NECROTISING ENTEROCOLITIS IN PREMATURE INFANTS AT CHRIS HANI BARAGWANATH HOSPITAL. WHAT ARE THE ASSOCIATED FACTORS?

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DECLARATION

I, Peter Angura declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in Epidemiology and Biostatistics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

30th day of January 2013

Dedicated to my parents,

Kevina Akello and John Angura

ABSTRACT

Introduction

Previous studies have found prematurity to be consistently associated with necrotising enterocolitis (NEC). At Chris Hani Baragwanath Academic Hospital (CHBAH) in 1994, prematurity, antepartum haemorrhage and positive blood culture at birth were associated with NEC. This study looked at the maternal and neonatal factors that are associated with NEC at CHBAH during the era of human immunodeficiency virus (HIV) infection.

Objectives

The primary objective was to determine maternal and infant characteristics or factors associated with NEC. The second objective was to determine factors associated with mortality and severe disease in infants with NEC.

Methods

This was a retrospective case control study.

Study population

A total of 110 infants of gestational age <37 weeks and birth weight <2500g, diagnosed with definite NEC between January 2005 and December 2008, were matched by birth weight, gestational and postnatal age with 220 infants who did not develop NEC. Overall, a total of 330 infants were studied.

Data collection

Data on maternal and infant characteristics, staging of NEC and outcome was collected from the mothers' and infants' medical records and analysed using STATA 8 statistical programs.

Results

Lack of maternal antenatal steroids (OR 2.35, 95% CI 1.25-4.42, p= 0.008), chorioamnionitis (OR 8.73, 95% CI 2.59-29.5, p< 0.0001), Apgar score <4 at 1st minute (OR 2.92, 95% CI 1.05-8.01, p= 0.038) and not mechanically ventilated at birth (OR 3.20, 95% CI 1.18-8.69, p< 0.023) were associated with necrotising enterocolitis. Mortality was higher in infants diagnosed with necrotising enterocolitis compared to that of infants without NEC (OR 4.95, CI 2,24-10.95, p< 0.001). Mortality increased progressively with increasing severity of the disease. Lack of antenatal care was associated with severe NEC (OR 4.31, 95% C1 1.46-12.73, p= 0.008) and mortality (OR 4.75, 95% CI 1.65-13.67, p= 0.004). Maternal HIV status was not associated with NEC or mortality.

Conclusions

Antenatal steroids have a protective effect against NEC. Infants who develop NEC are less likely to have received mechanical ventilation at birth, suggesting that they were less sick. Neonates with severe disease have a high mortality rate and likely to be born to mothers who had no antenatal care.

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LIST OF ABBREVIATIONS

ANC- antenatal care

ANS- antenatal steroids

APH- antepartum haemorrhage

CHBAH- Chris Hani Baragwanath Academic Hospital

CI- confidence interval

ELBW- extremely low birth weight

eNOS- endothelial nitric oxide synthase

ET- endothelin

GALT- gut associated lymphoid tissue

HIV- human immunodeficiency virus

IgA- immunoglobulin A

IL- interleukin

LBW- low birth weight

LPS- lipopolysaccharide

mRNA- messenger ribonucleic acid

NEC- necrotising enterocolitis

NO- nitric oxide

OR- odds ratio

PAF- platelet activating factor

PAMP- pathogen associated molecular pattern

PDA- patent ductus arteriosus

PIH- pregnancy induced hypertension

PROM- premature rupture of membranes

RR- relative risk

SGA- small for gestation

TLR- toll-like receptor

TNF- tumour necrosis factor

USA- United States of America

VLBW- very low birth weight

CHAPTER 1

1.0 INTRODUCTION

Necrotising enterocolitis is an acquired inflammatory disease of the intestine that causes necrosis and gangrene of the intestinal tissues. It is the leading cause of death from gastrointestinal disease in preterm neonates. The incidence ranges between 0.9 and 2.4 per 1000 live births. It occurs in approximately 1-5% of all admissions to the neonatal intensive care unit and in approximately 5-10% of all very low birth weight neonates.[1-3]

Prior to modern neonatal intensive care before the 1950s, NEC was not recognized as a disease that affected primarily premature infants, most likely because these infants were not surviving then. With advances in perinatal and neonatal care in the 1970s and 1980s, survival in preterm infants improved significantly.[1] For example, in 1997, the Victorian Infant Collaborative Study Group[4] (Australia) reported survival rates in extremely low birth weight (ELBW) infants of 25.4%, 37.9% and 56.2% during the periods of 1979-80, 1985-87 and 1991-92 respectively. The odds of survival increased as the era advanced (OR 1.76, 95% CI 1.32-2.33 and OR 2.09, 95% CI 1.62-2.69, between the era 1985-87 versus 1979-80 and 1991-92 versus 1985-87 respectively). Between 1986 and 1999, Darlow et al.[5] reported an increase in survival to discharge home from 81.8% to 90.3% ($p< 0.001$) in very low birth weight (VLBW) infants in neonatal intensive care units in New Zealand.

In less developed countries, a similar trend of declining mortality and improved survival was reported. In 2003, Bae and Bae[6] (North Korea) reported a progressive decline in mortality of VLBW infants over a period of 40 years: 66.2%, 56.8%, 50.8%, 32.9% and 11.7% in 1960s, 1970s, 1980s, 1990s and early 2000 respectively. In South Africa, Cooper et al.[7] reported a significant increase in survival from 64% to 79% between the years 1981-

82 and 1995-96 for infants with birth weight 1000-1499 g ($p< 0.01$), and from 14% to 32% for those with birth weight less than 1000 g ($p= 0.027$).

As survival increased, the incidence of NEC increased in most neonatal centers.

Incidences of 2.1 and 3.5 per 1000 live births were reported at CHBAH neonatal unit during the periods of 1988 and 1994 respectively.[8,9] In Melbourne Australia, incidence increased from 0.07% to 0.25% of all live births during the periods of 1971-1974 to 1984-1985 respectively.[10] Llanos et al.[11] reported an increase in incidence of NEC from 0.65 to 1.03 per 1000 live births between 1991 and 1997 in USA.

Several studies have found prematurity to be consistently associated with NEC. Other factors that have been studied are formula feeds, low birth weight, low five minute Apgar score, hyaline membrane disease (HMD), exogenous surfactant, small for gestation, patent ductus arteriosus (PDA) needing treatment, assisted ventilation, younger maternal age, antepartum haemorrhage, pregnancy induced hypertension and caesarean section.[9, 12,13]

At CHBAH in 1994, Velaphi[9] found prematurity, antepartum haemorrhage and positive blood culture at birth were associated with NEC. Desfrere et al.[14] suggested that HIV infection in mothers might be a risk for NEC in preterm infants. In South Africa, about 30% of pregnant mothers attending antenatal care are HIV positive,[15] therefore a large number of babies will be exposed to the virus. The study looked at the maternal and neonatal factors that are associated with NEC at CHBAH during the era of HIV infection.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pathophysiology of NEC

Necrotising enterocolitis classically affects the terminal ileum, although any segment of the gastrointestinal tract can be involved including the stomach. The histopathologic hallmark is coagulation and ischemic necrosis, acute and chronic inflammation, bacterial overgrowth and tissue repair suggesting that NEC is an evolving process rather than an acute event. In more advanced stages, pathological findings include gastrointestinal bleeding, intestinal distension with multiple dilated loops of small bowel, pneumatosis intestinalis and portal air, intestinal perforation, pneumoperitoneum and intra-abdominal fluid.[16,17]

Ballance et al.[18] reviewed pathology specimens from a cohort of 84 neonatal NEC patients and reported ischemic necrosis, inflammation, and bacterial overgrowth to be the most consistent features. Similar finding was made by Chan et al.,[19] who reported severe coagulative necrosis and extensive macrophage infiltration in intestinal tissue from acute-phase NEC.

The mechanisms implicated in the development of NEC include developmental immaturity of the gastrointestinal motility, digestive ability, circulatory regulation, intestinal barrier function and immune defense.[20,21]

2.1.1 Immature intestinal motility and digestion

Sase et al.[22] found that human fetal gastric emptying cycles are developmentally regulated, beginning by 12 weeks gestation and normalise during the third trimester. The proportion of foetuses demonstrating gastric emptying >10th percentile increased with gestational age.

Gastrointestinal migrating motor complexes appear as waves along the intestine by about 34 weeks gestation and are fully developed during the later part of the third trimester. Their action produces gastric emptying and coordinated peristaltic activity of the intestine, propelling luminal contents caudally along the length of the small intestine. Peristalsis reduces intestinal transit time thus prevents overgrowth of anaerobic bacteria in the small intestine, limits the amount of time during which antigens are able to interact with the apical surfaces of the enterocytes and speeds the process by which antigen-antibody complexes are eliminated. In the infants born preterm, the under-developed gastric emptying and peristalsis, results in noxious substances and antigen-antibody complexes accumulating in the intestines causing intestinal mucosal injury and breakdown in the gut barrier. Consequently, intestinal bacteria translocate and stimulate cells of the innate immune system, triggering systemic inflammatory response and damage to the intestine as seen in clinical NEC.[20,21]

Preterm infants also have low gastric and pancreatico-biliary secretions. Kelly et al.[23] showed that as infants became more mature, both in terms of gestation and postnatal age, there was a decrease in intra-gastric pH. Lebenthal and Lee[24] described that the function of the exocrine pancreas is limited in infants and the insufficiency may last until age of two years. Therefore, nutrients and other macromolecules, which are incompletely digested, interact with immature host defenses and other factors and may initiate an inflammatory cascade leading to NEC.[21]

2.1.2 Immature regulation of intestinal circulation

Nitric oxide (NO), a vasodilator stimulus and endothelin-1 (ET-1) a vasoconstrictor stimulus, dominate control of vascular resistance within the newborn intestine. Although constitutively produced, ET-1 production responds to a wide range of stimuli, including

rate of blood flow, hypoxia and inflammatory cytokines. NO production is determined by the level of expression of endothelial nitric oxide synthase (eNOS). Endothelial NOS is responsive to blood flow rate, hypoxia and nuclear transcription factors such as nuclear factor kappa B (NF-κB). Endothelial NOS expression is developmentally regulated and found in all tissues.[25] Rossmanith et al.[26] found eNOS mRNA was expressed nearly 30 times in placental tissue at third compared to first trimester (mean ratio 1.3 ± 1.2 versus 0.045 ± 0.02 respectively, $p < 0.05$).

