - 2.970; P < 0.0001), blood transfusion (OR 3.918, 95% CI 2.890 - 5.312; P < 0.0001), and respiratory distress syndrome (OR 1.802, 95% CI 1.121 - 2.896; P < 0.015) were significant risk factors for developing NEC.

Furthermore, after controlling for potential confounders, Multivariable logistic regression showed that the significant risk factors associated with NEC were, Oxygen given on day 36,

[1] The word neonatespecifically refers to a newborn baby in its first 4 weeks postbirth.

increasing maternal age, conventional ventilation, and blood transfusion. Patent ductus arteriosus (OR 1.41, 95% CI 0.945 – 2.101; P = 0.092) was found to be marginally significant.

Conclusion and recommendation- NEC remains predominant among VLBW neonates. Adopting preventive strategies like promoting breastfeeding, antenatal care attendance can help in the reduction of the incidence of NEC among VLBW neonates.

ACKNOWLEDGEMENTS

I would like to appreciate my supervisors Professor Daynia Ballot and Professor Tobias Chirwa for their relentless assistance and guidance that they have rendered to me in completing my research and research report despite their tight schedules. I also thank the CEO of Charlotte Maxeke Johannesburg Academic Hospital for making the data accessible.

I am grateful to my husband Ajayi Oluwaseun Damilola, you are the best, thank you for your support. To my daughters Oluwalonimi Ofure Ajayi and Oluwabori Tokunbo Ajayi, you keep me going. Finally, to my parents, Mr. and Mrs. Hammed Hassan Olajokun, you are rare gems, thank you for your support.

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LIST OF ABBREVIATIONS

APC- Annual Percent Change

APGAR- Appearance, Pulse, Grimace, Activity, and Respiration

CMJAH- Charlotte Maxeke Johannesburg Academic Hospital

HIV- Human Immunodeficiency Virus

MNNR- Malaysian National Neonatal Registry

MTCT- Mother-to-child-transmission

NCPAP- Nasal continuous positive airway pressure

NEC- Necrotizing Enterocolitis

NICHD- National Institute of Child Health and Human Development Neonatal Research Network

NICUs- Neonatal intensive care unit

PDA- Patent ductus arteriosus

RDS- Respiratory distress syndrome

REDCap-Research Electronic Data Capture

ROP- Retinopathy of prematurity

VLBW- Very low birth weight

PROM- Premature rupture of membrane

LIST OF DEFINITIONS

NEC- The invasion of bacteria into the intestinal wall, thereby destroying the intestine.

Nasal CPAP- The provision of steady flow of air to the lungs through the nose

Face mask ventilation- Giving positive pressure ventilation through bag and mask.

Oxygen- Provision of oxygen to sustain the life of neonates

RDS- A respiratory disease of a new-born baby

Gestational age- It is used in calculating the length of pregnancy

PDA- It is an opening between the two blood vessels from the heart

Antenatal steroids- It is given to mothers to speed up the development of the neonate`s lungs

CHAPTER 1.0: INTRODUCTION

This chapter summarizes the background of the study as well as the findings from past studies. The gaps and the significance of this study are also being established. This chapter also showcases the overall aim and the specific objectives that this study intended to achieve.

1.1. Background to the Study

Necrotizing Enterocolitis (NEC) is a devastating gastrointestinal disease that usually affects newborns (1). This disease is of utmost public health concern as it is one of those difficult-to-eradicate diseases (2). More so, it chiefly affects neonates who are preterm and born with a very low birth weight (VLBW)[2] (3–5). NEC is an important cause of neonatal[3] deaths in the neonatal intensive care unit (6). This condition is more predominant in babies with a smaller gestational age (7,8).

This condition commonly occurs in the second week of life following the commencement of enteral feeds and diagnosis is established based on findings during physical examination, laboratory studies and abdominal radiographs (6,9). NEC is said to have occurred when there is presence of mucosal or transmural necrosis and it is characterized by intestinal injury of varying degrees which could be caused by infection, enteral feeding, premature birth, intestinal ischemia However there is no clarity about how it occurs(7,9). It can also be referred to a syndrome that is characterized by vomiting, abdominal distension, gastro intestinal bleeding, apnea[4] and shock (10).

The prevalence of NEC among neonates admitted in the neonatal intensive care unit is approximately 5 to 10% globally, which is likely to increase to 12% in VLBW infants (11). In Australia there was an increase in the incidence of NEC from 0.07% during 1971-1974 to 0.25% from January, 1984 to July, 1985. In the United States the overall incidence of NEC is estimated to be 1-3 cases per 1000 live births (12). However in South Africa, studies from the Chris Hani

[2] Babies who weigh less than 1500g at birth.
[3] The word neonatespecifically refers to a newborn baby in its first 4 weeks postbirth.
[4] Apnea means cessation of breathing of longer than 20 seconds.

Baragwanath Hospital showed that the incidence in the years 1988 and 1994 was 2.1 and 3.5 for every 1000 live births respectively.

Most gastrointestinal emergencies reported among neonates are due to this condition and it is also the major cause of death needing gastrointestinal surgery (3,13,14). It is evident that the presence of micro-organisms in the intestine of the infants has a crucial role to play in the pathogenesis of NEC however it is not associated with any organism in particular (15). In 1985, during an epidemic in Australia, no microorganism was isolated. Previously *Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia* were reported to be the single organism responsible for the outbreak (16). A high level of fatality is associated with this condition and there is usually presence of ulceration mostly at the terminal ileum when an autopsy is carried out (17).

In recent years, breastfeeding, improvement in providing care to very ill neonates, using anti-infectious control measures has helped in controlling NEC epidemics and thus increased the chance of survival among the VLBW babies (16). It has been demonstrated from large observational studies that neonates who were given breast milk as their primary source of enteral feeding, were less likely to develop NEC (18). This has also been reiterated by the American Academy of Pediatrics (19).

Additionally, results for some randomized control trials and meta-analysis have proven that, the use of probiotics has some role to play in reducing the incidence of NEC (18). Results from the Sunny brooks health Sciences Centre in Canada showed that the incidence of NEC dropped from 4.4% to 1.7% during a probiotic intervention period February 1, 2015 and March 31, 2018 (20). For prebiotics, large randomized control trials are yet to be performed, to determine if it has an association in reducing the risk of NEC among VLBW neonates but some trials are in progress (18). However, there is still a rise in the incidence of NEC (21). Recently, the use of antibiotics together with pentoxifylline which has the ability to regulate the immune system has been studied but there is poor evidence that it is protective against NEC among VLBW infants(22).

The monetary cost of NEC is large; annually, the estimated total cost of treatment and management of infants with this medical condition in the United States ranges between $500 million and $1 billion (2).The critical role of both medical and surgical management in the treatment of NEC cannot be overemphasized however prevention seems to have the most effective influence on morbidity and mortality generally (6). Hence the aim of the study is to determine the factors associated with NEC among the VLBW infants in CMJAH in South Africa between 2013 and 2018.

1.2. Literature Review

1.2.1. Prevalence of NEC

In spite of increased knowledge in understanding the clinical presentation and pathophysiology of NEC, global prevention of this disease is still a big challenge even in the twenty-first century (23). Although data from the US reveals that there is a decrease in the incidence rate of NEC(1). The prevalence of NEC differs among neonatal intensive care units (NICUs). However studies from the National Institute of child health and human development (NICHD) Neonatal research Network revealed that the prevalence of NEC on average was 7% in VLBW neonates and further increases to 15% in neonates whose birth weights are less than 750g (19).

The neonatal network in Korea revealed the incidence of NEC to be 6.41% whereas in Sweden it was as low as 2.68%(24). Studies from a systematic review showed that the incidence of NEC among VLBW at the Polish neonatal surveillance network and the Malaysian National neonatal registry were 8.68% and 6.20% respectively(24). From the largest hospital in Singapore the incidence was found to be 6.98%(24). A Romanian study experienced a high incidence of NEC 17.08% whereas the Swiss neonatal network showed a prevalence OF 4.95%(24). In addition, there appears to be no data on incidence of NEC from regions like the North Africa, the Middle east or the Arab region except a by the lone study performed in UAE(24).

In the year 2000, the United States of America reported the rate of NEC-associated hospitalizations to be 109.9 per 100,000 live births(3). The incidence of NEC and its mortality rate increase are inversely proportional to gestational age and birth weight. It was reported in

Johannesburg between 2013 and 2015 that NEC was a predictor of poor survival among the VLBW neonates (25). In South African study, among 43 neonates that were admitted to the intensive care unit for NEC, 37 of them died indicating that a very high mortality in critically ill neonates with this condition (25).

1.2.2. Factors associated with NEC in VLBW neonates

The neonatal and maternal characteristics are important when assessing the rate of incidence of NEC among VLBW babies. Although many studies have been done in the context of neonatal morbidity and survival, it appears there still lack of data on factors associated with the occurrence of neonatal morbidity such as NEC. Therefore it is imperative that more studies are carried out on predictors related with these occurrences. Familial predisposition and genetic factors have been proposed to contribute to the incidence of NEC(9).

The context of the VLBW cannot be overemphasized as there appears to have been more recent studies in the specific area. Cases of morbidity among neonates have equally been reported in research. For instance, a study reported that there has been no significant increase in rate of morbidity among VLBW between 1997 and 2002. This study which was carried out in the NICHD, United States of America also suggested adoption of best practices in ameliorating the cases of morbidity. (26).

1.2.3. Neonatal characteristics associated with NEC

Retrospective studies from the United States Pediatrix Data Warehouse between 1997 to 2009 revealed that younger gestational age, lower birth weight and small for gestational age are related to an increased risk of developing NEC [(27),(1)]. However studies done in some hospitals in New York showed that there was no significant association between the incidence in NEC and gender which is in agreement with the literature (28). The black ethnicity and its strong association with NEC in comparison with the whites has been consistent over a period of years in the United States (1). Neonatal bacterial infection including sepsis was reported to have a strong association with the risk of developing NEC in Sweden between 1987-2009 (29).

Results from several studies showed that congenital heart disease is a major predisposing factor to developing NEC in VLBW infants (30). Congenital heart diseases such as patent ductus arteriosus (PDA) have also been assessed to determine its contribution see if it contributes to the risk of developing NEC. More than 30% of babies who are born before 30 weeks are usually diagnosed with PDA. PDA can lead to the "steal" phenomenon, which entails the flow of blood to organs that are below the PDA which includes the gastrointestinal tract, potentially making the neonate susceptible to developing NEC (15). From a study that was done in the United States, it was reported that there was a significant relationship between lower 5min APGAR score and NEC as well as the male gender (31).

1.2.4. Maternal and antenatal characteristics associated with NEC

The state of health of a pregnant woman and circumstances around the process of labor and delivery can possibly play a key role in the development of NEC in neonates. If complications occur during pregnancy, it is a likely source of contamination to the fetus. This was confirmed by a study that reported that early rupture of membranes, chorioamnionitis, fever just before and during delivery are potential causes of neonatal NEC (10).

Studies from a case control carried out in Italy from 2002-2016 reported that there was no association between maternal age, maternal diabetes and NEC however among the NEC group was a higher prevalence of preeclampsia and chorioamnionitis (32). In Sweden a case control study that was carried out which revealed that there is an independent and positive relationship between Caesarean section and NEC. However it was also reported that the risk of developing NEC decreased with increase in maternal weight, preeclampsia and maternal urinary tract infection (29). Two other studies that were done showed that Caesarean section had a protective effect against developing NEC and this was possibly due to less stress of labor during delivery (33).

A meta-analysis of 10 randomized control trial showed that the risk of developing NEC was reduced by 50% if antenatal steroids were administered prior to delivery (1). However, in the United States it was reported that there was no significant benefit in the administration of antenatal steroids before delivery (31). Administration of magnesium sulphate just before

delivery showed no significant increase in the risk of developing NEC especially among infants born between 24-31 weeks of gestation (1,31). In the local context there is a knowledge gap association between maternal characteristics and NEC due to limited data.

The pandemic of Human Immunodeficiency Virus (HIV) is highest in South Africa with an antenatal prevalence of 31% (34). Mothers who are HIV infected have a higher risk of delivering VLBW infants and/or preterm infants hence contributing to the prevalence of NEC(34,35). HIV-exposed VLBW infants are at a higher risk of mother-to-child transmission (MTCT) of HIV during the peripartum period. Previously, instead of breast milk they were given formula in order to prevent transmission of HIV via the breast milk. They have been deprived of the advantages of breast milk which includes protection of the mucosal intestinal wall thus increasing the chances of developing NEC in the infant (34).

1.2.5. Clinical characteristics associated with NEC

Based on the clinical characteristics that are associated with NEC, a systematic review of prognostic studies proved that surfactant therapy reduced the risk of developing NEC in VLBW (36,37). In the United States studies have shown that the use of mechanical ventilation increases the risk of developing NEC in VLBW and from their study they also proved that the risk of developing NEC from the use of antenatal steroids was independent of birth weight (37). In South Africa, less than 5% use of antenatal steroids was reported in 1996 (38). However in 2001 more than 60% of women who had preterm labor at the Chris Hani Baragwanath Hospital were given a minimum of one dose of antenatal steroid (38).

Another study also showed that VLBW infants are more likely to develop NEC from assisted ventilation (13). More recently, many studies have however proposed that there is an increasing risk of developing NEC with blood transfusion (19,23). It is also a matter of interest that there is no relationship between perinatal asphyxia, intraventricular hemorrhage and the risk of developing NEC (39). It has been observed that there is a strong association between babies who developed NEC and had mothers who had gestational diabetes (16).

Oxygen therapy improves the survival rate of infants however excess administration was found to be associated with retinopathy of prematurity (40). In a meta-analyses that was done, neonates that were given oxygen had lesser occurrence of NEC compare to neonates that were not given (40). Some studies have also reported that there is an association between the use of nasal continuous positive airway pressure (NCPAP) and increased risk of developing NEC as a result of increased supply of oxygen and pressure straight to the digestive tract (41). Usually, infants who are supported with NCPAP have reduced blood flow to the superior mesenteric artery compare to those who are not on NCPAP (41).

1.3. Problem Statement

The importance of historical data in estimating and reducing the occurrence of NEC is essential. Some researchers affirmed this assertion in their own research that the menace of neonatal surgical diseases like NEC in Africa is not properly documented (42). Over the years it has been difficult to identify consistent factors that are associated with NEC apart from preterm birth and low birth weight (16). More so, we do not have a good understanding of predictors of NEC due to the absence of robust data before the surfactant era (27,28). It is imperative for such data to be available even in emerging economies like South Africa; it is assumed that this will assist in the development of strategies as regards making efforts to reduce the NEC incidence.

There are continuously changing patterns in reproductive health and associated diseases. Thus, findings from previous studies will appear not to be sufficient for new strategies in reducing the occurrence of this health menace.

1.4 Justification of the Study

We need to determine if there are consistent factors associated with neonatal NEC in CMJAH or if there have been changing patterns over the years as it differs across geographical locations. This will enable stakeholders (Health institutions, Departments of health and Government) to create sustainable best practices and strategies to be put in place to aid the reduction in the incidence of neonatal NEC in South Africa.

1.5 Research Questions

1. Are there changes in the prevalence of NEC among VLBW neonates in CMJAH, Johannesburg, South Africa over time?

2. What are the factors associated with the occurrence of NEC among VLBW neonates in CMJAH, Johannesburg, South Africa?

1.6 Aim

The aim of the study is to examine the factors associated with the occurrence of NEC at CMJAH Johannesburg, South Africa.

1.7 Objectives

i. Describe the demographic and clinical characteristics of VLBW neonates at Charlotte Maxeke Johannesburg Academic hospital between 1 January, 2013 and 31 December, 2018.
ii. Determine the level of NEC prevalence and the changes in the prevalence of NEC in VLBW neonates annually at Charlotte Maxeke Johannesburg Academic hospital between 1 January, 2013 and 31 December, 2018.
iii. Determine factors associated with NEC at Charlotte Maxeke Johannesburg Academic hospital between 1 January, 2013 and 31 December, 2018.

CHAPTER TWO: 2.0 METHODS

This chapter provides details of all the processes and methods that were used in conducting this study. It elaborates on the study design, the study site, the study population, the sampling strategy, how the data were collected, how the collected data was being managed, the variables that were explored, and the statistical analysis methods that were used.

2.1 Study design

An institutional based retrospective cross-sectional study was conducted using records from January 1, 2013 to 31 December, 2018. This study was a secondary analysis of an existing neonatal database at the CMJAH; hence this proposed study used the secondary data from the same facility. There is weekly routine collection of hospital records of VLBW babies admitted to CMJAH for the purpose of clinical audit.

2.2 Study site

The CMJAH was used for the purpose of the research; this was because of the availability of current data. The CMJAH is an accredited tertiary referral hospital in Parktown, Johannesburg, South Africa. The hospital has a quaternary neonatal intensive care unit which provides surgical and medical services for newborns. The pediatric and the NICU is shared with 14 beds for babies requiring ventilation, 35 beds at the high care unit, 24 bed at the low care unit and 8 beds at the Kangaroo Mother Care (KMC) unit.

2.3 Study population

The study population was all VLBW babies (<1500g at birth) born at CMJAH and admitted to the neonatal unit between 1 January, 2013 and 31 December, 2018.

2.4 Sampling

No sampling strategy was conducted. All available medical records of VLBW neonates that fulfilled the inclusion criteria were included in the study, which is roughly an estimate of a minimum of 2,300 VLBW babies.

2.5 Data source and collection

Data was extracted from the VLBW database administered by the unit. This is a standard database used for registering all VLBW neonates who are born and/or admitted within the first 28 days (neonatal period) of life. The database was created in January 2013 and is usually updated as soon as eligible neonates are born or admitted. The creation of the database is for the purpose of clinical audit and quality improvement. Skilled staff members in the unit collect and capture relevant information on the mother and neonate into the database which is usually verified and certified by the head of department. Information captured on the database includes demographic and clinical characteristics of the neonates and their mothers are captured in the database.

2.6 Data management

Data was captured on a secure web-based database system using Research Electronic Data Capture (REDCap), hosted by University of Witwatersrand(43). Data was extracted by the gate keeper, obtained as an excel file from the database and imported into Stata statistical software for data management and analysis. Data inputting and cleaning was done in STATA 15.0 to check for missing data and errors. Data was made available after ethical approval.

Medical records of neonates with missing clinical outcomes, those who died within the first week and neonates with a birth weight of <500g were excluded from the study. This is because deaths resulting from NEC usually occur in the second week of life.

2.6.1 Dependent, independent Variables and their measurement

Dependent variables

The primary outcome of interest was the neonatal NEC status whether present or absent. The diagnosis of NEC was founded on the presence of clinical, radiological and /or histopathological evidence that is present in Bells stage II or III. NEC status is a binary variable where present is tagged as 1 (Yes) and absent is tagged as 0 (NO).

Independent Variables

The exposure variables were selected based on prior knowledge and biological plausibility. It includes neonatal characteristics such as gender, birth weight, gestational age (in weeks), APGAR score (< 6 after 5 minutes), race, face mask ventilation, Temperature, oxygen on day 36, Nasal continuous positive airway pressure (Nasal CPAP), Conventional ventilation, Oxygen, Respiratory distress syndrome, Patent ductus arteriosus, Retinopathy of prematurity (ROP).

Maternal characteristics includes maternal age (in years), antenatal care, antenatal steroids, Chorioamnionitis, antenatal Mg SO4, hypertension, HIV, syphilis, diabetes, tuberculosis. Clinical characteristics include NCPAP, surfactant therapy, conventional ventilation, oxygen therapy, and mode of delivery.