The low vascular resistance characteristic of newborn intestinal circulation reflects a balance of NO and ET-1 that favours vasodilatation. In a preterm infant however, because of deficiency in eNOS, exposure to factors such as hypoxia, inflammatory cytokines and low blood flow rate will result in production of more ET-1 than NO, altering the balance to favour vasoconstriction and ischaemia. Ischaemic injury to the gastrointestinal tract causes disruption to the epithelial integrity and sets up a cascade of inflammation, ischaemia and necrosis resulting into necrotising enterocolitis.[25]

2.1.3 Immature intestinal barrier function

The intestinal epithelial barrier function consists of structural component (tight junctional complexes and goblet cells), biochemical component (paneth cells) and gut associated lymphoid tissues (GALT).

The intestinal epithelial barrier, through the tight junctional complex, allows selective permeability to small ions, absorption of nutrients, and control of bi-directional fluid flow and flushing of the unwanted pathogens or toxins from the intestinal lumen. The intestinal mucosa is protected by a thick layer of mucus gel (produced by goblet cells), which hampers direct microbial epithelial binding, aggregates adherent bacteria and dietary antigens and enhances their removal.

Biochemical substances (lysozymes, phospholipase A_2) and the small antimicrobial peptides (α and β-defensin) produced by Paneth's and epithelial cells, have bioactivity against a wide range of microbes including: bacteria, viruses, fungi, protozoa and spirochaetes and regulate the composition and distribution of microbial populations.[3,20,21]

The GALT consist of mucosal and sub mucosal Peyer's patches and lymphoid tissues that is comprised predominantly of B-lymphocytes and some T-lymphocytes. M cells, local dendritic cells and macrophages transport a variety of protein and peptide antigens from the lumen and present them to the under lying GALT. Subsequently the B cells are signalled to produce secretory IgA, which is then transported through the epithelial cells into the lumen while the T cells are signalled to migrate into lamina propria and the epithelium where they mature to cytotoxic T cells. The secretory IgA interferes with adhesion and invasion of bacteria, which provide another mechanism for containing microbial assault.[27]

Studies have shown that each component of the intestinal barrier function develops with increasing gestation.[28-31] Preterm infants are therefore vulnerable to invasion by pathogens because of immature epithelial barrier function.

2.1.4 Immature intestinal innate immunity

The intestinal epithelial cells have over 10 pattern recognition receptors (PRRs), called toll-like receptors (TLRs), which play a key role in the maintenance of intestinal innate immunity. TLR-2 and TLR-4 are expressed on the surface of crypt enterocytes and also to a lesser degree, on the surface of microvillous and villous epithelium. Bacterial pathogen-associated molecular pattern (PAMPs) have specific regions (such as lipopolysaccharide (LPS) of bacterial cell wall) by which it binds to and activates a corresponding TLRs pathway, which in turn activates nuclear factor kappa B (NF- κB). When activated, NF- κB

translocates into the cell's nucleus and switches on the genes responsible for expression of pro-inflammatory mediators (cytokines) such as platelet activating factor (PAF), tumor necrosis factor-α (TNF-α), interleukin-1, 6, 8, 12, 18 (IL-1, IL-6, IL-8, IL-12, IL-18) and anti-inflammatory mediators such as PAF-acetylhydrolase (PAF-AH), IL-10, IL-11 and growth factors. Subsequently, the action of the pro-inflammatory cytokines causes up-regulation of metalloproteinases (MMPs; zinc dependant endopeptidases which degrades extracellular matrix), infiltration and activation of inflammatory cells such as macrophages, neutrophils, monocytes and release by the inflammatory cells of cytopathic oxidants, proteases and associated cytokines. A cascade of inflammation, cell death, tissue degradation and necrosis is therefore set. Concurrently, up-regulation of anti-inflamatory cytokines and release of TIMP-1 (natural tissue inhibitor of metalloproteinases which inhibits MMPs) occurs and down-regulates inflammatory cascade.

In a preterm immature intestine, the TLRs pathway is excessively activated by specific PAMPs or stress stimuli while the TLR-negative signal intermediates that inhibit inflammation are downgraded. This results into excessive pro-inflammatory response.[21,27,32] Nanthakumar et al.[33] demonstrated higher mRNA levels of TLR-2, TLR-4 and NF-κB in immature versus mature human foetal intestines ($p< 0.01$ each). They also found higher levels of IL-8 mRNA in preterm versus mature human foetal intestines, $p< 0.01$. Negative regulators such as single immunoglobulin IL-1-related receptor (SIGIRR) and toll interacting protein (TOLLIP), were reduced by 80% and 90% respectively in foetal versus older child intestine ($p< 0.01$ each), and were further decreased by approximately 95% in NEC versus mature intestine ($p< 0.001$ each). Soliman et al.[34] demonstrated a dose and time dependant increase of TLR-4 mRNA expression in human intestinal epithelial cells (IEC) treated with PAF versus the untreated controls ($p< 0.01$). They also demonstrated that human IEC secreted more IL-8 when treated (primed) with PAF and

then exposed to LPS, compared to when the cells are unexposed to PAF, or exposed to either PAF or LPS alone ($p < 0.05$).

Increased recognition of TLR-4 ligands at the intestinal epithelial surface after treatment with PAF and subsequently increased production of inflammatory cytokines, demonstrates the role PAF plays in the inflammatory process seen in NEC.

2.1.5 Impaired healing of intestinal epithelium

The epidermal growth factor (EGF) and intestinal trefoil factor (ITF) are expressed within the intestine and are produced in salivary glands, duodenum and in exocrine pancreas. These factors cause intestinal epithelium healing by enhancing migration and proliferation of enterocytes, thereby reducing the severity of intestinal injury caused in NEC. Levels of EGF and ITF are low in infants with NEC. [35,36] The other factors that contribute to the impaired intestinal healing in NEC include high levels of cytotoxic NO (produced as a result of increased expression of inducible NOS (iNOS) during inflammation) and the inhibitory effect of LPS on restitution and proliferation of the enterocytes and its pro-inflammatory effect on the TLR pathway.[35-37]

2.1.6 Genetic susceptibility

Treszl et al.[38] found that VLBW infants with NEC were less likely to possess the IL-4 receptor α-chain mutant allele ($A^{1902}G$) compared to infants without NEC, suggesting a genetic susceptibility for reduced IL-4 mediated modulation of intestinal injury. IL-4 regulates T-helper (Th) cell differentiation and favours production of Th2 over Th1 thereby reducing the Th1 mediated intestinal injury seen in inflammatory bowel disease.[32]

The risk of NEC has also been associated with the frequency of the IL-18^{607} AA genotype. The frequency of the AA genotype is significantly higher in infants with stage 3 NEC

compared to stages 1 and 2. Thus, the presence of the AA genotype may adversely affect the outcome of NEC through altered IL-18 levels. IL-18 induces interferon-γ (IFN-γ) and amplifies Th1 cytokine production and IL-8 accumulation.[39]

Nadler et al.[40] found IL-8 and IL-11 mRNA were up regulated in patients with acute NEC compared with those with other inflammatory conditions or those without disease. These levels returned to baseline at the time of stoma closure. Increased level of IL-11 mRNA decreased the likelihood of pan-necrosis (OR 0.93, p= 0.002) and increased IL-12 levels showed a protective trend against pan-necrosis (OR 0.70, p= 0.06). Henderson et al.[41] studied variant alleles for the following cytokines: TNF, IL-1β, IL-4 receptor-α, IL-6, IL-8 and IL-18. They found no association between cytokine genetic polymorphism and NEC. They suggested that lack of association did not rule out the possibility that cytokines were important in the pathogenesis of NEC in preterm infants. True associations may exist but with very modest effect sizes too small to be excluded by the available data.

2.2 Risk factors for NEC

A number of risk factors have been implicated in the development of NEC. They include prematurity, hypoxic-ischaemic injury to the intestine, enteral feeding and alteration in the normal microbiological flora of the intestine[2].

2.2.1 Prematurity

Over 90% of cases occur in premature infants, making prematurity the single most independent risk factor for NEC.[1,20,21] Llanos et al.[11] found 93% of NEC occurred in infants born at <37 weeks gestation. Hallstrom et al.[12] reported infants who developed NEC had smaller mean gestational age (in weeks) than the controls (26 ± 2.0 versus 29 ± 2.6 respectively, p< 0.001). Luig et al.[13] demonstrated a declining risk of NEC with increasing gestational age (8%, 6%, 4%, and 2% in gestational age group 24-25, 26-27, 28-

29 and 30-31 weeks respectively, p <0.001). They also showed that the odds (calculated for each increase of one week gestational age) of developing NEC declined by 1.2 times when gestational age increases by one week (OR 0.82, CI 0.77-0.89, p< 0.001). Fitzgibbons et al.[42] found an inverse relation between risk of NEC and birth weight.

These studies consistently showed that the risk of developing NEC is inversely related to birth weight and gestational age of the infant.

In a premature infant, factors such as formula feeding, abnormal bacterial colonisation and hypoxic-ischaemic injury, abnormally activates TLRs pathway and triggers inflammatory cascade more readily.

2.2.2 Hypoxic-Ischaemic Injury

Any process that results in hypoperfusion and subsequent hypoxia (such as patent ductus arteriosus (PDA), treatment with indomethacin, severe pre-eclampsia), may disrupts endothelial function in a manner that alters the ET-1 and NO balance, and would favour vasoconstriction and ischaemic injury. Ischaemic injury to the gastrointestinal tract cause disruption to the epithelial integrity and set up a cascade of inflammation, ischaemia and necrosis.[16]

2.2.2.1 Patent Ductus Arteriosus

The presence of a PDA with significant left to right shunt has been considered a risk factor for the development of necrotising enterocolitis. The proposed mechanism is the ‘‘diastolic steal’’ whereby there is a decrease in blood flow from the aorta to the mesenteric arteries during diastole, creating an intestinal hypoperfusion state, thus compromising the gut blood flow.[43]

Mossali et al.,[44] in a systematic review and meta-analysis of randomised control trial, reported that ELBW infants with PDA who had no ligation, were four times at risk for

developing NEC compared to those who had PDA ligation (RR 0.25, 95% CI 0.08-0.83, p= 0.02). Their finding was however in contrast to that of Brooks et al.,[45] who observed no difference in the odds of NEC between three groups of infants ≤28 weeks gestation. The infants were: those diagnosed without or with insignificant PDA (control), compared to those diagnosed with significant PDA which closed after medical treatment or to those diagnosed with significant PDA which failed to close after medical treatment (OR 1.1, CI 0.31-3.94, p= 0.888 and OR 1.57, CI 0.36-6.83, p= 0.546, respectively).