Table 2.1 ***Independent variables***

Variable **Neonatal Characteristics**	**Type of Variable**	**Labels (Values)**
Gender	Categorical	Female = 0: Male = 1
Birth weight (in grams)	Continuous	>=1000g = 0: <1000 = 1
Gestational age (in weeks)	Continuous	>=28weeks = 0: <28weeks = 1
APGAR score (after 5mins)	Categorical	>5mins = 0:<5mins = 1
Face mask ventilation	Categorical	No = 0: Yes = 1
Temperature within 1hr	Categorical	No = 0: Yes = 1
Oxygen on day 36	Categorical	No = 0: Yes = 1
Nasal continuous positive airway pressure (nasal CPAP)	Categorical	No = 0: Yes = 1
Conventional ventilation	Categorical	No = 0: Yes = 1
Respiratory support at 36weeks (oxygen)	Categorical	No = 0: Yes = 1
Respiratory distress syndrome	Categorical	No = 0: Yes = 1
Patent ductus arteriosus	Categorical	No = 0: Yes = 1
Blood transfusion	Categorical	No = 0: Yes = 1
Retinopathy of prematurity	Categorical	No = 0: Yes = 1

Maternal Characteristics	Type of variable	Labels (Values)
Maternal age (in years)	Continuous	
Antenatal care	Categorical	N0 =0 : Yes = 1
Antenatal steroids	Categorical	N0 =0 : Yes = 1
Chorioamnionitis	Categorical	N0 =0 : Yes = 1
Hypertension	Categorical	N0 =0 : Yes = 1
HIV	Categorical	N0 =0 : Yes = 1
Syphilis	Categorical	N0 =0 : Yes = 1
Diabetes	Categorical	N0 =0 : Yes = 1
Tuberculosis	Categorical	N0 =0 : Yes = 1
Caesarian section	Categorical	N0 =0 : Yes = 1
Mode of delivery	Categorical	Vaginal delivery = 0: Caesarian section = 1

2.7 Data Analysis

The collected data was cleaned, coded and exported to STATA 15 for further cleaning and analyses. Appropriate recoding was done for example using gender as a categorical variable (males were coded as 0 while females were be coded as 1), for birth weight (babies with a weight of <1000g were coded as 0, while those with a weight of >1000g were coded as 1).

The demographic and clinical characteristics of study participants were described using frequencies, proportions and charts for categorical variables such as gender, and mode of delivery. For continuous variables such as birth weight, gestational age, and maternal age were summarized using median and interquartile range because they were not normally distributed.

Comparisons were done for clinical and demographic data between infants that had NEC and those who did not have NEC.

Bivariate analysis was conducted to assess the relationship between demographic and clinical variables and the outcome (NEC). The association between categorical variables such as gender and the development of NEC was assessed using the Pearson‟s chi- square test. While the association between continuous variables e.g. gestational age and NEC was assessed using Student‟s t-test variables that are normally distributed and Mann Whitney U test for variables that are not normally distributed.

In determining the trends in the prevalence of NEC, the proportion of neonates with NEC was computed for each year and represented using line graphs to display the trends in prevalence over the six year period. Trends analysis was done using joint point regression and comparison was done annually.

2.7.1. Logistic regression

To determine the relationship between the dependent variable which is NEC and each of the independent variables [whether continuous such as birth weight, estimated gestational age, Apgar score, length of hospital stay or categorical such as gender, place of birth and race] univariate logistic regression modeling was conducted.. Stepwise backward multivariable regression modeling was conducted by using variables that have P-value <0.10 at the univariate analysis level. Some variables were also be selected *a priori* to be included in the model based on their importance after reviewing literature e.g. gender, birth weight and gestational age.

Two tailed test of hypothesis was assumed and statistically significant level was set at 5% level. Given $X = (X_1, X_2, X_3 \ldots X_P)$ as a set of independent variables comprising of both continuous and categorical. The relationship between NEC and the Independent variables is better described by the non-linear equation;

( )

The value of *E(Y)* in the equation above provides *probability* that Y = 1given a particular set of values for the independently variables. $X_1, X_2 \ldots X_p$.

The logistic equation below will be adopted for the bivariate analysis given that the model involves only one independent variable *X*;

( ) ( )

Potential confounder like gender was adjusted for.

2.8 Ethical consideration

Ethical approval was sought from the Human Research Ethics Committee of the University of the Witwatersrand in September, 2019 and unconditional approval was granted on 20th November, 2019 with reference no- M191062. The data was utilized for solely academic purposes with strict confidentiality. Data was made accessible to only the principal investigator and supervisors.

CHAPTER 3.0: RESULTS

This chapter gives the results of the data analysis that was performed with the use of the analysis techniques indicated in chapter 2. It shows the descriptive statistics using graphs and tables. The results are displayed chronologically based on the objectives of the study.

3.1 Demographic and clinical characteristics

Describing the demographic and the clinical characteristics of the VLBW neonates is of utmost importance. This section gives a summary of the characteristics among the study participants which includes the VLBW neonates and the mothers but reported separately.

3.1.1 Demographic and clinical characteristics of VLBW neonates

Figure 3.1 below shows that the maximum birth weight across the years was about 1500grams. However there was little variation across the years for the median birth weight (in grams) as shown by the overlaps of the box plots over time which was about 1200g. The values of the minimum birth weight across the years were all greater than 500 grams. They range between 570 grams and 650 grams.

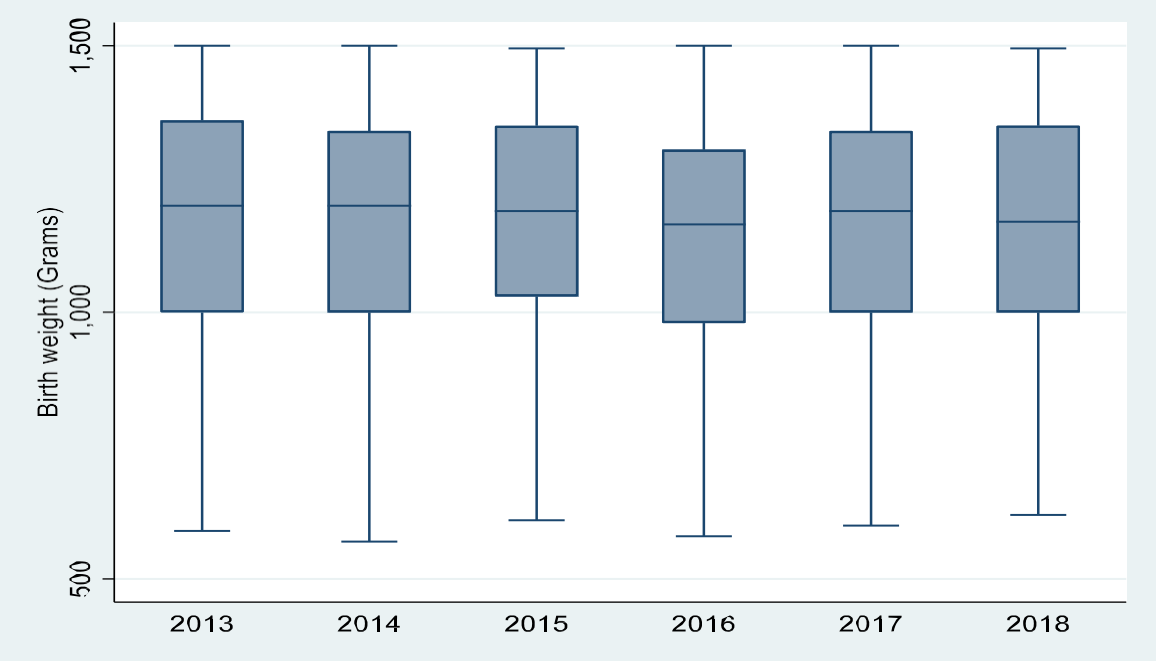


Figure 3.1 Boxplot showing the birth weight (in grams) of VLBW neonates at CMJAH between 2013 and 2018

From table 3.1 below, the results show that there were about 2,114 VLBW babies involved in this study between 2013 and 2018. Number of VLBW babies for each year does not add up to the variable because of deficiency of the database. Across the years, more females were recorded on average except in 2016 where the proportions of males were higher (51.1%). The highest number of extremely low birth weight was recorded in 2013 (30.2%) and 25.2% were born with gestational age of <28 weeks. About 90.7% of neonates had an APGAR score of > 6 at 5mins. Most of the neonates were on Nasal CPAP 72% in 2013, 74.6% in 2015, and 73.3% in 2018.

More than half of the neonates were initially resuscitated at the delivery room during the study period. But a few needed respiratory support at 36 weeks 12.9% in 2013, 30.2% in 2015, and 22.3% in 2018. An appreciable number of neonates had surfactant therapy at some point during admission 87.1% in 2013, 72.4% in 2015, and 67.4% in 2018. NEC of stage II and III was reported in 226 neonates (7.5%) with the highest incidence observed in 2017, 49 (12.6%). On average, over 80% of the neonates had RDS however less than one-quarter of the neonates had PDA and ROP.

3.1.2 Demographic and clinical characteristics of mother of VLBW neonates

The demographic and clinical characteristics of mothers of the VLBW neonates are presented in table 3.2. Within the six year period the median maternal age was about 28 years. On average, more than 80% of mothers attended antenatal care and about half of them were placed on antenatal steroid during these six year study period. Very few women had tuberculosis, diabetes mellitus, syphilis, chorioamnionitis with a low prevalence of less than 5%. However, more women presented with HIV and hypertension, with a similar prevalence ranging from 26.6% - 35.2% and 25.2% - 33.5% respectively. More women had deliveries via caesarian section compared to those who had deliveries vaginally, 65.2% in 2013, 61.7% in 2014, and 66% in 2018.

3.1.3. Clinical and demographic characteristics among VLBW neonates with and without NEC at CMJAH from 2013 – 2018.

Univariate analysis showed that infants with NEC had a lower birth weight and gestational age compared to those without NEC although these differences were not statistically significant (Table 3.3). Variables such as NCPAP, conventional ventilation, delivery of oxygen on day 28 and 36, initial resuscitation of neonates in the delivery room, respiratory distress syndrome, patent ductus arteriosus, blood transfusion and surfactant therapy were significantly more common among infants with NEC compare to infants without NEC.