2.2.2.2 Indomethacin and NEC

Indomethacin competitively inhibits both cyclooxygenase (COX-1 and COX-2) iso-enzymes. These enzymes catalyze the conversion of arachidonic acid to prostaglandins. Prostaglandins are vasodilators and regulate the intestinal epithelial tight junction assembly. Therefore indomethacin, an anti-prostaglandin, causes vasoconstriction and disrupts the intestinal epithelial barrier, consequently increases the risk for development of NEC in exposed infants.[36,46]

Amin et al.[47] performed a meta-analysis of data from observational studies and found that preterm neonates <37 weeks gestation who were exposed to recent (within 48 hours to birth) antenatal indomethacin had more than twice the odds of developing NEC compared to those who were not exposed (OR 2.23, 95% CI 1.16-4.25). Conversely however, some investigators observed no increased risk of developing NEC in infants who were post-natally exposed to indomethacin compared to those who were not exposed.[48,49]

O'Donovan et al.[48] showed that NEC occurred equally in preterm VLBW infants (gestational age 23-32 weeks and birth weight 320-1450g) with PDA and treated with indomethacin, or indomethacin and ligation, or ligation alone (13.0%, 12.0% and 14.0%, respectively). Similarly, Madan et al.[49]observed that definite NEC developed equally in

ELBW infants (birth weight 401-1000g) in three PDA treatment groups. The treatment groups were: surgery versus indomethacin only (OR 1.22, CI 0.67-2.24, p= 0.43), indomethacin plus surgery versus indomethacin only (OR 0.94, CI 0.67-1.31, p= 0.65) and no treatment versus indomethacin only (OR 1.16, CI 0.75-1.79, p= 0.42).

2.2.2.3 Severe pre-eclampsia and NEC

In severe pre-eclampsia, the foetal umbilical arterial blood flow may reduce, become absent or reverse because of placental failure and increased placental vascular resistance. The foetus adapts to the hypoperfusion state by initiation of the Hering-Breur or "diving reflex", whereby blood get shunted to critical organs such as the brain and the heart away from less important organs such as the intestine. This process may result in hypoxic ischemic injury of the intestine and trigger development of NEC.[2,35]

Kamoji et al.[50] found that preterm infants of gestational age ≤32 weeks born to mothers diagnosed with pre-eclampsia and had absent or reversed end diastolic flow (AREDF) in umbilical vessels, were nearly six times more likely to develop NEC compared to infants born to mothers with normal flow (14/24 versus 35/182, OR 5.88, 95% CI 2.41-14.34, p< 0.0001). Manogura et al.[51] reported a higher antenatal umbilical arterial pulsatility index (measure of placental resistance and/ or insufficiency) in infants who developed NEC than in those who did not develop NEC. Luig et al.[13] on the contrary, found maternal hypertensive disease with placental insufficiency to be associated with a reduced risk of NEC in infants between 24 and 31 weeks gestation (OR 0.61, 95% CI 1.27-3.49, p= 0.02).

2.2.3 Enteral feeding

Introduction of enteral substrate such as infant formula or breast milk into neonatal intestinal lumen causes a disruption of mucosal integrity, blood flow and motility. In addition, the incompletely digested molecules interact with immature host defenses and

other factors and may initiate an inflammatory cascade leading to NEC.[43] Ninety percent of preterm infants who develop NEC do so after being fed whereas only 10% develop NEC before feeding.[52]

Yu et al.[53] randomised infants of birth weight <1200g into enteral and total parenteral feeding and studied their outcome within 14 days while admitted in intensive care unit. Infants fed orally developed NEC (4 out of 17 infants) while no infant in the parental fed group developed NEC. Their finding was supported by that of Eyal et al.[54] They investigated the occurrence of NEC in VLBW infants who were either fed early within 24 hours of life or had delayed feeding, feeds started at 14-21 days after birth. NEC occurred only during the period infants were fed, at 7-24 days in the early fed group and at 23-60 days in the group that had delayed feeding ($p< 0.05$). Although these studies were small, they showed the role of enteral substrate in the development of NEC.

2.2.3.1 Type of milk

A substantially lower incidence of NEC has been reported in exclusively breast-fed infants compared to formula-fed infants. This is attributed to presence of unique immunoprotective properties in breast milk such as secretory IgA, specific macrophages and lymphocytes, presence of non-pathogenic bacteria such as bifidobacteria and secretory molecules with antibacterial properties.[35,55]

Quigley et al.[56] performed systematic review and meta-analysis of five randomised control trials that determined the effect of formula milk compared with donor human breast milk. They found 2.5 times risk of developing necrotising enterocolitis in the formula-fed group compared to donor human milk group (typical relative risk 2.5, 95% CI 1.2-5.1; typical risk difference 0.03, 95% CI 0.01-0.06). Sisk et al.[57] demonstrated that the protective effect of human milk against NEC is dose related. They reported six-fold decrease in odds of

NEC (OR 0.17, 95% CI 0.04-0.68) in infants whose enteral feed had a proportion of ≥50% human milk compared to those whose enteral feeds had <50% human milk. This finding was supported by that of Meinzen-Derr et al.,[58] who reported that the likelihood of NEC or death was decreased by 1.2 times for each 10% increase in the proportion of total intake as human milk (OR 0.83, 95% CI 0.72-0.96).

2.2.3.2 Feeding schedule

Studies which looked at the effect of the time of initial enteral feeding or rate of advancement of feeds on the incidence of NEC, showed contrasting results.[59-65] Differences in characteristics of infants in the studies may explain the contrasting observations.

2.2.3.2.1 Time of initiating enteral feeds

Anderson and Kliegman[59] investigated the relationship of neonatal feeding practices to the occurrence of NEC in low birth weight (LBW) infants weighing < 2000g at birth (19 cases versus 38 controls). They observed that feeding was initiated earlier in infants who developed NEC compared to controls (mean age of 4.1 ± 2.5 versus 7.1 ± 8.8 days, $p < 0.05$). But O Flidel-Rimon et al.,[60] in a larger study comprising of infants weighing <1500g, found no difference (despite smaller infants than that in the study of Anderson et al.[59]) in the timing of initiation of enteral feeds in infants who developed NEC compared to those who did not develop NEC (mean post natal age 3.1 ± 2 versus 3.7 ± 3 days, $p= 0.28$). Morgan et al.[61] did a systematic review and meta-analysis of five randomized control trials (600 infants) and found no statistically significant effects on the risk of NEC in infants who had delayed introduction of feeds (later than 5-7 days after birth) compared to those whose feeds were introduced early (≤4 days after birth) (typical relative risk 0.89, 95% CI 0.58-1.37). Bombell and McGuire[62] also performed a systematic review and meta-analysis

of nine randomised or quasi-randomised control trials (754 VLBW infants). They looked at the effect of early trophic feeding (feeding started within 96 hours postnatal age at volume of 24mls/kg/day for one week after which feeds were increased progressively) compared with delayed enteral feeding (feeds started at one week post natal age and progressively increased) on NEC. There was no statistically significant effect on the incidence of necrotising enterocolitis (typical relative risk 1.07, 95% CI 0.67-0.70).

2.2.3.2.2 Slow versus fast feeding

Rayyis et al.[63] randomised VLBW infants into "slow" feeding group (feeds were started at 20ml/kg/day and advanced by increments of 15ml/kg/day to a maximum of 160ml/kg/day) and "fast" feeding group (feeds were started at 35ml/kg/day and advanced by increments of 35ml/kg/day to a maximum of 160ml/kg/day). Both groups were fed formula milk. They found no difference in the incidence of definite NEC between both groups (slow 13%, fast 9%, p= 0.5). Morgan et al.[64] did a systematic review and meta-analysis of four randomized control trials, which studied the incidence of NEC in slow versus fast feeds advancement. The daily rates of feeds given were: 15ml/kg/day versus 35ml/kg/day, 20ml/kg/day versus 35ml/kg/day, 15ml/kg/day versus 30ml/kg/day and 20ml/kg/day versus 30ml/kg/day. They demonstrated no effect of slow versus fast feeds advancement on NEC risk (typical relative risk 0.91, 95% CI 0.47-1.75 and typical risk difference -0.01, 95% CI -0.05-0.04). There was no statistical evidence of heterogeneity in the meta-analysis implying similar characteristics of the study population. In contrast, although in a smaller case control study, Anderson et al.[59] observed that feeds were increased faster in neonates with NEC than those without NEC (56.7 ± 19.4 versus 44.6 ± 26.2 ml/kg/day, p< 0.05). Bersth et al.[65] randomized VLBW infants into trophic feeding group (infants were fed 20ml/kg/day for the first 10 days of life) and advancing feeding group (infants were fed on day one of life and feeding volume was increased by 20ml/kg/day up to a maximum of 140ml/kg/day)

groups. The study was stopped prematurely because more infants developed NEC in the advancing feeding group compared to those in the trophic feeding group (10% versus 1.4% respectively, p= 0.03).

2.2.4 Bacterial colonisation of the neonatal gut

The newborn gut is sterile at birth but natural bacterial colonisation begins on the first day of life. Initially, both breast-fed and formula-fed infants have similar flora. By the end of the first week of life, the gut of term breast-fed neonate is colonised by predominantly *Bifidibacteria* over other bacteria such as *Lactobacilli*, *Escherichia coli* and other *Enterococci.* In contrast, the intestine of a term formula-fed neonates is colonised with *Bacteroids, Clostridia, Bifidobacteria*, *Lactobacilli*, gram-positive cocci and coliforms with no predominant organism at the end of the first week of life. A healthy breast-fed term neonate has *Bifidobacteria* predominant intestinal flora, considered normal by convention.[35,55,66]

But the intestinal bacterial colonisation of a preterm differs from that of a term neonate. Most preterm neonates are admitted in intensive care units that have relatively sterile environment (such as incubators), enteral feeds are usually delayed and broad-spectrum antibiotics are administered frequently. Therefore the natural colonisation process tends to be delayed and impaired. These infants are colonised by fewer bacterial strains that are more likely predominated by pathogenic ones such as *Klebsiella, Enterobacter* and *Clostridium* organisms. Even human milk-fed preterm infants are slower to acquire *Bifidobacteria* as compared with their term counterparts, and in many cases *Bifidobacteria* reach predominance only after the peak period of susceptibility to NEC, which is the first 2-3 weeks of life.[55,66]

Weistra-Meijer et al.[67] investigated stool colonization with *Klebsiella* in preterm infants of gestational age <34 weeks and birth weight <2000g and were diagnosed with NEC, compared to those without NEC. Stool cultures were positive of *Klebsiella* in 71% of infants diagnosed with NEC and in 37% of infants without NEC, p= 0.01. In similar studies, Franz et al.[68] isolated *Klebsiella* species from the stool of 38% versus 17% of infants with and without NEC respectively (p= 0.03), while Bell et al.[69] isolated *Klebsiella* species from the stool of 58% versus 16% of infants with and without NEC respectively (p= 0.005). Bell et al.[69] also isolated *Escherichia coli* in 50% versus 29% of infants with and without NEC respectively (p< 0.05). Further, they found that the incidence of NEC was directly related to the degree of predominance of *Escherichia coli* and *Klebsiella* isolates from the faeces or stomach of the infants. The incidence of NEC decreased from 4-7% to 0% with a decrease in colonisation with *Escherichia coli* and *Klebsiella* from 82% to 11% and 88% to 47% respectively. The incidence increased to 4.4% with increase in colonisation with *Escherichia coli* and *Klebsiella* from 11% to 55% and 47% to 56% respectively. Hoy et al.[70] investigated quantitative changes in the faecal flora preceding the clinical onset of necrotising enterocolitis. They observed considerable decline in the numbers of bacteria such as *Streptococcus faecalis, Staphylococcus* species but considerable rise in numbers of *Klebsiella, Escherichia coli* and *Enterococcus cloacae* from72 hours before the clinical onset of NEC.