The proportion of mothers who attended antenatal care and took antenatal steroids were significantly lower among infants with NEC. Additionally, a significantly lower proportion of neonates with NEC had mothers who had HIV, hypertension, syphilis, and chorioamnionitis compared to neonates without NEC.

Table 3.1 ***Clinical and demographic characteristics among VLBW neonates at Charlotte Maxeke Johannesburg Academic Hospital from 2013 -2018***.

Characteristics	2013 (N=255) (%)	2014 (N=399) (%)	2015 (N=384) (%)	2016 (N=372) (%)	2017 (N=388) (%)	2018 (N=316) (%)
Gender						
Female	139 (54.51)	205 (51.38)	198 (51.56)	182 (48.92)	222 (57.22)	181 (57.28)
Male	116 (45.49)	194 (48.62)	186 (48.44)	190 (51.08)	166 (42.78)	135 (42.72)
Birth weight(in grams)						
>= 1000g	178 (69.80)	309 (77.19)	308 (80.21)	272 (73.12)	301 (77.58)	239 (75.63)
<1000g	77 (30.20)	91 (22.81)	76 (19.79)	100 (26.88)	87 (22.42)	77 (24.37)
Gestational age (in weeks)						
>= 28 weeks	187 (74.80)	320 (81.01)	305 (80.05)	292 (79.13)	309 (80.47)	243 (77.88)
<28 weeks	63 (25.20)	75 (18.99)	76 (19.95)	82 (20.87)	75 (19.53)	69 (22.12)
Apgar score (>6 at after 5mins)						
>= 5mins	223 (94.09)	333 (94.60)	339 (96.03)	313 (93.99)	321 (93.86)	266 (94.33)
< 5mins	14 (5.91)	19 (5.40)	14 (3.97)	20 (6.01)	21 (6.14)	16 (5.67)
Oxygen on day 36						
Yes	30 (12.88)	60 (23.62)	55 (30.22)	65 (28.14)	75 (24.27)	60 (22.30)
No	203 (87.12)	194 (76.38)	127 (69.78)	166 (71.86)	239 (75.73)	209 (77.70)
Conventional ventilation						

Yes	54 (23.28)	97 (26.36)	81 (21.72)	92 (25.99)	101 (28.45)	84 (29.47)
No	178 (78.72)	271 (73.64)	292 (78.28)	262 (74.01)	254 (71.55)	201 (70.53)
Nasal continuous positive airway pressure (nasal CPAP)						
Yes	173 (72.03)	266 (70.93)	279 (74.60)	258 (73.50)	257 (72.19)	209 (73.33)
No	67 (27.92)	109 (29.07)	95 (25.40)	93 (26.50)	99 (27.81)	76 (26.67)
Initial resuscitation in delivery room						
Yes	180 (74.38)	254 (70.36)	269 (76.64)	238 (70.00)	240 (69.97)	195 (70.14)
No	62 (25.62)	107 (29.64)	82 (23.26)	102 (30.00)	103 (30.03)	83 (29.86)
-Necrotizing Enterocolitis						
Yes	28 (11.02)	35 (8.79)	38 (9.90)	30 (8.06)	49 (12.66)	48 (15.24)
No	226 (88.98)	363 (91.21)	346 (90.10)	342 (91.94)	338 (87.34)	267 (84.76)
Respiratory distress syndrome						
Yes	222 (87.06)	346 (87.82)	347 (90.84)	293 (80.05)	332 (86.68)	266 (85.53)
No	33 (12.94)	48 (12.18)	35 (9.16)	73 (19.95)	51 (13.32)	45 (14.47)
Patent ductus arteriosus						
Yes	35 (13.78)	52 (13.13)	47 (12.27)	45 (12.13)	44 (11.43)	39 (12.42)
No	219 (86.22)	344 (86.87)	336 (87.73)	326 (87.87)	341 (88.57)	275 (87.58)
Blood transfusion						
Yes	95 (38.31)	186 (47.94)	192 (49.87)	197 (53.10)	184 (47.92)	167 (53.35)
No	153 (61.69)	202 (52.06)	191 (50.13)	174 (46.90)	200 (47.92)	146 (46.65)

Retinopathy of prematurity						
Yes	14 (9.86)	26 (11.76)	22 (11.17)	26 (11.76)	18 (7.50)	22 (11.22)
No	128 (90.14)	195 (88.24)	175 (88.83)	195 (88.24)	222 (92.50)	174 (88.78)
Surfactant therapy at any time						
Yes	183 (73.79)	273 (70.91)	279 (72.44)	244 (67.40)	265 (71.43)	203 (67.44)
No	65 (26.21)	112 (29.09)	105 (27.56)	118 (32.60)	106 (28.57)	98 (32.56)

Table 3.2 ***Maternal demographic and clinical characteristics of VLBW neonates at Charlotte Maxeke Johannesburg Academic Hospital from 2013 -2018***.

Characteristics	2013 (N=407) (%)	2014 (N=361) (%)	2015 (N=354) (%)	2016 (N=333) (%)	2017 (N=338) (%)	2018 (N=280) (%)
Median Age (in years)	28	28	28	29	29	29
Antenatal care						
Yes	190 (77.87)	298 (80.98)	315 (84.45)	297 (85.59)	298 (83.01)	247 (84.30)
No	54 (22.13)	70 (19.02)	58 (15.55)	50 (14.41)	61 (16.99)	46 (15.70)
Antenatal steroids						
Yes	109 (48.02)	165 (48.82)	204 (55.89)	171 (50.74)	180 (53.10)	143 (53.76)
No	118 (51.98)	173 (51.18)	161 (44.11)	166 (49.26)	159 (46.90)	123 (46.24)
Chorioamnionitis						
Yes	9 (3.96)	16 (4.65)	14 (3.83)	15 (4.44)	13 (3.77)	12 (4.24)
No	218 (96.04)	326 (95.32)	352 (96.17)	323 (95.56)	332 (96.23)	271 (95.76)
HIV						
Yes	66 (26.72)	114 (29.61)	127 (33.16)	128 (35.16)	130 (34.39)	83 (26.60)
No	181 (73.28)	271 (70.39)	256 (66.84)	236 (64.84)	248 (65.61)	229 (73.40)
Hypertension						
Yes	56 (25.23)	95 (27.70)	100 (27.32)	92 (26.82)	100 (28.41)	94 (33.45)
No	166 (74.77)	248 (72.30)	266 (72.68)	251 (73.18)	252 (71.59)	187 (66.55)
Syphilis						
Yes	3 (1.30)	8 (2.19)	9 (2.42)	7 (2.02)	8 (2.23)	5 (1.68)

No	228 (98.70)	357 (97.81)	363 (97.58)	339 (97.98)	351 (97.77)	293 (98.32)
Diabetes						
Yes	2 (0.86)	0 (0)	1 (0.27)	4 (1.15)	3 (0.83)	2 (0.68)
No	231 (99.14)	357 (100)	371 (99.73)	343 (98.85)	357 (99.17)	292 (99.32)
Tuberculosis						
Yes	2 (0.87)	3 (0.85)	0 (0)	10 (2.91)	2 (0.56)	2 (0.68)
No	229 (98.99)	348 (99.15)	367 (100)	334 (97.09)	354 (99.44)	291 (99.32)
Mode of delivery						
Vaginal delivery	87 (34.80)	145 (38.26)	154 (40.85)	155 (43.30)	160 (43.48)	102 (34)
Caesarian section	163 (65.20)	234 (61.74)	223 (59.15)	203 (56.70)	208 (56.52)	198 (66)

Table 3.3 ***Clinical and demographic characteristics among VLBW neonates with and without NEC at Charlotte Maxeke Johannesburg Academic Hospital from 2013 -2018***.