More recently in 2009, Wang et al.[71] found that infants with NEC had a significantly lower diversity of intestinal microflora than those without NEC (diversity index 1.19 ± 0.62 versus 1.99 ± 0.55, p= 0.005 respectively). Four phyla were present in infants without NEC: *Proteobacteria* 35% (such as *Klebsiella, Escherichia coli, Enterobacter, Shigella* etc), *Firmicutes* 57.8% (such as *Lactobacillus, Staphlococcus, Streptococcus, Clostridium, Enterococcus*), *Bacteroidetes* 2.45% (such as *Acidophilus, Bifidobacterium*), *Fusobacteria*

0.54% and unclassified bacteria 4.25%). Infants with NEC had only two phyla (*Proteobacteria* 90.72%, *Firmicutes* 9.12% and unclassified bacteria 0.16%). The average proportion of *Proteobacteria* significantly increased while the average proportion of *Firmicutes* significantly decreased in infants with NEC, p= 0.001. Smith et al.[72] in 2011 in a similar study, investigated the microbial composition and the relative number of bacteria in inflamed intestinal tissue surgically removed from neonates diagnosed with NEC. Of the tissue specimen studied, 71% had moderate to high densities of bacteria. The phyla detected were *Proteobacteria* (49.0%), *Firmicutes* (30.4%), *Actinobacteria* (17.1%) and *Bacteroidetes* (3.6%). These studies consistently found abnormal bacterial colonisation of the gut, characterised by reduced intestinal microbial diversity and changes in bacterial proportions predominated by pathologic bacteria in infants with NEC.[66-72]

The abnormal colonisation of premature neonates (with pathogenic organism), together with poor intestinal motility and immaturity of intestinal barrier function, predispose the gut to bacterial overgrowth and translocation, triggering cascade of events leading to NEC.[20,55,66]

2.2.4.1 Antibiotics and NEC

Wang et al.[71] found infants with NEC had a history of significantly more days of antibiotics (antibiotic days counted were for days prior to the diagnosis of NEC) compared to infants without NEC (13.7 ± 10.2 days versus 3.7 ± 3.0 days respectively, p= 0.005). Their finding was supported by that of Cotten et al.,[73] who retrospectively studied a cohort of ELBW infants whose blood cultures were negative and were empirically given broad-spectrum antibiotics within three days postnatal age for prolonged duration (≥5 days). They found that longer duration of initial empirical antibiotic treatment was more likely to be associated with NEC and/ or death, compared with shorter durations of initial empirical

antibiotic treatment. They also observed about four percent increase in the odds of an infant having NEC or dying with each additional day of initial empirical antibiotic treatment (OR 1.04, 95% CI 1.02-1.06, p< 0.01). Kuppula et al.[74] in a similar study, found that empirical antibiotics for duration ≥5 days in VLBW infants of gestation ≤32 weeks, was associated with late-onset sepsis, NEC, or death (OR 2.66, 95% CI 1.12-6.3).

Antibiotic exposure results in decreased anaerobic species, delayed colonisation with *Lactobacillus* species and increased colonisation with pathogenic *Enterobacteriaceae* and *Staphlococcus* species. Colonisation of a preterm gut with these pathogenic bacteria species may trigger the cascade that results to NEC.

Evidence indicates that the administration of prophylactic oral vancomycin can reduce the incidence of NEC.[75,76,77] Ng et al.[75] found that one of 84 infants who received oral vancomycin compared to 19 of 92 infants who did not receive oral vancomycin, developed NEC (p= 0.001). Similarly, in a double blind randomised placebo control trial, Siu et al.[76] found that nine of 71 infants who received oral vancomycin and 19 of 69 infants who received the placebo solution developed necrotising enterocolitis (p=0.035). Bury and Tudehop[77] performed a systematic review of randomized or quasi-randomized controlled trials where enteral antibiotics were used as a prophylaxis against NEC in LBW (<2500g) and/or preterm (<37 weeks gestation) infants. They found that administration of prophylactic enteral antibiotics resulted in a statistically significant reduction in NEC (RR 0.47, CI 0.23-0.98). However, a significant increase in the incidence of colonization with resistant bacteria was also shown (RR 1.73, CI 1.00-2.97). Therefore, widespread implementation of this preventive measure is not recommended. Its use should be restricted to a high prevalence nursery for a short and well-defined period in a selected group of high-risk patients.[76,77]

2.2.5 HIV-positive mothers

Desfrere et al.[14] found seven of 79 infants with NEC versus two of 158 infants without NEC, were born to HIV-positive mothers, p= 0.01. All the infants except one case had received zidovudine and no infant was infected with HIV. They observed an imprecise association between maternal HIV-positive status and increased risk of NEC in preterm infants (OR 6.63, 95% CI 1.26-34.8, p= 0.025). Their finding although not conclusive, suggested that HIV infection in the mother and exposure to ziduvudine might predispose the infant to developing NEC.

Infants of HIV-positive mothers may be more prone to infection and possibly develop NEC because of immunological deficiencies including that of cytokines production. Impaired interleukin-12 production has been demonstrated at birth in uninfected infants born to HIV-positive mothers and may persist after birth. Decreases in production and mRNA expression of interleukin-12 in gut mucosa may contribute to the pathogenesis of NEC, whereas increased interleukin-12 levels appear to protect against pan-necrosis in infants with NEC.[78] In addition, the antiretroviral ziduvudine used for prevention of mother to child transmission of HIV, might increase the risk of NEC by inducing mitochondrial damage and dysfunction.[14]

2.3 Clinical presentation

Bell et al.[79] classified infants with NEC into three stages, according to signs and symptoms and degree of systemic, intestinal and radiological signs. Kliegman and Walsh[80] modified Bell's staging by breaking each stage into two subcategories and included signs that differentiate between milder and more severe courses of disease (Table 2.1).

Table 2.1 Modified Bell´s staging criteria for NEC

Stage	Systemic signs	Abdominal signs	Radiographic signs
1a Suspected	Temperature instability, apnea, bradycardia, lethargy	Gastric retention, abdominal distention, emesis, heme-positive stool	Normal or intestinal dilation, mild ileus
1b Suspected	Same as above	Grossly bloody stool	Same as above
2a Definite, mildly ill	Same as above	Same as above, plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
2b Definite, moderately ill	Same as above, plus mild metabolic acidosis and thrombocytopenia	Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as 2a, plus ascites
3a Advanced, severely ill, intact bowel	Same as 2b, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, and neutropenia	Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention	Same as 2a, plus ascites
3b Advanced, severely ill, perforated bowel	Same as 3a	Same as 3a	Same as above, plus pneumoperitoneum

NEC presents between one and two weeks of life and onset may be sudden or insidious.[35] Yaseen et al.[81] reported early onset of NEC in more mature than premature infants (35.4 ± 2.5 weeks versus 27.7 ± 2.8 weeks, $p< 0.0001$) and in bigger than smaller infants (2204 ± 660 gram versus 940 ± 304 grams, $p< 0.0001$). This finding was supported by Gonzalez et al.[82] who observed an inverse relation between gestational age at birth and postnatal age of presentation of NEC (median postnatal ages at onset of NEC were 25, 18, 13, 6, 6, 5 days in infants of <25, 25-26, 27-28, 29-30, 31-32 and ≥33 weeks respectively. The explanation for this difference in time of onset is not clear.

2.4 Outcome of NEC

NEC is a highly fatal disease with mortality ranges of between 30 and 50%.[1,12] Buch et al.[83] reported an overall mortality of 45% with most death occurring in infants with severe stage of NEC (67%, 36% and 20% in NEC stage 3, stage 2 and stage 1 respectively). Fitzgibbons et al.[42] reported an inverse relation between mortality from NEC and birth weight of the infant (mortality of 42%, 29%, 21% and 16% in infants of birth weight 501-750g, 751-1000g, 1001-1250g and 1251-1500g respectively). Luig et al.[13] found that in infants of gestation between 24 and 31 weeks with NEC, 39% required surgical treatment and of those, 36% die. Buch et al.[83] reported 60% and 37% mortality in those treated surgically and medically respectively. This difference in the mortality probably reflects the differences in neonatal practices at the different units.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Research questions

What are the factors associated with development of NEC in infants born preterm and what are the factors associated with severe form of the disease and mortality?

3.2 Objectives

3.2.1 Primary objective

To determine maternal and infant characteristics associated with the development of necrotising enterocolitis in infants born preterm and admitted at Chris Hani Baragwanath Academic Hospital neonatal unit.

3.2.2 Secondary objective

To determine factors associated with severe disease and/ or mortality in preterm infants diagnosed with NEC.

3.3 Methods

3.3.1 Study design

This was a retrospective case control study.

3.3.2 Study population

The study population comprised of infants admitted at CHBAH neonatal unit between January 1, 2005 and December 31, 2008. Participants were selected by review of the infants' medical records. Cases were defined as those infants who were diagnosed with NEC stage 2 and 3 according to what was recorded in the infants' files.

The diagnosis of NEC in the CHBAH neonatal unit is based on Modified Bell's staging criteria,[80] which considers clinical features such as increased nasogastric aspirates, abdominal distension, bloody stools, radiographic evidence of pneumatosis intestinalis, metabolic acidosis and full blood count changes such as leucopenia, leucocytosis and/or thrombocytopenia. Controls were defined as preterm infants who were admitted at CHBAH and were discharged with a diagnosis other than NEC during the same period. For each case, two matched controls were selected until the required sample size was obtained. The controls were matched with cases for gestational age (had to be within two weeks of gestational age of cases), birth weight (to be within 100 grams of birth weight of cases) and postnatal age (to be within a week of postnatal age of cases). Considering that NEC is a rare condition, a ratio of one case to two controls was used in order to get an adequate sample size.