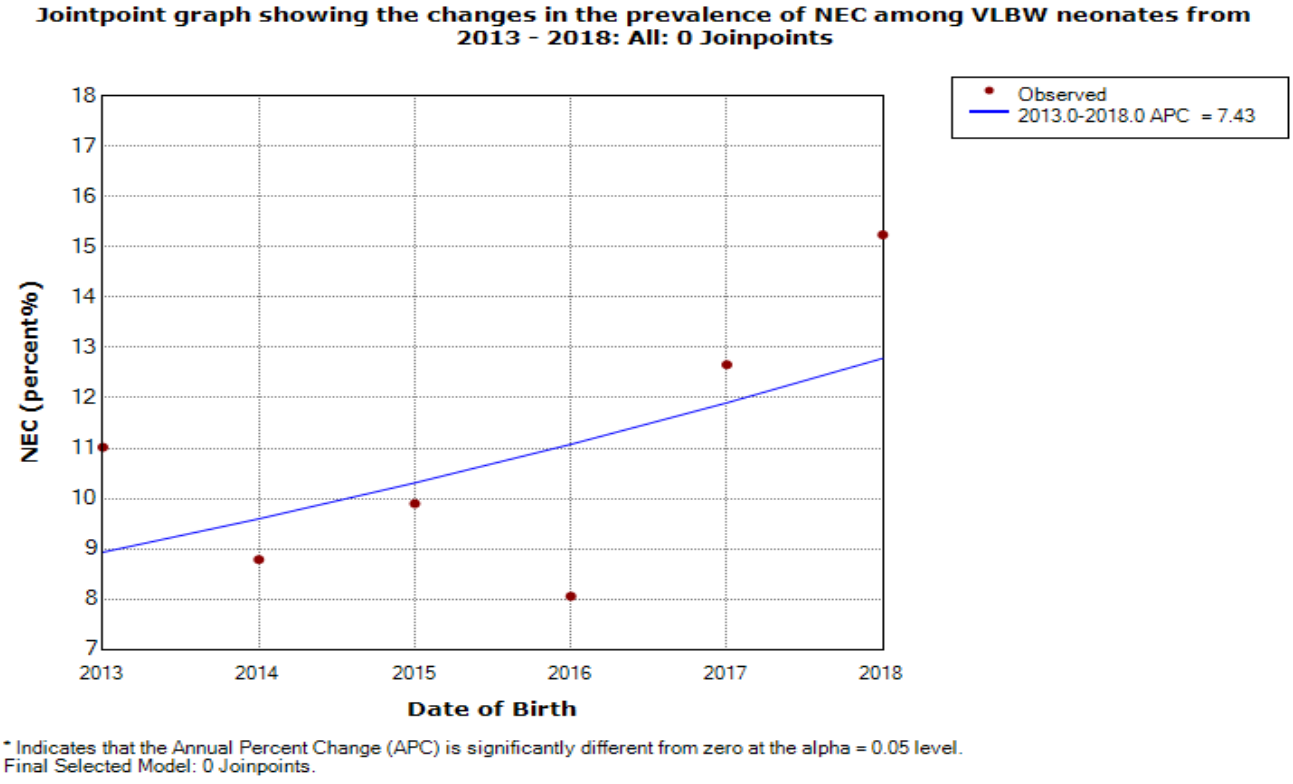
Characteristics	No. (%)			p-value
	Total	With NEC (n = 246)	Without NEC (n = 2075)	
Neonatal Factors				
Male gender	1124	113 (45.9)	1011 (48.72)	0.694
Birth weight >=1000g	1780	184 (74.79)	1596 (76.92)	0.756
Gestational age >=28weeks	1834	191 (77.64)	1643 (79.18)	0.324
APGAR score >6 @5mins	1965	171 (69.51)	1794 (86.46)	0.015
Nasal continuous positive airway pressure (nasal CPAP)	1578	162 (65.85)	1416 (68.24)	<0.001
Conventional ventilation	556	159 (64.63)	397 (19.13)	<0.001
Oxygen on day 36	359	67 (27.24)	292 (14.07)	<0.001
Initial resuscitation in delivery room	1538	142 (58.94)	1396 (67.28)	<0.001
Respiratory distress syndrome	1985	215 (87.39)	1770 (85.30)	<0.001
Patent ductus arteriosus	283	50 (20.33)	233 (11.23)	<0.001
Blood transfusion	1078	182 (73.98)	896 (43.18)	<0.001
Retinopathy of prematurity	136	14 (5.69)	122 (5.88)	0.062
Surfactant therapy at any time	1582	158 (64.23)	1424 (68.63)	<0.001
Maternal factors				

Age 28+years	1144	118 (47.97)	1026 (49.45)	0.106
Antenatal care	1791	160 (65.04)	1631 (78.60)	<0.001
Antenatal steroids	1054	95 (38.62)	959 (46.23)	<0.001
Chorioamnionitis	84	13 (5.29)	71 (3.42)	<0.001
HIV	720	87 (35.37)	633 (30.51)	<0.001
Hypertension	594	47 (19.11)	547 (26.36)	<0.001
Syphilis	44	4 (1.63)	40 (1.93)	<0.001
Diabetes	13	0	13 (0.63)	<0.001
Tuberculosis	21	0	21 (1.01)	<0.001
Mode of delivery				
Vaginal delivery	889	93 (37.81)	796 (38.36)	<0.001
Caesarian section	1341	112 (45.53)	1229 (59.23)	<0.001

3.2. Changes in the prevalence of NEC among VLBW neonates at CMJAH between 2013-2018.

Figure 3.2 depicts the most recent level of prevalence and the trends in the prevalence of NEC over the six year study period. A decrease in the prevalence of about 6% was observed from 2013 (11%) to 2014 (8.8%). There was also a slight decrease in prevalence of NEC from 2015 (9.9%) to 2016 (8.1). Subsequently, there was a spike in the prevalence in 2016 (8.1%) to 15.2% in 2018. The prevalence of NEC was relatively unstable in the first four years. Overall, there was a rise in the prevalence of NEC over the years compared to the initial prevalence (2013, 11%).

Figure 3.2 ***Joint point regression showing changes in the prevalence of NEC among VLBW neonates at Charlotte Maxeke Johannesburg Academic Hospital from 2013 -2018.***



3.3 Risk factors associated with NEC among VLBW neonates at CMJAH from 2013 -2018.

VLBW neonates can develop NEC depending on their predisposition to neonatal, clinical and maternal factors. To further determine the factors that are significantly associated with NEC, separate logistic regression analysis will be performed.

3.3.1 Bivariate logistic regression analysis of risk factors associated with NEC among VLBW neonates at CMJAH from 2013 - 2018.

Table 3.4 shows results from the fitted logistic regression model of factors associated with NEC. From the results, the odds of females developing NEC were 16% higher compared to males but the observed difference was not statistically significant (OR= 1.16, 95% CI: 0.88 - 1.52); P = 0.284). We can infer that the likelihood of developing NEC decreases by -0.001for every unit increase in birth weight however this is not a significant finding (P = 0.12). There was a similar observation for gestational age, for every unit increase in gestational age, the likelihood of developing NEC lessened by -0.03 (OR= 0.97, 95% CI: 0.92 - 1.02; P = 0.231).

Those who were conventionally ventilated were about 8 times more likely to develop NEC (OR= 7.96, 95% CI: 0.92 - 1.02; P < 0.001). The odds of developing NEC among those who had oxygen on day 36 are significantly higher compare to those who were not given oxygen on day 36 (OR= 2.8, 95% CI: 2.01 - 3.91; P < 0.001).

The presence of respiratory distress syndrome (OR= 1.8, 95% CI: 1.12 - 2.89; P = 0.015, patent ductus arteriosus (OR= 2.12, 95% CI: 1.51 - 2.97; P < 0.001) as well as if the neonate had been transfused with blood (OR= 3.92, 95% CI: 2.89 - 5.31; P < 0.001), significantly poses the neonate at a higher risk of developing NEC compared to those without respiratory distress syndrome, patent ductus arteriosus and those who were never transfused with blood respectively. Increasing maternal age significantly increases the odds of NEC in neonates by about 39% (OR= 1.03, 95% CI: 1.00 - 1.05; P = 0.03).

Antenatal care attendance and the antenatal steroids use significantly reduced the odds of NEC by 18% and 3% respectively. However the observed difference is not statistically significant.

The presence of HIV (OR= 1.23, 95% CI: 0.98 - 1.72; P = 0.068) and chorioamnionitis (OR = 1.83, 95% CI: 0.99 - 3.37; P = 0.052) in the mother marginally increases the odds of NEC. Delivery via caesarian section was found to be protective therefore reducing the chance of developing NEC in neonates (OR= 0.79, 95% CI: 0.59 - 1.05; P= 0.099). However the observed difference was marginally significant.

3.3.2 Multivariable logistic regression analysis of risk factors associated with NEC among VLBW neonates at CMJAH from 2013 - 2018.

The significant risk factors that were found to be associated with NEC among VLBW neonates after controlling for potential confounders were the following: increasing maternal age, delivery of conventional ventilation, oxygen delivery on day 36, and blood transfusion (Table 3.5). However the association between NEC and patent ductus arteriosus was found to be marginally significant. Variables such as birth weight and gestational age were not significant risk factors.

Table 3.4 ***Bivariate logistic regression analysis of risk factors associated with NEC among VLBW neonates at Charlotte Maxeke Johannesburg Academic Hospital from 2013 -2018.***

Risk Factors	Coefficient (β)	OR (90% CI)	p-value
Female gender	0.149	1.161 (0.884 – 1.523)	0.284
Birth weight	-0.001	0.999 (0.999 – 1.000)	0.12
Birth weight <1000g	0.116	1.123 (0.829 – 1.520)	0.455
Gestational age	-0.034	0.967 (0.915 – 1.021)	0.231
Gestational age <28weeks	0.055	1.056 (0.762 – 1.465)	0.742
APGAR score <5mins	0.597	1.817 (1.061 – 3.114)	**0.03**
Nasal continuous positive airway pressure (nasal CPAP)	0.019	1.019 (0.749 – 1.388)	0.903
Conventional ventilation	2.074	7.962 (5.944 – 10.665)	**<0.001**
Oxygen on day 36	1.029	2.799 (2.005 – 3.907)	**<0.001**
Initial resuscitation in delivery room	0.052	1.053 (0.755 – 1.469)	0.756
Respiratory distress syndrome	0.589	1.802 (1.121 – 2.896)	**0.015**
Patent ductus arteriosus	0.75	2.119 (1.511 – 2.970)	**<0.001**
Blood transfusion	1.366	3.918 (2.890 – 5.312)	**<0.001**
Retinopathy of prematurity	0.025	1.025 (0.572 – 1.838)	0.933
Surfactant therapy at any time	0.189	1.209 (0.880 – 1.659)	0.241
Maternal age	0.026	1.026 (1.003 – 1.051)	**0.03**
Maternal age (28+years)	0.328	1.389 (1.022 – 1.886)	**0.035**

Antenatal care	-0.019	0.825 (0.577 – 1.181)	0.294
Antenatal steroids	-0.034	0.967 (0.717 – 1.303)	0.825
Chorioamnionitis	0.605	1.832 (0.995 – 3.374)	0.052*
HIV	0.262	1.229 (0.981 – 1.721)	0.068*
Hypertension	-0.229	0.795 (0.566 – 1.117)	0.186
Syphilis	-0.082	0.921 (0.326 – 2.601)	0.877
Caesarian section	-0.241	0.785 (0.589 – 1.0468)	0.099*

Table 3.5 Multivariable logistic regression analysis of risk factors associated with NEC among VLBW neonates at Charlotte Maxeke Johannesburg Academic Hospital from 2013 -2018.

Risk Factors	Coefficient (β)	OR (95% CI)	p-value
Birth weight (in grams)	-0.0003	0.999 (0.999 – 1.001)	0.631
Gestational age (in weeks)	0.011	1.011 (0.922 – 1.109)	0.81
Maternal age (in years)	0.032	1.033 (1.005 – 1.062)	**0.021**
Conventional ventilation	1.566	4.787 (3.291 – 6.962)	**<0.001**
Oxygen on day 36	0.153	1.165 (1.019 – 1.333)	**0.026**
Patent ductus arteriosus	0.343	1.41 (0.945 – 2.101)	0.092*
Blood transfusion	0.507	1.66 (1.104 - 2.494)	**0.015**

CHAPTER 4.0: DISCUSSION

The focus of chapter one was to briefly introduce NEC and report findings on NEC from past studies. The purpose was to determine the factors associated with NEC and also evaluate the changes in the prevalence of NEC among VLBW neonates at CMJAH between 1 January 2013 and 31 December 2017. Furthermore the methods and the results were in chapter two and three of this research report respectively.

The intent of this chapter is to discuss findings, describe how they relate to existing knowledge and previous studies. It will also elaborate on the contributions that this study makes to the public health field.

4.1. Changes in the prevalence of NEC among VLBW neonates at CMJAH between 2013 - 2018.

From the findings in this study, NEC remains a major risk factor that contributes to the neonatal morbidity and mortality burden. Despite the surfactant era and introduction of modern facilities, there seems to be an increase in the prevalence of NEC among the VLBW neonates over the six year study period from 11% (2013) to 15.2% (2018). This means that, the more we have neonates who are delivered as VLBW, the more we expect cases of NEC.

Interesting findings were the similar trends of decrease in the prevalence of NEC in 2014 and 2016 by 6% from the previous years. A spike in the prevalence of NEC was also observed in 2017. Various methods of feeding, control of infection and various proportions of neonates who were VLBW admitted in the different years appear to be a cause for the variations in the incidence of NEC at CMJAH. Other reasons for these findings can be better understood with further research.

Some studies have suggested that the incidence of NEC ranges between 1% and 15% of NICU admissions(4,8). Going forward, this study has shown that the prevalence of NEC as at 2018 was 15.2% which was higher than three cohort studies carried out in Italy (3.1%), Australia (3.8%),

and the United States (2.6%) (44). Records from the Canadian Neonatal Network indicated that the incidence of NEC among Canadian infants with gestational age <33weeks is 5.1% (13,14). Recently, the incidence of NEC has been on the rise in Canada and the United Kingdom due to the increase in preterm births (13,14). However the rate of developing NEC differs across geographic and ethnic distribution, with fewer occurrences in Japan, Switzerland, Austria and a high rate of instance in Northern America, the UK, Ireland and Australia(9) (8,13,14).

More recent studies from the Pediatrix Neonatal Network showed that after the reorganization the NICUs and intensive health promotion, there was a decreased in the incidence of NEC from from 6.6% (2007) to 3.9% (2013) (8). This degree of prevalence (in South Africa) could be as a result of increasing patient numbers, overcrowding, lack of donor breast milk, increasing sepsis and antibiotic use. Several reports have suggested that using donor breast can potentially lead to a decrease in the incidence of NEC (22). Statistics on the prevalence of NEC in most emerging countries is still lacking.

4.2. Risk factors associated with NEC among VLBW neonates at CMJAH from 2013 - 2018

The odds of developing NEC among VLBW were found to reduce with increasing birth weight (P = 0.631) and gestational age (P = 0.81) in this study although not statistically significant. Whereas data from MNNR for 2017 identified decreasing birth weight as a significant risk factor for developing NEC (36,45). Multivariate analysis in this study showed that increasing maternal age was a significant risk factor for NEC which is consistent with another study (36). However, contrary to the finding in this study, reports from a case control study in the United States showed that women <19 years of age were associated with developing NEC among VLBW infants(45). Another study reported that certain risk factors were peculiar to certain birth weight(9).

With respect to the clinical factors, surfactant therapy in this study proved to be a positive predictor for NEC. Whereas, from a systematic review it was a positive predictor for NEC in one

study and a negative predictor in another study (36,46). An explanation that the oxygen perfusion of the gut is improved, hence preventing gut ischemia was given by the authors of the latter study. There was a significant association between respiratory distress syndrome and NEC in this study. This is contrary to the finding in a study where they reported that there is no etiological relationship between neonatal hypoxia and NEC (36,46). Notwithstanding, a US study proved that RDS and other respiratory problems had the highest percentage of diagnoses listed together with NEC (47).

The positive association that was shown between blood transfusion and NEC in this study is contrary to findings in other studies. It suggested that severe anemia and not red blood cell transfusion was associated with a higher risk of developing NEC (46,48). Another randomized control trial revealed that there was no effect of red blood cell transfusion on incidence of NEC among ELBW neonates (18). Furthermore, a case report and a retrospective chart review suggested that presence of gut injury leading to NEC in VLBW neonates is associated with the risk of developing NEC (19). However, another author suggested that there is a relationship between NEC and elective transfusion of packed red cells, in which there might be an association between transfusion and altered intestinal blood flow (2). Summary from a case report suggested that children who had NEC after being transfused with packed red cell possibly had a smaller birth weight and gestational age(49).

From previous studies, the association between patent ductus arteriosus as a risk factor and NEC has been inconsistently reported (18). However, contrary to the study carried out in Malaysian NICUs (36), patent ductus arteriosus was a significant independent risk factor associated with NEC in CMJAH. Additionally, reports from the Children‟s National Medical Centre in the US suggests an inverse relationship between NEC and PDA (41,50). Recently, the use of NCPAP to aid the respiration of VLBWs has been on the increase (41). Many observational studies have reported that instead of doing an initial intubation, early use of NCPAP can reduce the incidence of retinopathy of prematurity and chronic lung disease (41). However, some studies reported that there is an increased risk of NEC with NCPAP use, although randomized controlled trials are yet to be performed to confirm that finding (41).

As regards maternal factors, other maternal factors including hypertension, syphilis, antenatal care and use of antenatal steroids did not increase the risk of NEC among VLBW neonates in this study. Similarly, some studies did not find a difference between hypertensive disorders, diabetes mellitus, intrahepatic cholestasis, heart disease, hypothyroidism, PROM, placenta abruptio, use of antenatal steroids between NEC group and control group (51). In literature, there is some conflict about establishing an association between maternal hypertension and NEC (51). However, NEC was reported as an independent risk factor from a study that involved 211 low birth weight infant between 1995-1998(9). From this study caesarian section was found to be protective which is similar to results from a systematic review that was carried out (3). From the systematic review two studies had similar odds ratio of 0.6 (46). Their reason being that the neonates were subjected to fewer rigors during the process of delivery (46).

4.4. Strengths and limitations

The strengths of this study are the prominent clinical and radiological criteria that were used in diagnosing NEC (3) as well as having a large and balanced dataset which is likely to give accurate results. Nevertheless, selection of our participants has been influenced by some limitations. Some variables such as breastfeeding, enteral feeding, antepartum hemorrhage, premature rupture of membrane, sepsis as well as antibiotic use were not included in the neonatal database. We recommend that subsequent studies should include those variables. Another important limitation in this study is that the findings are limited to CMJAH and there are were no observations for long term outcomes (11). A cohort study would have given allowance timely follow up.

CHAPTER 5.0: CONCLUSION AND RECOMMENDATION

From this study we have been able to determine the prevalence of NEC and the significant factors associated with NEC in CMJAH. Due to the high fatality associated with NEC, novel treatment strategies are not likely to proffer lasting solutions to reduce the morbidity and deaths associated with it (2). Presently, the key modifiable risk factor that is associated with NEC development among the VLBW neonates is the preventive practice of enteral feeding (18). The incidence of NEC can be reduced by evaluating more effective strategies like introducing the use prebiotics and probiotics (18).

Lifestyle factors of the mothers (such as drinking alcohol and smoking) and intrapartum antibiotics could be important risk factors for developing NEC; hence studies should be conducted to explore this further. Inclusion of other factors like breast feeding and enteral feeding in future studies at CMJAH could assist clinicians in adopting better preventive strategies that will help to mitigate the incidence of NEC among VLBW neonates (36).

However, active surveillance of NEC and quality improvement projects will be of great benefit (19). It will help reduce the incidence of morbidity and mortality associated with NEC. More so, data from multi-centre studies should be incorporated in upcoming research (11).This will give a better and fairer assessment of the risk factors that are associated with NEC.

Furthermore, Some researchers have suggested that the use of amniotic fluid stem cells, growth factors, human milk oligosaccharides, and lactoferrin among VLBW infants have the potential of altering the trend of NEC(22).