3.3.3 Sample size

The sample size was estimated based on a similar previous study,[13] which looked for factors associated with NEC. Any risk factor was used to calculate the estimated power for two-sample comparison of proportions. An estimate of a total of 300 infants, 100 cases and 200 controls was made because it was the largest sample size (using proportions 15.4% cases and 4.4% controls associated with the risk factor) found to be adequate to show results with power of over 80% and p-value of <0.05. It was estimated that 10% of patients would have missing data. Therefore, in order to compensate for this, enrolment of 110 cases of definite NEC was planned.

3.3.4 Sources of data and data collection

3.3.4.1 Sources of data

The medical records (bed letters) of the selected infants (cases and controls) and that of their mothers were reviewed. The feeding charts (stapled on the infants' bed letter) on which the nursing personnel recorded the type of feeds given to the infants were also reviewed. If the feeding charts were missing, the type of milk prescribed by the clinician in the infants' medical records was recorded. Data on maternal demographics, infant characteristics, and outcome was collected and recorded in appropriately designed data sheets (see appendix A).

3.3.4.2 Infants' characteristics

Demographics: gestational age, birth weight, small for gestation, and gender.

Clinical status before development of NEC: Apgar score, respiratory distress requiring oxygen, need for ventilation or intensive care and use of antibiotics for suspected or proven infection before diagnosis of NEC or before discharge or death in controls.

Feeding: postnatal age at first enteral feeds, types of milk feeds, largest volume of feeds increased within ten days of diagnosis of NEC or within ten days of getting full feeds in controls.

3.3.4.3 Maternal characteristics

Maternal age, mode of delivery, antenatal steroids, pregnancy induced hypertension, antepartum haemorrhage, multiple gestation, HIV status, premature rupture of membranes (PROM) >18 hours and chorioamnionitis.

3.3.4.4 Outcome of NEC

Stages or severity of NEC and mortality.

3.3.5 Definitions and measurements of main exposure variables

Preterm infant: infants born before 37 weeks gestational age.

Gestational age was based on what the clinician recorded, which was either based on Ballard score by the admitting neonatal clinicians (this was used where Ballard score sheets were stapled in the infants' bed letters) or in infants where there were no Ballard score sheets in the bed letters, the assumption was that the neonatal clinician recorded the estimated gestational ages based on mothers' bed letters.

LBW infants were those who weighed <2500g at birth.

Use of antibiotics was defined as antibiotic administered for suspected or proven infection, before the diagnosis of NEC or before discharge or death in controls.

The type and volume of milk feeds was that which was recorded in the infants' feeding chart by nursing personnel and/ or that which was prescribed by the attending clinicians and recorded in the infants' bed letters.

The largest volume of feeds increased was defined as the largest volume in milliliters per kilograms per day given to the infant within 10 days of diagnosis of NEC or within 10 days of getting full feeds in controls.

Mothers were considered to have received antenatal steroids (ANS) if they received at least one dose of steroid before delivery. If no record of administration of steroids was found, it was assumed that the mothers did not receive antenatal steroids.

Chorioamnionitis was based on the diagnosis that the obstetric clinicians recorded in the mothers' bed letters.

Surgery was any surgical procedure (pencil drains and/or laparotomy) for NEC.

3.3.6 Inclusion and exclusion criteria:

Inclusion:

- Cases were all infants of gestation ≤36 weeks who were admitted at CHBAH neonatal unit between January 1, 2005 and December 31, 2008 and were diagnosed with definite NEC.
- Controls were those who were within two weeks, one week and 100 grams for gestational age, postnatal age and birth weight respectively.

Exclusion:

- Infants born at gestational ages of >36 weeks.
- Infants admitted from other hospitals with a diagnosis of NEC were excluded because the record of their progress after discharge from CHBAH and that of their mothers would not be available at CHBAH.

3.3.7 Data processing and analysis

Data from the data collection sheets were entered in STATA 8 statistical programs for analysis. Summary statistics was used to describe the baseline characteristics of participants. Chi Square and Fischer's exact test were used to compare categorical data while continuous variables were compared using Student's t test. Logistic regression analysis was used to determine independent factors associated with NEC. Odds ratios were reported with 95% confidence intervals. A significant difference and/ or association was taken as a p-value <0.05 and a confidence interval not including one.

3.3.8 Ethics

3.3.8.1 Permission

Permission to perform the study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand and from the Protocol Review Committee of the CHBAH (see appendix B).

3.3.8.2 Confidentiality

A list, which contained the names and hospital numbers of the selected infants and their mothers, was kept confidential by the author. This list was used only during retrieving of the medical records. Entry of data on the collection data sheets and on STATA statistical programme was coded and anonymous.

CHAPTER FOUR

4.0 RESULTS

4.1 Enrolment

There were a total of 110 infants who were diagnosed with definite NEC between January 2005 and December 2008. They were identified as cases. These infants were matched by birth weight, gestational age and postnatal age with 220 infants who were identified as controls. Overall, 330 infants were enrolled in the study.

4.2 Description of mothers' and infants' characteristics

4.2.1 Description of the characteristics and diagnoses in mothers whose infants were diagnosed with NEC

The median age of mothers was 26 years. Forty eight percent of mothers delivered by caeserean section, 41% were HIV positive, a majority (81%) attended antenatal care, 63% received antenatal steroids and 17% had chorioamnionitis (Table 4.1).

Table 4.1 Characteristics and diagnoses in mothers whose infants were diagnosed with NEC

Maternal characteristics and diagnoses	Number (%)
*Median maternal age (yrs) (n= 110)	26 (16-42)
Mother's HIV status (n= 109)	
Positive	45 (41)
Negative	64 (59)
Mode of delivery (n= 110)	
Vaginal	57 (52)
Caeserean	53 (48)
Attended antenatal care	88 (81)
Received antenatal steroids	67 (63)
Multiple gestation	24 (22)
PIH	23 (22)
APH	11 (11)
PROM	12 (12)
Chorioamnionitis	18 (17)

*Measurement in median (range)

4.2.2 Description of demographic and perinatal factors in infants diagnosed with NEC (See Table 4.2)

The infants who were diagnosed with NEC had median birth weight of 1370 grams, median gestational age of 31 weeks and 19% were small for gestational age. Fifty three percent of them were females. Their median Apgar scores were 8 and 9 at 1st and 5th minutes respectively. The median postnatal age at which NEC was diagnosed was 10 days.

Table 4.2 Demographic and perinatal factors in infants diagnosed with NEC (n= 110)

Demographic and perinatal factors	Median (range)
Median birth weight (grams)	1370 (900-1760)
Median gestational age (weeks)	31 (25-35)
Median Apgar score:	
1 minute	8 (1-9)
5 minute	9 (4-10)
*Not recorded	6 (5%)
*Gender:	
Female	58 (53%)
Male	52 (47%)
*Small for gestation	21 (19%)
Postnatal age at diagnosis of NEC	10 (3-38)

*Measurement in number (%)

4.2.3 Demographic characteristic of infants diagnosed with NEC stratified by birth weight and gestational age categories

Table 4.3 shows nearly 64% of infants who were diagnosed with necrotising enterocolitis were of birth weight <1500g and 71% of them were less than 33 weeks gestation. Less than 5% of the infants were ELBW or were of gestational age ≤27 weeks. About two thirds of infants (64.6%) were diagnosed with necrotising enterocolitis stage 2a. The remaining proportion were diagnosed with stage 2b (18.2%), stage 3b (14.5%) and stage 3a (2.7%). Severe necrotising enterocolitis (stages 3a and 3b) was diagnosed in infants of all birth weight and gestational age categories except in those infants of birth weight categories <1000g and 1650-1799g and gestational age category 25-27 weeks.

Table 4.3 Demographic characteristic of infants diagnosed with NEC stratified by birth weight and gestational age categories (n= 110)

Demographic characteristics	Stages of necrotising enterocolitis; n (%)				Total
Birth weight (g)	**2a**	**2b**	**3a**	**3b**	
<1000	4 (80.0)	1 (20)	0	0	5 (100)
1000-1249	19 (57.6)	9 (27.3)	1 (3)	4 (12.1)	33 (100)
1250-1499	17 (53.1)	6 (18.7)	2 (6.3)	7 (21.9)	32 (100)
1500-1649	20 (76.9)	1 (3.9)	0	5 (19.2)	26 (100)
1650-1799	11 (78.6)	3 (21.4)	0	0	14 (100)
Gestational age (weeks)					
25-27	3 (100)	0	0	0	3 (100)
28-30	24 (60)	8 (20)	1 (2.5)	7 (17.5)	40 (100)
31-32	24 (68.6)	5 (14.3)	1 (2.9)	5 (14.3)	35 (100)
33-35	20 (62.5)	7 (21.9)	1 (3.1)	4 (12.5)	32 (100)

4.2.4 Ventilation, feeding and antibiotic use before diagnosis of NEC

Among the infants who had NEC only 11% required ventilation at birth. None of the infants were exclusively fed breast milk, they were either formula-fed (42%) or had mixed feeding (58%). A majority of infant (84.6%) were fed on or before 2 days of life. The mean largest volume of feeds increased was 29mls/kg/day. Nearly 24% of the infants received antibiotics before diagnosis of NEC and of these infants, 46% received antibiotics between three and seven days before diagnosis of NEC, 39% between one and two days and 15% over seven days before diagnosis of NEC (Table 4.4).

Table 4.4 Ventilation, feeding and antibiotic use before diagnosis of NEC

(n= 110)

Infants' ventilation, feeding and use of antibiotics	**Number (%)**
Ventilation at birth:	
No	98 (89.1)
Yes	12 (10.9)
Type of milk feeds:	
Breast milk only	0
Mixed	64 (58.2)
Formula	46 (41.8)
*Mean largest increase of volume of feeds (mls/ kg/ day)	29.3 ± 17.6
Postnatal age at first milk feeds:	
≤2 days	93 (84.6)
>2 days	17 (15.4)
Use af antibiotics before diagnosis of NEC:	
No	84 (76.4)
Yes	26 (23.6)
Last day on antibiotics before NEC:	
>7 days	4 (3.6)
3-7 days	12 (10.9)
1-2 days	10 (9.1)
No antibiotics before NEC	84 (76.4)

* Measurement in mean ± standard deviation

4.3 Outcome in infants with necrotising enterocolitis

4.3.1 Death and/ or surgery in infants with NEC

Nineteen percent of infants with NEC died. Seventy four percent of those with stage 3 died compared to about 8% among those with stage 2. Twelve (11%) of infants had surgery. Two thirds (67%) of infants who had surgery died compared to 13% among those who did not have surgery (Table 4.5).

Table 4.5 Outcome in infants with necrotising enterocolitis.

Outcome	**No; n(%)**	**Yes; n(%)**
Surgery	98 (89)	12 (11)
Deaths	89 (81)	21 (19)
Deaths according to NEC stage:		
Stage 2a (n= 71)	69 (97)	2 (3)
Stage 2b (n= 20)	15 (75)	5 (25)
Stage 3a (n= 3)	1 (33)	2 (67)
Stage 3b (n= 16)	4 (25)	12 (75)
Deaths among those who had surgery	4 (33)	8 (67)
Deaths among those who did not have surgery	85 (87)	13 (13)

4.3.2 Odds of death in infants diagnosed with necrotising enterocolitis according to severity of the disease

The odds of dying increased progressively with increasing severity of the disease. The odds ratios were: 11.5, 69 and 103 for stages 2b, 3a and 3b respectively compared to infants with NEC stage 2a. See table 4.6.

Table 4.6 Odds of death in infants diagnosed with necrotising enterocolitis according to severity of the disease (n= 110)

NEC stages	Odds ratio	95% confidence interval	P-value
‡2a	Reference	-	-
2b	11.5	2.03-65.01	**0.006**
3a	69.0	4.27-1114.2	**0.003**
3b	103.0	17.02-629.07	**<0.001**

‡NEC stage 2a was used as a baseline for within the group comparison

4.3.3 Odds of death in infants diagnosed with severe necrotising enterocolitis

The odds of death in infants diagnosed with severe necrotising enterocolitis were 33.6, 95% CI 9.35-120.8, $p< 0.001$ compared with those diagnosed with NEC stage 2.

4.4 Comparison of factors in cases and controls

4.4.1 Comparison of maternal and infants' factors in cases and controls

Infants who developed NEC were more likely to be born to mothers who did not receive antenatal steroids (36.8% versus 22.3%, $p= 0.007$), and who developed chorioamnionitis (16.8% versus 2%, $p< 0.0001$) (Table 4.7). More controls (32%) were ventilated as compared to cases (11%), $p< 0.0001$. (Table 4.8). There were no differences in other maternal and infants' factors in cases compared to controls (Tables 4.7 and 4.8).

Table 4.7 Comparison of maternal characteristics between cases and controls

Maternal factors	**Number (%)**		
	Cases	**Control**	**P-value**
*Median Age (yrs) (n= 329)	26 (16-42)	26 (14-44)	0.905
Mode of delivery (n= 330)			
Vaginal	57 (51.8)	124 (56.4)	0.434
Caesarean	53 (48.2)	96 (43.6)	
Mother's HIV status (n= 327)			
Negative	64 (58.7)	134 (61.5)	0.631
Positive	45 (41.3)	84 (38.5)	
Attended ANC (n= 324)			
No	21 (19.3)	46 (21.4)	0.655
Yes	88 (80.7)	169 (78.6)	
Received ANS (n= 317)			
No	39 (36.8)	47 (22.3)	**0.007**
Yes	67 (63.2)	164 (77.7)	
Multiple gestation (n= 330)			
No	86 (78.2)	188 (85.4)	0.099
Yes	24 (21.8)	32 (15.6)	
PIH (n= 312)			
No	82 (78.1)	149 (72.0)	0.245
Yes	23 (21.9)	58 (28.0)	
APH (n= 307)			
No	94 (89.5)	185 (91.6)	0.553
Yes	11 (10.5)	17 (8.4)	
PROM (n= 306)			
No	92 (88.5)	190 (94.1)	0.090
Yes	12 (11.5)	12 (5.9)	
Chorioamnionitis (n= 307)			
No	89 (83.2)	196 (98.0)	**<0.0001**
Yes	18 (16.8)	4(2.0)	

*Measurement in median (range)

Table 4.8 Comparison of the infants' characteristics between cases and controls

(n= 330)

Characteristics	**Number (%)**		
	Cases	**Controls**	**P-value**
*Median birth weight (grams)	1370 (900-1760)	1380 (800-1795)	0.928
*Median gestational age (weeks)	31 (25-35)	31 (25-35)	0.895
*Median Apgar score			
1 minute	8 (1-9)	8 (1-10)	0.365
5 minute	9 (4-10)	9 (2-10)	0.142
Unrecorded	6 (5.5)	18 (8.2)	0.828
Gender			
Female	58 (52.7)	107 (48.6)	0.484
Male	52 (47.3)	113 (51.4)	
Small for gestation			
No	89 (80.9)	176 (80.0)	0.845
Yes	21 (19.1)	44 (20.0)	
Ventilation at birth			
No	98 (89.1)	150 (68.2)	**<0.001**
Yes	12 (10.9)	70 (31.8)	
Use of antibiotics :			
No	84 (76.4)	164 (74.6)	0.719
Yes	23 (23.6)	56 (25.4)	

* Measurement in median (range)

4.4.2 Comparison of infants' feeding between cases and controls

Table 4.9 shows that more infants received the first feeds on or before day two of life in cases compared to controls (84.6% and 66.4% respectively, p= 0.001). More infants in cases (41%) than in controls (24%) had their feeds increased at volume ≥24.5mls/kg/day (p= 0.001). The rate of increase of feeds was greater in cases than in controls (29.3ml/kg/day and 25.2mls/kg/day respectively, p= 0.032). The types of milk feeds were similar in cases and controls.

Table 4.9 Comparison of infants' feeding (n= 330)

Feeding characteristics	Number (%)		
	Cases	**Controls**	**P-value**
Types of milk feeds:			
Mixed	64 (58.2)	134 (60.9)	0.634
Formula	46 (41.8)	86 (39.1)	
*Mean postnatal age at first milk feeds	1.85 ± 1.12	2.21 ± 1.14	**0.007**
Postnatal age at first milk feeds:			
≤2 days	93 (84.6)	146 (66.4)	**0.001**
>2days	17 (15.4)	74 (33.6)	
*Mean largest increase of volume of feeds (mls/ kg/ day)	29.3 ± 17.6	25.3 ± 13.1	**0.032**
Increase of feeds stratified by volume:			
<24.5 mls/kg/d	67 (60.9)	177 (77.7)	**0.001**
≥24.5 mls/kg/d	43 (39.1)	49 (22.3)	

*Measurement in mean ± standard deviation

4.5 Factors associated with necrotising enterocolitis and with mortality

4.5.1 Factors associated with necrotising enterocolitis

On univariate logistic regression, the following maternal and infants' factors were associated with NEC: non-exposure of infants to maternal antenatal steroids (OR 2.0, 95% CI 1.21-3.38, p= 0.007), chorioamnionitis (OR 9.91, 95% CI 3.26-30.13, p< 0.0001), Apgar score <4 at first minute (OR 2.48, 95% CI 1.06-5.75, p= 0.034), not ventilated at birth (OR 3.81, 95% CI 1.96-7.40, p< 0.0001), postnatal age at first feeds ≤2 days (OR 2.77, 95% CI 1.54-5.00, p= 0.001) and increase of feeds at a volume ≥24.5mls/kg/day (OR 2.24, 95% CI 1.36-3.68, p= 0.001). See table 4.10. However, on multivariate logistic regression of the above factors, non-exposure of infants to maternal antenatal steroids (OR 2.35, 95% CI 1.25-4.42, p= 0.008), chorioamnionitis (OR 8.73, 95% CI 2.59-29.5, p< 0.0001), Apgar score <4 at 1st minute (OR 2.92, 95% CI 1.05-8.01, p= 0.038) and not ventilated at birth (OR 3.20, 95% CI 1.18- 8.69, p< 0.023) were the only factors that remained significantly associated with necrotising enterocolitis (Table 4.11).

Table 4.10 Factors associated with necrotising enterocolitis on univariate analysis.

Mothers' and infants' factors	Odds ratio	95% confidence interval	P-value
Mothers' age (yrs)	1.05	0.85-1.29	0.639
Caeserian mode of delivery	1.20	0.76-1.90	0.434
Mothers' HIV status	1.12	0.70-1.79	0.631
Mother did not attend ANC	0.88	0.49-1.56	0.655
Mother did not receive ANS	2.03	1.21-3.38	**0.007**
Multiple gestation	1.64	0.91-2.95	0.099
PIH	0.72	0.41-1.25	0.245
APH	1.27	0.57-2.82	0.553
PROM	2.06	0.89-4.77	0.090
Chorioamnionitis	9.91	3.26-30.13	**<0.0001**
Apgar score at 1st minute <4	2.48	1.06-5.75	**0.034**
Apgar score at 5th minute <7	2.26	0.96-5.32	0.061
Gender	0.85	0.54-1.34	0.484
Small for gestation	0.94	0.53-1.68	0.845
Not ventilated at birth	3.81	1.96-7.40	**<0.0001**
Formula milk feeds	1.12	0.70-1.78	0.634
Postnatal age at first feeds ≤2 days	2.77	1.54-5.00	**0.001**
Increase of feeds ≥24.5mls/kg/day	2.24	1.36-3.68	**0.001**
Use of antibiotics	0.90	0.53-1.55	0.719

Table 4.11 Factors associated with necrotising enterocolitis on multivariate analysis.

Mothers' and infants' factors	Odds ratio	95% confidence interval	P-value
Mother did not receive ANS	2.35	1.25-4.42	**0.008**
Chorioamnionitis	8.73	2.59-29.5	**<0.0001**
Apgar score <4 in 1st minute	2.92	1.06-8.01	**0.038**
Not ventilated at birth	3.20	1.18-8.69	**0.023**
Increase of feeds ≥24.5mls/kg/day	1.09	0.60-2.02	0.759
Postnatal age at first feeds ≤2 days	1.43	0.58-3.50	0.435

4.5.2 Factors associated with severe necrotising enterocolitis and with mortality in infants with NEC

Lack of antenatal care was associated with severe NEC (OR 4.31, 95% C1 1.46-12.73, p= 0.008) and with mortality (OR 4.75, 95% CI 1.65-13.67, p= 0.004) in infants. All other factors were not associated with severe NEC or with mortality (Tables 4.12 and 4.13).