REFERENCES

1. Rose AT, Patel RM. A critical analysis of risk factors for necrotizing enterocolitis. Semin Fetal Neonatal Med [Internet]. 2018;23(6):374–9. Available from: https://doi.org/10.1016/j.siny.2018.07.005
2. Puri P. Newborn surgery, Fourth edition. Newborn Surgery, Fourth Ed. 2017;364(3):1–1237.
3. R.C. H, B.J. S, A.T. C, K.L. Y, C.A. S, L.B. S. Necrotising enterocolitis hospitalisations among neonates in the United States. Paediatr Perinat Epidemiol [Internet]. 2006;20(6):498–506. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L44581549%0Ahttp://dx.doi.org/10.1111/j.1365-3016.2006.00756.x%0Ahttp://library.deakin.edu.au/resserv?sid=EMBASE&issn=02695022&id=doi:10.1111%2Fj.1365-3016.2006.00756.x&atitle=Necrot
4. Robertson NJ, Kuna J, Cox PM, Lakhoo K. Spontaneous intestinal perforation and Candida peritonitis presenting as extensive necrotizing enterocolitis. 2003;258–61.
5. Outcomes M. Development of a Gestational Age–Specific Case Definition for Neonatal Necrotizing Enterocolitis. 2017;
6. Berman L, Moss RL. Necrotizing enterocolitis: An update. Semin Fetal Neonatal Med [Internet]. 2011;16(3):145–50. Available from: http://dx.doi.org/10.1016/j.siny.2011.02.002
7. Choi YY. Necrotizing enterocolitis in newborns: Update in pathophysiology and newly emerging therapeutic strategies. Korean J Pediatr. 2014;57(12):505–13.
8. Robinson JR, Rellinger EJ, Hatch LD, Weitkamp H, Speck KE, Danko M, et al. HHS Public Access. 2018;41(1):70–9.
9. Ahle M, Andersson R, Drott P, Smedby Ö, Ringertz H, Wester T, et al. Necrotising Enterocolitis epidemiology and imaging [Internet]. 2017. 98 p. Available from: files/984/Ahle et al. - 2017 - Necrotising Enterocolitis epidemiology and imaging.pdf
10. Necrotizing enterocolitis in premature infants and newborns. J Neonatal Perinatal Med. 2016;9(3):233–42.
11. Lin H, Mao S, Shi L, Tou J, Du L. Clinical characteristic comparison of low birth weight

and very low birth weight preterm infants with neonatal necrotizing enterocolitis: a single tertiary center experience from eastern China. Pediatr Surg Int [Internet]. 2018;34(11):1201–7. Available from: http://dx.doi.org/10.1007/s00383-018-4339-9

12. Lin PW, Stoll BJ. Necrotising enterocolitis. Lancet. 2006;368(9543):1271–83.
13. Reed BD, Schibler KR, Deshmukh H, Ambalavanan N, Morrow AL. The Impact of Maternal Antibiotics on Neonatal Disease. J Pediatr [Internet]. 2018;197:97-103.e3. Available from: https://doi.org/10.1016/j.jpeds.2018.01.056
14. Zani A, Pierro A. Necrotizing enterocolitis : controversies and challenges [version 1 ; referees : 3 approved] Referee Status : 2016;4(0).
15. Rasiah V, Yajamanyam PK, Ewer AK. Necrotizing enterocolitis: current perspectives. Res Reports Neonatol. 2014;(March 2016):31.
16. Leong M. Necrotizing Enterocolitis: A 15-Year Experience. 1987;40–4.
17. Paridah M., Moradbak A, Mohamed A., Owolabi F abdulwahab taiwo, Asniza M, Abdul Khalid SH. We are IntechOpen , the world " s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. Intech. 2016;i(tourism):13.
18. McGuire W, Young L, Morgan J. Preventing necrotising enterocolitis in very preterm infants: Current evidence. Paediatr Child Heal (United Kingdom) [Internet]. 2015;25(6):265–70. Available from: http://dx.doi.org/10.1016/j.paed.2015.02.007
19. Huda S, Chaudhery S, Ibrahim H, Pramanik A. Neonatal necrotizing enterocolitis: Clinical challenges, pathophysiology and management. Pathophysiology [Internet]. 2014;21(1):3–12. Available from: http://dx.doi.org/10.1016/j.pathophys.2013.11.009
20. Rolnitsky A, Ng E, Asztalos E, Shama Y, Karol D, Findlater C, et al. A Quality Improvement Intervention to Reduce Necrotizing Enterocolitis in premature infants with Probiotic Supplementation. Pediatr Qual Saf. 2019;4(5):e201.
21. Matson A. Synonyms of Necrotizing Enterocolitis Signs & Symptoms. 1–6 p.
22. Zubarioglu U, Uslu S, Bulbul A. New Frontiers of Necrotizing Enterocolitis : From Pathophysiology to Treatment. 2017;106–23.
23. Sharma R, Hudak ML. A Clinical Perspective of Necrotizing Enterocolitis: Past, Present, and Future. Clin Perinatol [Internet]. 2013;40(1):27–51. Available from: http://dx.doi.org/10.1016/j.clp.2012.12.012
24. Alsaied A, Islam N, Thalib L. Global incidence of Necrotizing Enterocolitis: A systematic

review and Meta-analysis. BMC Pediatr. 2020;20(1):1–15.

25. Ballot DE, Davies VA, Cooper PA, Chirwa T, Argent A, Mer M. Retrospective cross-sectional review of survival rates in critically ill children admitted to a combined paediatric/neonatal intensive care unit in Johannesburg, South Africa, 2013-2015. BMJ Open. 2016;6(6):1–7.
26. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, et al. Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol. 2007;196(2):147.e1-147.e8.
27. Clark RH, Gordon P, Walker WM, Laughon M, Smith PB, Spitzer AR. Characteristics of patients who die of necrotizing enterocolitis. J Perinatol [Internet]. 2012;32(3):199–204. Available from: http://dx.doi.org/10.1038/jp.2011.65
28. Moss ME, Kendig JW, Llanos AR, Pinzòn MC, Dye T, Sinkin RA. Epidemiology of neonatal necrotising enterocolitis: a population-based study. Paediatr Perinat Epidemiol [Internet]. 2002;16(4):342–9. Available from: http://www.mendeley.com/c/6678096921/p/122340771/llanos-2002-epidemiology-of-neonatal-necrotising-enterocolitis--a-population--based-study/
29. Ahle M, Drott P, Elfvin A, Andersson RE. Maternal, fetal and perinatal factors associated with necrotizing enterocolitis in Sweden. A national case-control study. PLoS One. 2018;13(3):1–13.
30. Mcelhinney DB, Hedrick HL, Bush DM, Pereira GR, Stafford PW, Gaynor JW, et al. Necrotizing Enterocolitis in Neonates With Congenital Heart Disease : Risk Factors and Outcomes. 2019;D(5).
31. Stafford IA, Rodrigue E, Berra A, Adams W, Heard AJ, Hagan JL, et al. European Journal of Obstetrics & Gynecology and Reproductive Biology The strong correlation between neonatal early-onset Group B Streptococcal disease and necrotizing enterocolitis $. Eur J Obstet Gynecol [Internet]. 2018;223:93–7. Available from: http://dx.doi.org/10.1016/j.ejogrb.2018.02.024
32. Duci M, Frigo AC, Visentin S, Verlato G, Gamba P, Fascetti-Leon F. Maternal and placental risk factors associated with the development of necrotizing enterocolitis (NEC) and its severity. J Pediatr Surg [Internet]. 2019;54(10):2099–102. Available from: https://doi.org/10.1016/j.jpedsurg.2019.04.018

33. Uauy RD, Fanaroff AA, Korones SB, Phillips EA, Phillips JB, Wright LL. Necrotizing enterocolitis in very low birth weight infants: Biodemographic and clinical correlates. J Pediatr. 1991;119(4):630–8.
34. Coetzee M, Delport SD. Peripartum HIV infection in very low birth weight infants fed „raw" mother"s own milk. South Afr J HIV Med. 2019;20(1):1–7.
35. Van Niekerk E, Autran CA, Nel DG, Kirsten GF, Blaauw R, Bode L. Human Milk Oligosaccharides Differ between HIV-Infected and HIV-Uninfected Mothers and Are Related to Necrotizing Enterocolitis Incidence in Their Preterm Very-Low-Birth-Weight Infants. J Nutr. 2014;144(8):1227–33.
36. Boo NY, Cheah IGS. Risk factors associated with necrotising enterocolitis in very low birth weight infants in Malaysian neonatal intensive care units. Singapore Med J. 2012;53(12):826–31.
37. Guthrie SO, Gordon P V., Thomas V, Thorp JA, Peabody J, Clark RH. Necrotizing enterocolitis among neonates in the United States. J Perinatol. 2003;23(4):278–85.
38. NECROTISING ENTEROCOLITIS IN PREMATURE INFANTS AT CHRIS HANI BARAGWANATH HOSPITAL . WHAT ARE THE ASSOCIATED FACTORS ? Peter Angura A research report submitted to the Faculty of Health Sciences , University of the Witwatersrand , Johannesburg , in partial . 2013;(January).
39. Clark DA, Munshi UK. Feeding associated neonatal necrotizing enterocolitis (Primary NEC) is an inflammatory bowel disease. Pathophysiology [Internet]. 2014;21(1):29–34. Available from: http://dx.doi.org/10.1016/j.pathophys.2013.11.006
40. Manja V, Lakshminrusimha S, Cook DJ. Oxygen saturation targetrange for extremely preterm infants: A systematic review and meta-analysis. JAMA Pediatr. 2015;169(4):332–40.
41. Aly H, Massaro AN, Hammad TA, Narang S, Essers J. Early nasal continuous positive airway pressure and necrotizing enterocolitis in preterm infants. Pediatrics. 2009;124(1):205–10.
42. Chirdan LB, Ngiloi PJ, Elhalaby EA. Neonatal surgery in Africa. Semin Pediatr Surg [Internet]. 2012;21(2):151–9. Available from: http://dx.doi.org/10.1053/j.sempedsurg.2012.01.007
43. Harris PA, Ph D, Taylor R, Thielke R, Ph D, Payne J, et al. NIH Public Access.

2010;42(2):377–81.

44. Boo NY, Cheah IGS. Risk factors associated with necrotising enterocolitis in very low birth weight infants in Malaysian neonatal intensive care units. Singapore Med J. 2012 Dec;53(12):826–31.

45. Seeman SM, Mehal JM, Haberling DL, Holman RC, Stoll BJ. Infant and maternal risk factors related to necrotising enterocolitis- associated infant death in the United States. 2016;240–6.

46. Samuels N, van de Graaf RA, de Jonge RCJ, Reiss IKM, Vermeulen MJ. Risk factors for necrotizing enterocolitis in neonates: A systematic review of prognostic studies. BMC Pediatr. 2017;17(1):1–9.

47. Holman RC, Stoll BJ, Curns AT, Yorita KL, Steiner CA, Schonberger LB. Necrotising enterocolitis hospitalisations among neonates in the United States. Paediatr Perinat Epidemiol. 2006;20(6):498–506.