Table 4.12 Factors associated with severe necrotising enterocolitis

Mothers' and infants' factors	Odds ratio	95% confidence interval	P-value
Mothers' age (yrs)	1.17	0.73-1.89	0.518
Caeserian section delivery	0.74	0.27-2.02	0.561
Mothers' HIV status	2.00	0.72-5.55	0.183
Mother did not attend ANC	4.31	1.46-12.73	**0.008**
Mother did not receive ANS	1.11	0.39-3.15	0.840
Multiple gestation	0.95	0.28-3.17	0.929
PIH	1.11	0.33-3.84	0.860
APH	3.56	0.91-13.9	0.067
Chorioamnionitis	0.27	0.03-2.16	0.217
Birth weight	0.90	0.58-1.42	0.664
Gestational age	0.95	0.54-1.69	0.865
Apgar score at 1^{st} minute <4	0.39	0.05-3.22	0.383
Apgar score at 5^{th} minute <7	0.43	0.05-3.59	0.437
Gender	1.27	0.48-3.49	0.608
SGA	0.76	0.20-2.89	0.688
Not ventilated at birth	0.59	0.14-2.40	0.457
Formula milk feeds	2.20	0.81-6.00	0.124
Postnatal age at first feeds ≤2 days	1.68	0.35-8.03	0.517
Increase of feeds ≥24mls/kg/day	0.67	0.23-1.93	0.462
Use of antibiotics	0.33	0.07-1.53	0.156

Table 4.13 Factors associated with death in infants with NEC.

Mothers' and infants' factors	Odds ratio	95% confidence interval	P-value
Mothers' age (yrs)	0.90	0.57-1.43	0.666
Caeserean mode of delivery	0.36	0.13-1.00	0.051
Mothers' HIV status	1.20	0.45-3.20	0.709
Mother did not attend ANC	4.75	1.65-13.67	**0.004**
Mother did not receive ANS	2.22	0.81-6.07	0.119
Multiple gestation	1.58	0.54-4.6	0.407
PIH	0.62	0.16-2.34	0.480
APH	1.98	0.47-8.31	0.353
PROM	0.40	0.48-3.32	0.397
Chorioamnionitis	1.41	0.41-4.88	0.588
Birth weight	0.78	0.50-1.21	0.265
Gestational age	0.97	0.56-1.69	0.927
Apgar score at 1^{st} minute <4	0.36	0.04-3.00	0.346
Apgar score at 5^{th} minute <7	0.40	0.05-3.32	0.397
Gender	1.29	0.50-3.34	0.603
SGA	1.43	0.46-4.46	0.542
Not ventilated at birth	0.68	0.16-2.75	0.583
Formula milk feeds	1.34	0.51-3.48	0.550
Postnatal age at first feeds ≤2 days	1.12	2.9-4.32	0.869
Increase of feeds ≥24.5mls/kg/day	0.74	0.27-2.00	0.549
Use of antibiotics	1.01	0.33-3.02	0.983

4.5.3 Odds of death in infants diagnosed with NEC compared to those without NEC

The odds of death was five times higher in infants diagnosed with necrotising enterocolitis compared to that of infants without NEC. The odds of death increased progressively with increasing severity of the disease (OR: 7, 42, 63 for stages 2b, 3a, 3b respectively). There was however no difference in odds of death between infants with NEC grade 2a and infants without NEC (Tables 4.14).

Table 4.14 Odds of death in infants diagnosed with necrotising enterocolitis, combined and stratified by grades, compared to those not diagnosed with necrotising enterocolitis (n= 330)

NEC grades	Odds ratio	95% confidence interval	P-value
Combined	4.95	2.24-10.95	**<0.001**
2a	0.61	0.13-2.80	0.528
2b	7.0	2.12-23.1	**0.001**
3a	42.0	3.51-503	**0.003**
3b	63.0	17.2-230	**<0.001**

CHAPTER FIVE

5.0 DISCUSSION

The study showed an inverse relationship between the development of NEC and birth weight or gestational age of the infants, a relationship similarly observed in previous studies.[9,11-13,42] However, this relationship did not include infants of birth weight <1000g and of gestational age 25-27 weeks. A small proportion of these infants developed NEC since they may have died before the disease was clinically manifest.

Infants were either formula or mixed-fed. There was no difference between the type of milk feeds between cases and controls. The proportion of infants who were formula-fed (42%) was similar to that of infants whose mothers were HIV positive (41%), a reflection that infants with HIV-positive mothers were routinely fed formula milk as one of the strategy for prevention of mother to child transmission of the disease. It is possible that no infant received breast milk exclusively because firstly, mothers who were HIV negative were not consistently able and/ or available to provide breast milk to their infants and secondly, no expressed donor breast milk was available for the infants because of limited funding. Therefore, the association of exclusive breast milk feeds with NEC was not studied.

Previous studies showed the protective effect of human milk against NEC was directly related to the proportion of human milk in mixed feeds.[56,57] But in this study, the protective effect of mixed feeds was not observed. The proportions of the types of milk in the mixed feeds were not quantified because in infants who were breast-fed, measurement of the proportions of human milk that they received was not possible. Also, in infants who received mixed feeds and had missing feeding charts, the type of milk prescribed by the clinician in the infants' medical records was described. However, clinicians frequently

prescribed both expressed breast milk and formula milk, thus the type of milk feed administered could not be determined.

Infants who developed NEC were more likely to have had their feeds initiated on or before day two of life and/ or had their feeds increased more rapidly than those who did not develop the disease. However, after multiple logistic regression analysis, these factors were no longer associated with NEC probably implying that a combination of factors contribute to predispose the infants to develop NEC. Consistent with observation in this study, several previous studies did not find any association between development of NEC in infants and age at initiation of feeds or rate of advancement of the feeds.[60-64]

Several studies reported inconsistent association between NEC and hypoxia/ischaemic clinical conditions.[13,44,45,47-51] Hypoxia probably acts on the premature gut together with other risk factors, to predispose the infants to NEC. The difference in characteristics and/ or presence of different risk factors in infants in various studies, may explain the inconsistent observations. This study looked at pre-eclampsia, antepartum haemorrhage and Apgar score and observed associations inconsistent with those of other studies.

Infants were more likely to develop NEC if the 1-minute Apgar score was <4, consistent with findings by Powell et al.[84] They found infants were more likely to develop NEC if the 1-minute Apgar score was ≤6 compared to score >6 (82% versus 31% respectively, p< 0.028). Acunas et al.[85] observed an inverse relationship between 1-minute Apgar score and development of NEC (OR -0.3, 95% CI 0.67-0.98, p= 0.03). Other studies however reported no association between low Apgar scores and NEC.[9,51,53] Apgar score is an expression of the infant's physiologic condition that has a limited period, and includes subjective components. The biochemical disturbance must be significant before the score is affected. A number of factors such as prematurity, maternal drugs and conditions

associated with hypoxia such as pre-eclampsia and antepartum haemorrhage may affect the Apgar score.[85] A low Apgar score therefore may indicate a significant hypoxic and biochemical disturbances inflicted on the infant as a result of these factors.

Manogura et al.[51] studied mothers with severe pre-eclampsia. They found their infants were more likely delivered preterm, at lower birth weights, and had a low Apgar score. Infants were more likely to develop NEC if their mothers had a higher placental resistance and/ or insufficiency. Kamoji et al.[50] found that preterm infants of gestational age ≤32 weeks who were born to mothers diagnosed with severe pre-eclampsia with absent or reversed end diastolic flow (AREDF) in umbilical vessels, had six-folds odds of development of NEC compared to those born to mothers with less severe pre-eclampsia. Luig et al.[13] on the contrary found maternal hypertensive disease with placental insufficiency was associated with reduced risk of NEC in infants between 24 and 31 weeks gestation. Velaphi[9] observed no association between pre-eclampsia and NEC in infants, consistent with finding in this study. Severity of pre-eclampsia and related complications such as abruptio-placenta and prematurity probably differed between various studies, thus explaining the inconsistent observations.

Sanchez et al.[87] reported four times risk of abruptio-placenta in mothers with severe pre-eclampsia (OR 3.7, 95% CI 2.2-6.3). Previous study at CHBAH, reported that a higher proportion of infants who developed NEC were born to mothers who had antepartum haemorrhage compared to controls (10.8% versus 2.3%, p= 0.003).[9] The finding was supported by that of another study which found infants had two times odds of development of NEC if they were born to mothers whose pregnancies were complicated by antepartum haemorrhage (OR 2.09, 95% CI 1.30-3.35, p= 0.002).[13] This study however did not show any association between antepartum haemorrhage and development of NEC. There were

8.7 times odds of NEC in infants born to mothers who developed chorioamnionitis (OR 8.73, 95% CI 2.59-29.5). However, the association was imprecise probably because a small number of infants was studied (18 cases and 4 controls), thus the finding may be inconclusive. Several studies we reviewed reported contrasting results. These studies investigated larger number of patients and found no association between chorioamnionitis (diagnosed clinically or histologically) with development of NEC in infants.[88,90] Chorioamnionitis has been associated with preterm births and early neonatal sepsis, both factors are also associated with NEC.[88,91] Therefore, chorioamnionitis probably plays an indirect role in predisposing infants to the development of NEC.

Some studies have reported that infants whose blood cultures were negative at birth and were empirically given broad-spectrum antibiotics within three days postnatal age for prolonged duration (≥5 days), were more likely to develop NEC compared to those who received shorter durations (<5 days) of initial empirical antibiotic treatment.[71,73,74] This study investigated infants who were exposed to antibiotics at any postnatal age until one to two days prior to the diagnosis of NEC and found no association with NEC. However, the data may not be precisely comparable with that of the studies reviewed. Exposure to antibiotics alters intestinal microbial diversity and changes bacterial proportions, therefore predisposing infants to develop NEC.[71,73,74]

The risk of developing NEC was two times more in infants whose mothers did not receive antenatal steroids compared to infants whose mothers received antenatal steroids. The protective effect of antenatal steroids against NEC was also consistently observed in other studies.[92,93] Halac et al.[92] found three times more infants who were not exposed to antenatal steroid compared to those who were exposed, had developed NEC (10.4% versus 3.4% respectively, $p< 0.01$). Roberts and Dalziel[93] did a systematic review and meta-analysis of

eight randomised control trials and observed that antenatal steroids were protective against NEC (RR 0.46, 95% CI 0.29-0.74). Previously in 1988 and 1994 at CHBAH,[8,9] the association between antenatal steroids and NEC was not studied probably because of low use of antenatal steroids then. Davies[94] in a review reported less than 5% use of antenatal steroids in South Africa in 1996. In 2001[95] and in this study however, over 60% of women in preterm labour at CHBAH received at least one dose of antenatal steroid.

Steroids accelerate maturation of digestive function, epithelial proliferation and differentiation. They strengthen the intestinal barrier to macromolecules thus reducing the potential for translocation of bacterial and their toxins and exert protective anti-inflammatory effect on the gastrointestinal tract.[2]

There were 3.8 times odds of NEC in infants not ventilated at birth compared to those ventilated at birth, suggesting a protective association between ventilation at birth and NEC. The finding agrees with that of a previous study at this unit, which found four-folds less risk of NEC in infants ventilated at birth compared to those who were not ventilated at birth (RR 0.26, 95% CI 0.10-0.72, p= 0.006). The author suggested that infants on a ventilator may have experienced less episodes of hypoxia and probably had delayed initiation of oral feeds. This may have allowed their gut to recover from hypoxic/ ischaemic insults that occurred at birth, hence a reduction in the risk for NEC.[9] In addition, this study suggest that infants who were not ventilated at birth were probably clinically stable therefore fed earlier and more rapidly than those who were ventilated at birth. Although early initiation and rate of advancement of oral feeds may not be independent risk of NEC,[60-64] a combination of these factors, acting on premature gut may predispose the non-ventilated infants to develop NEC.

Studies in other units however reported contrasting results. Guthrie et al.[96] reported 3.5 times odds of NEC in infants who were ventilated at birth compared to those not ventilated at birth, OR 3.5, 95% CI 2.5-4.7, p< 0.001. Similarly, Carter and Holdich-Davis[97] reported increased odds of NEC in ventilated infants (OR 1.053, p= 0. 0.047). They also reported a negative correlation between birth weight and need for mechanical ventilation (r -0.49, p< 0.0001). The mean birth weight of ventilated infants was 989.0g ± 304. These infants were smaller than infants that had NEC in this study. Our unit offered mechanical ventilation to infants of birth weight >1000g due to limited resources.[98] The differences in birth weight of ventilated infants may explain the contrasting observations.

There was no difference in the odds of NEC between infants born to HIV-positive and HIV-negative mothers. One hundred twenty nine infants (39% of total) were born to HIV-positive mothers. Desfrere et al.[14] observed an imprecise association between maternal HIV-positive status and increased risk of NEC in preterm infants (OR 6.63, 95% CI 1.26-34.8, p= 0.025). However, their study comprised of nine infants born to HIV-positive mothers compared to 129 infants born to HIV-positive mothers in this study. A small sample size in the study by Desfrere et al.,[14] probably explains the imprecise association observed.

Severe NEC was not described in infants of birth weight <1000g and 1650-1799g and gestation 25-27 weeks. The distribution of severe disease was similar in other birth weight or gestation categories. Previous studies showed no association between birth weight and gestational age with severity of the disease, [96,99] supporting finding in this study.

Non-attendance of antenatal care was the only factor associated with severe NEC. The reason for this observation is not clear. It is possible that infants whose mothers did not attend antenatal care were a high-risk population with multiple risk factors for

development of severe NEC. Mothers who did not attend antenatal care missed opportunity for screening and treatment for maternal conditions such as pre-eclampsia, chorioamnionitis and antepartum haemorrhage.[100,101] These conditions also predispose to premature births, therefore contribute to the development of NEC.

There was no association between ventilation at birth with severe NEC. However, Guthrie et al.[96] found ventilation was independently associated with severe NEC. Carter et al.[97] reported inverse relationship between birth weight and gestational age with ventilation, implying ventilated infants were smaller, of lower gestational age and were critically ill and probably more prone to severe disease.

The odds of severe NEC were not different in infants born to HIV-positive mothers and HIV-negative mothers. Consistent to our finding, Karpelowsky et al.[102] found no difference in the rate of pan-intestinal necrosis between infants born to HIV-positive and HIV-negative mothers (p= 0.19). Similar finding was made by Arnold and Moore.[103]
Chougnet et al.[78] reported impaired interleukin-12 production at birth in uninfected infants born to HIV-positive mothers. They hypothesised that decreases in production and mRNA expression of interleukin-12 in gut mucosa may contribute to the pathogenesis of NEC and since increased interleukin-12 levels appear to protect against pan-necrosis in infants with NEC, decreased levels as in HIV-exposed infants, may be associated with severe disease.

The overall mortality associated with NEC was 19%, a decline from 34% that was reported in 1994 at CHBAH neonatal unit[9]. The finding was comparable with that of recent studies from the USA which reported an overall mortality from NEC of 15-20%.[104,105]
The decline of mortality at our unit may be attributed to improved availability and use of antenatal services by high-risk pregnant women, advances in obstetric care, appropriate training of staff, especially medical and nursing, improvements in all levels of neonatal

care including intensive care, progress in technology and equipment and increased use of antenatal steroids.[7] Over 60% of mothers in this study received antenatal steroids, an increase from five percent estimated in 1990 for the whole of South Africa.[94] Antenatal care attendance by mothers of infants born preterm increased to 81% in this study, from 71% and 79% in 2001 and 2005, respectively.[95,98]

Mortality increased with advancing stage of the disease at 3%, 25%, 67% and 75% for stages 2a, 2b, 3a and 3b respectively, supporting observation previously made at this unit. The mortality reported then was 16%, 29% and 61% in NEC stages 1, 2 and 3 respectively.[9] Similarly elsewhere, Buch et al.[83] observed mortality of 20%, 36% and 67% in disease stages 1, 2 and 3 respectively. Mortality in medically treated infants declined to 13% from 27% in 1994,[9] suggesting improved medical treatment of the disease. Mortality in surgically treated infants increased to 67% from 50% in 1994,[9] probably a reflection of the difference in the infants' clinical condition at surgery. Luig et al.[13] reported 36% mortality in infants treated surgically while Buch et al.[83] reported 60% mortality, probably reflecting differences in neonatal practices at different units.

The odds of death from NEC were increased by 4.8 times if the mothers did not attend antenatal care. The reason for this association is not known, but it may be similar to the previous suggestion that infants whose mothers did not attend antenatal care were a high-risk population with multiple risk factors for development of severe NEC and consequently risk of death. Holman et al.[106] however reported no association between non-attendance of antenatal care and death from NEC, probably because the characteristics of infants in their study differed from those in this study.

Birth weight was not associated with death from NEC. There was severe disease in all birth weight groups except in birth weight groups <1000g and 1650-1799g. Conversely

however, previous studies found an inverse relation between mortality from NEC and birth weight of the infants.[42,104,106] These infants were probably smaller, more critically ill and were more likely ventilation at birth compared to those in this study.

Clark et al.[105] reported that infants who died from NEC were more likely ventilated at birth (71.1% versus 46.7% respectively, $p< 0.001$), were smaller (median birth weight of 820g (550-1600) versus 1320g (720-2500), $p< 0.001$) and more often critically ill compared to those who were not ventilated at birth. In contrast to this study, there was no association between ventilation and death from NEC probably because bigger infants of birth weight >1000g were ventilated. There was no association between mothers' HIV status and death from NEC, consistent with observation by Arnold et al.[103] Karpelowsky et al.[102] on the contrary found increased odds of death from severe NEC if their mothers were HIV positive compared to those of HIV-negative mothers (OR 4.8, CI 1.7-14.2).

Strengths and limitations of the study

The strengths of this study were that presence and lack of association of antenatal steroids and HIV status of mothers with development of NEC respectively were reported for the first time at this unit. A large proportion (63%) of mothers received antenatal steroids; therefore an adequate sample size for this association was obtained. The proportion of mothers who tested HIV positive in our unit was high (39%), thus the number of infants studied for this association was large (129). Therefore, the study probably observed a true association. Desfrere et al.[14] investigated nine infants whose mothers were HIV positive and found an imprecise association.

The limitation of the study however was that estimations of gestational ages and Apgar scores, diagnosis of maternal conditions such as antepartum haemorrhage and chorioamnionitis, may have been inconsistent since they were performed by several

attending clinicians or nurses (for Apgar scores) with varying degrees of experience. This may have resulted in presumably inaccurate estimation of association of NEC with these factors.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The study showed multiple factors were associated with NEC. Infants who developed NEC were predominantly VLBW and were of gestational age ≤32 weeks. Ventilation and antenatal steroids were protective against NEC and are modifiable factors, whereas low 1-minute Apgar score and chorioamnionitis were associated with increased odds of development of NEC. Although a predominant proportion of infants who developed NEC were fed earlier or rapidly, infants' feeding was not independently associated with NEC.

HIV status of the mothers and other factors that were studied showed no association with NEC. Non-attendance of antenatal care increased the odds for severe NEC and mortality.

Compared to the previous finding at our unit, the overall mortality from NEC declined. Fewer infants were treated surgically, however their mortality increased. Mortality was inversely related to the severity of NEC.

Recommendations

The following recommendations are suggested:

- Pregnant mothers should be encouraged to attend antenatal clinics.
- All mothers presenting in preterm labour should be given antenatal steroids in order to prevent the risk of developing NEC.
- Strategies or interventions that have been shown to reduce NEC, namely breast milk feeding should be implemented, as NEC is associated with high mortality.

APPENDIX A

DATA COLLECTION SHEET

Enrollment no:……

Infant's information

GA:…….wks; BW:…….g; Small for gestation: Y/ N;

Apgar score: 1st min ……..5th min…….; Gender: M/ F

Mother's information

Age:……yrs; MOD: Vaginal/ Caesarian/ Assisted; ANC: Y/ N/ NK

Any antenatal steroids: Y/ N; If yes, nature of exposure: complete/ partial

PIH: Y/ N; APH: Y/N; Multiple gestation: Y/ N

HIV status: positive/ negative/ unknown

PROM > 24hrs: Y/ N; Chorioamnionitis: Y/ N

Infant's clinical care and progress

Respiratory distress at birth requiring oxygen: HMD/ TTN/ Congenital pneumonia/

other (specify)…….; If HMD, surfactant given: Y/ N

Other cause(s) of respiratory distress requiring oxygen after birth……

Age(s) at diagnosis……days

Need for resuscitation: Y/ N

If yes, method(s) of resuscitation: Oxygen/ bag & mask/mechanical ventilation/

Other (specify)……..

Age at resuscitation:…….

Age at 1st oral feeds:……days;

Prescribed type(s) of milk feed: formula/ BM/ mixed (BM & formula)

Rate of increase of milk feeds:……(kcal/ kg body wt/ day)

Rate of increase of milk feeds:……(mls/ kg body wt/ day)

Aggressive increase of milk feed: Y/ N

PDA needing treatment: Indocid/ surgery; Age at treatment……..days

Infections: No/ culture pos/ culture neg; Site:……..; Organism:……

Age at diagnosis of infection:……days.

NEC: Y / N. If yes, stage:……. Age at diagnosis…….days;

Surgery; Y / N; Age at surgery……..days

Outcome: discharge/ death

APPENDIX B

ETHICS CLEARANCE CETIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Angura

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M070629**
PROJECT	Necrotising Enterocolitis in Premature Infants at CH Baragwanath Hospital. What Are the Associated Factors?
INVESTIGATORS	Dr P Angura
DEPARTMENT	School of Public Health
DATE CONSIDERED	07.06.29
DECISION OF THE COMMITTEE* collection sheet and using age only	APPROVED subject to removing d.o.b from the data

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.07.02 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr SC Velaphi

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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