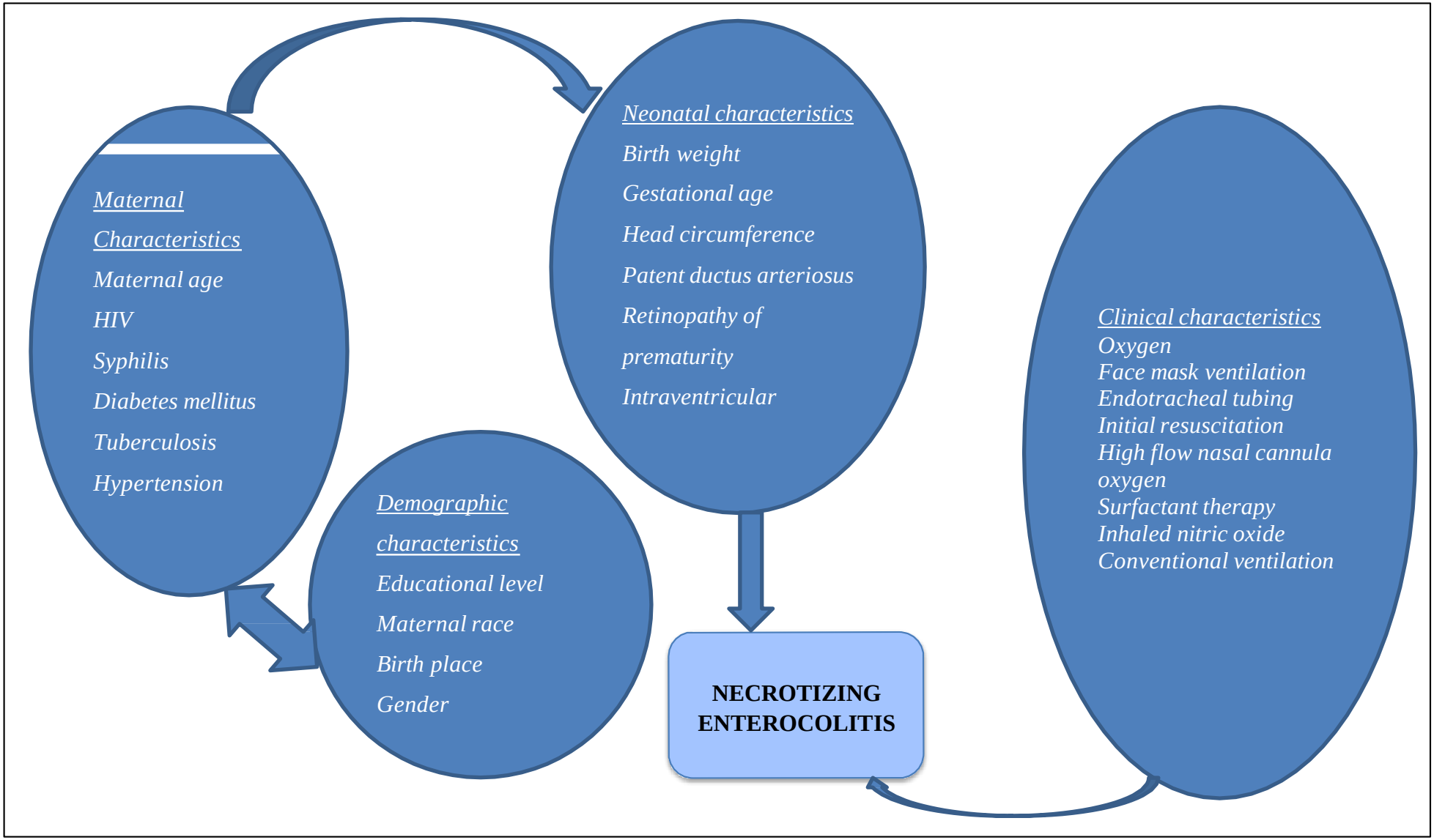
48. Patel RM, Knezevic A, Shenvi N, Hinkes M, Keene S, Roback JD, et al. Association of Red Blood Cell Transfusion, Anemia, and Necrotizing Enterocolitis in Very Low-Birth-Weight Infants. 2016;30322(9):889–97.

49. Sellmer A, Tauris LH, Johansen A, Henriksen TB. Necrotizing enterocolitis after red blood cell transfusion in preterm infants with patent ductus arteriosus : a case series. 2012;570–2.

50. Leung MP, Chau K, Hui P. Necrotizing enterocolitis in neonates with symptomatic congenital heart disease. 1988;(December):1988.

51. Irles C, González-Pérez G, Muiños SC, Macias CM, Gómez CS, Martínez-Zepeda A, et al. Estimation of neonatal intestinal perforation associated with necrotizing enterocolitis by machine learning reveals new key factors. Int J Environ Res Public Health. 2018;15(11).

CONCEPTUAL FRAMEWORK- showing the factors associated with Necrotizing enterocolitis



APPENDICES

Appendix 1- Descriptive statistics of Neonatal characteristics

Variable	*Type of Variable*	*Labels (Values)*
Gender	***Categorical***	***Female = 0 : male = 1***
Birth weight (in grams)	***Continuous***	
Gestational age (in weeks)	***Continuous***	
Apgar score (after 5mins)	***Categorical***	***No = 0 : Yes = 1***
Face mask ventilation	***Categorical***	***No = 0 : Yes = 1***
Temperature (in degree celsius)	***Categorical***	***No = 0 : Yes = 1***
Oxygen on day 28	***Categorical***	***No = 0 : Yes = 1***
Nasal continuous positive airway pressure (nasal CPAP)	***Categorical***	***No = 0 : Yes = 1***
Conventional ventilation	***Categorical***	***No = 0 : Yes = 1***
Respiratory support at 36weeks (oxygen)	***Categorical***	***No = 0 : Yes = 1***
Respiratory distress syndrome	***Categorical***	***No = 0 : Yes = 1***
Patent ductus arteriosus	***Categorical***	***No = 0 : Yes = 1***

Blood transfusion	***Categorical***	***No = 0 : Yes = 1***
Retinopathy of prematurity	***Categorical***	***No = 0 : Yes = 1***

Appendix 2- Descriptive statistics of maternal characteristics

Maternal Characteristics	**Type of variable**	**labels (values)**
Maternal age (in years)	Continuous	
Antenatal care	Categorical	N0 =0 : Yes = 1
Antenatal steroids	Categorical	N0 =0 : Yes = 1
Chorioamnionitis	Categorical	N0 =0 : Yes = 1
Hypertension	Categorical	N0 =0 : Yes = 1
HIV	Categorical	N0 =0 : Yes = 1
Syphilis	Categorical	N0 =0 : Yes = 1
Diabetes	Categorical	N0 =0 : Yes = 1
Tuberculosis	Categorical	N0 =0 : Yes = 1

Caesarian section	Categorical	N0 =0 : Yes = 1

Appendix 3

Clinical and Demographic Characteristics among VLBW Neonates at Charlotte Maxeke Johannesrburg Academic Hospital between 2013 -2018.

Characteristics	**2013 (N=) (%)**	**2014 (N=) (%)**	**2015 (N=) (%)**	**2016 (N=) (%)**	**2017 (N=) (%)**	**2018 (N=) (%)**
Gender						
Birth weight(in grams)						
Gestational age (in weeks)						
Apgar score (after 5mins)						
Face mask ventilation						
Temperature (in degrees celsius)						
Oxygen on day 28						
Nasal continuous positive airway pressure						

(nasal CPAP)						
Conventional ventilation						
Necrotizing Enterocolitis						
Respiratory distress syndrome						
Patent ductus arteriosus						
Blood transfusion						
Retinopathy of prematurity						
Major birth defect						

Appendix 4

Socio-demographic characteristics of the VLBW neonates

Characteristics	**Frequency**	**Percent**	**Mean +/- SD**

Sex	Male Female Total			
Gestational Age	<28weeks >28weeks			
Birth weight	<1000g >1000g			
Mode of delivery	Vaginal delivery Assisted Vaginal delivery Caesarian section			

Appendix 5

Factors associated with NEC among VLBW neonates at CMJAH.

Characteristics	**Odds ratio**	**95% CI**	**P-value**
Birth weight			
Gestational age in weeks			

Gender

Males

Apgar score(after 5mins)

>5

Mode of delivery

Caesarian

Blood transfusion

Yes

Conventional Ventilation

Yes

Retinopathy of Prematurity

Yes

Oxygen

Yes

Antenatal care

Yes

Maternal age

Maternal syphilis

Yes

Appendix 6

Bivariate and multivariable analysis of factors associated with NEC among neonates born and admitted to CMJAH.

		NEC(yes)	NEC(no)	COR (CI, 95)	AOR (CI, 95)	P-value
		No (%)	No (%)			
Educational level	Primary					
	Secondary					
HIV	Yes					
	No					
Syphilis	Yes					
	No					
Diabetes mellitus	Yes					
	No					
Tuberculosis	Yes					
	No					
Hypertension	Yes					
	No					
Chorioamnionitis	Yes					
	No					
Conventional Ventilation	Yes					
	No					
Retinopathy of Prematurity	Yes					
	No					

Oxygen	Yes					
	No					
Antenatal care	Yes					
	No					
Face mask ventilation	Yes					
	No					
Endotracheal tubing	Yes					
	No					
Initial resuscitation	Yes					
	No					
High flow nasal cannula oxygen	Yes					
	No					
Surfactant therapy	Yes					
	No					

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

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FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2019/11/20

REF: *R14/49*

PROTOCOL NO: **M191062** *(*This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study*)*

PROJECT TITLE: *Factors associated with necrotizing enterocolitis in a public hospital in Johannesburg, South Africa, 2013-2018: a cross-sectional study*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr OM Ajayi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M191062

NAME: (Principal Investigator)	Dr OM Ajayi
DEPARTMENT:	School of Public Health Division of Epidemiology and Biostatistics Medical School University
PROJECT TITLE:	Factors associated with necrotizing enterocolitis in a public hospital in Johannesburg, South Africa, 2013-2018: a cross-sectional study
DATE CONSIDERED:	2019/10/25
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor D Ballot
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2019/11/20

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore reports and re-certification will be due early in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

2019/11/20
Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES