UNIVERSITY OF THE WITWATERSRAN

SCHOOL OF CLINICAL MEDICINE

DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH

RESEARCH REPORT

TITLE: Retrospective review of neonates with Persistent Pulmonary Hypertension of the Newborn at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)

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Research report submitted in partial fulfilment of requirements for a Master of Medicine degree in Paediatrics and Child Health (MMed).

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DECLARATION

I, Innocent HARERIMANA, declare that this research report is my own original work. It is being submitted for the degree of Master of Medicine in Paediatrics and Child Health at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signed….....

The.....day of 2014

DEDICATION

To my wife Jeanne d’Arc and my son Maxime who bring so much joy to my life. Your love and support are invaluable.

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This work would not have been successful without the guidance, help and support of number of individuals. I would like to express my profound thanks to them.

I would like to express my sincere gratitude to Professor Daynia Ballot who suggested the research topic to me and accepted to supervise this research. Her commitment, guidance and support have been invaluable.

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ABSTRACT

Background: Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome characterised by high pulmonary pressure, low systemic pressures and severe hypoxemia due to failure of circulation transition after birth.

Objective: The aim of the study was to determine the incidence, describe the risk factors, patient's characteristics, and treatment strategies for PPHN at CMJAH over the last 8 years and discuss the possible need of ECMO treatment in our settings.

Patients and methods: This was a retrospective descriptive study. I reviewed the computer database and medical records of infants who had a discharge diagnosis of PPHN from January 2006 to December 2013. The study included term and preterm, inborn and outborn infants. PPHN diagnosis was mainly based clinical suspicion. Patients with congenital cyanotic heart defect were excluded.

Results: The incidence of PPHN was estimated at 0.33 per 1000 live births in our unit. Out of 81 infants who had a discharge diagnosis of PPHN 72 patients were included in the study. Of the 72 patients 37(51.4%) were female, 38 (52.8%) born by vaginal delivery and 44(61.1%) were inborn. Most of them (75%) were born at term and had an appropriate weight for gestation age. The mean birth weight was 2.94 kg (SD 0.69) while mean gestation age was 38.2 weeks (SD3.3). Meconium aspiration syndrome (MAS) seen in 43 patients (59.7%) was the most frequent underlying disease followed by pneumonia that was seen in 9 patients (12.5 %). Of the 72 patients 67(93.1%) were treated with mechanical ventilation and only18.1% of them required high frequency oscillatory ventilation. Magnesium sulfate and Sildenafil were used in 12 patients (16.7%) and 9 patients (12.5%) respectively, while inhaled nitric oxide and extracorporeal membrane oxygenation were not available. Of the 72 patients

25(34.7%) died. The patients' characteristics were similar between survivors and non-survivors. The need for inotropic support was associated with a poor outcome.

Conclusion: PPHN was uncommon in our unit, but its management is still a challenge since it was associated with a high mortality. The leading cause of PPHN was MAS which can possibly be prevented by improving both antenatal and intrapartum obstetric care by good management of at-risk pregnancies. In our settings, the reduction of MAS incidence, adequate neonatal resuscitation, surfactant replacement therapy and early initiation of assisted ventilation for depressed infants with MAS could be cost- effective measures in preventing PPHN. ECMO therapy is very expensive and labour intensive, thus its use is limited in low- and middle- income countries including South Africa.

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ABREVIATIONS

AAP: American Academy of Paediatrics

ACOG: American college of obstetrics and gynaecology

CDH: Congenital diaphragmatic hernia

CMJAH: Charlotte Maxeke Johannesburg academic hospital

CMV: Conventional mechanical ventilation

ECMO: Extracorporeal Membrane Oxygenation

HMD: Hyaline membrane disease

HFOV: High frequency oscillatory ventilation

iNO: Inhaled nitric oxide

LGA: Large for gestation age

MAP: Mean airway pressure

MAS: Meconium aspiration syndrome

MSAF: Meconium stained amniotic fluid

NICU: Neonatal intensive care unit

NO: Nitric oxide

NO_2: Nitrogen dioxide

NRP: National Resuscitation Programme

NSAID: Non-steroidal anti-inflammatory drugs

PAP: Pulmonary artery pressure

PDA: Patent ductus arteriosus

PFO: Patent foramen ovale

PIP: Peak inspiratory pressure

PPHN: Persistent pulmonary hypertension of the newborn

PVR: Pulmonary vascular resistance

RDS: Respiratory distress syndrome

SGA: Small for gestational age

$SpO_{2:}$ Oxygen saturation

SVR: Systemic vascular resistance

TXA2: Thromboxane- 2

USA: United States of America

ET-1: Endothelin-1

1. INTRODUCTION

1.0. Background

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical condition characterised by severe respiratory failure and hypoxemia (1). Its incidence is estimated about 2 per 1000 live births across the world and it is associated with a high morbidity and mortality (2, 3). Despite the progress made in treatment of PPHN, it is still, not infrequently, a fatal disease especially in resource – limited settings (4). Walsh-Sukys et al. (2) reported an overall mortality of 11% (range 4%-33%) in a multicentre study in USA, Razzaq et al. (5) reported a mortality of 26.6% at Multan Children's Hospital in Parkistan and Abdel et al. (6) reported a mortality of 25% at Al-Minya University Hospital in Egypt.

In South Africa, previous studies reported the incidence of PPHN to be 1.1%, with a mortality rate of 31% at Tygerberg Children's Hospital as reported by Smith et al. (7) and 48% at Chris Hani Baragwanath Hospital as reported by Velaphi et al. (8). PPHN is due to failure of circulatory transition at birth, when pulmonary artery pressure (PAP) remains higher than systemic pressures (9) and the consequent right- to- left shunting of blood through patent foramen ovale (PFO) and/ or patent ductus arteriosus (PDA) results in severe hypoxemia (1, 10, 11) . PPHN usually affects term or near- term newborn babies though preterm babies can also be affected (1). This clinical condition was initially called persistent foetal circulation (PFC), but it was later named persistent pulmonary hypertension of the newborn to better describe its pathophysiology (11). PPHN is most commonly secondary to an underlying pulmonary pathology although primary or idiopathic PPHN is also frequent (1, 10, 11). Meconium Aspiration Syndrome (MAS) was the leading cause of PPHN (42%), followed by idiopathic PPHN (27%), respiratory distress syndrome (RDS) (17%), pneumonia/sepsis (13%) and less frequently lung hypoplasia as reported by Konduri et al.

(11) in a multicentre trial of inhaled nitric oxide (iNO). The other perinatal conditions that are potential risk factor of PPHN include perinatal asphyxia, polycythemia, acidosis and hypothermia (3).

PPHN is suspected when there is a significant difference between pre-ductal and post-ductal oxygen saturation, in combination with severe hypoxemia that does not improve when the infant is put on 100% supplemental oxygen. However it is difficult to accurately differentiate PPHN from cyanotic congenital heart disease only by clinical examination, so echocardiography is usually required to confirm a diagnosis of PPHN (1, 3).

Remarkable progress has been made in the management of this condition even though it frequently remains fatal in poorly-resourced facilities (4). The survival of infants suffering from PPHN has been improved by new medical technologies such as high frequency oscillatory ventilation (HFOV), selective pulmonary vasodilators such as iNO and phosphodiesterase inhibitors (Sildenafil and milrinone), surfactant and extracorporeal membrane oxygenation (ECMO) (3, 10-15). In resource-limited facilities, sildenafil and magnesium sulphate have been shown to be safe and effective pulmonary vasodilators and to improve oxygenation when iNO is not available (16-19). Adjuvant treatments such as inotropic support, correction of metabolic disturbances and minimal handling also play an invaluable role in the treatment of these infants (11, 15). Alkalinisation either by alkali infusion or hyperventilation has been abandoned because of consequent neurological complications and increased risk of developing chronic lung disease (11, 15). The current mainstay of PPHN treatment consists of a combination of HFOV and iNO. This combination treatment has been shown to reduce the need for ECMO rather than using each one of them separately (10, 13). However it does not reduce the mortality or duration of hospitalisation (13), and there is still unresolved debate and controversies among neonatologists regarding the appropriate time and dosage of initiation of the iNO treatment in newborn (10).

ECMO is used as a rescue therapy for infants in respiratory failure, unresponsive to other therapies (20, 21). Though it is very expensive and labour intensive, its introduction has remarkably changed the outcome of infants suffering from PPHN in well- equipped centres (22, 23). ECMO is generally not offered at CMJAH due to resource constraints; however, the cardiothoracic unit does offer ECMO to certain neonates. Locally it is worth considering whether we have the capacity to offer this therapy – would it be appropriate or required?

PPHN is a fatal clinical syndrome and MAS, the leading cause of PPHN, is more frequent in our settings. However there is paucity of data in literature regarding the management of PPHN in resource-limited settings. Therefore we undertook a retrospective review of the CMJAH computer database to determine the incidence, describe the risk factors, the patients' characteristics and treatment modalities for PPHN at CMJAH over the last 8 years and discuss the possible need of ECMO treatment in our settings.

1.1. Literature review

The literature review was based on the premise that persistent pulmonary hypertension of the newborn is a clinical syndrome resulting from failure or maladaptation of foetal pulmonary circulation at birth (4, 24).

1.1.1 Foetal circulation

The foetal circulation is characterised by high pulmonary vascular resistance (PVR) and low systemic vascular resistance (SVR). Elevated PVR is due to factors like low oxygen tension, elevated levels of vasoconstrictor mediators such as endothelin-1(ET-1) and Thromboxane A_2 (TXA_2), and low levels of vasodilators such as nitric oxide and prostacyclin (PGI_2) during foetal life. The foetal lungs are fluid filled and do not participate in gas exchange: they only receive 5-10% of the right ventricle output, and the gas exchange function is performed by the placenta (1, 10, 25). Recent studies have shown that pulmonary blood flow varies with

gestation age : it increases from 13% to 25% of the cardiac output at 20 and 30 weeks of gestation respectively and decreases to 21% at 38 weeks of gestation (26).

1.1.2. Adaptation of pulmonary circulation at birth

At birth, clamping of the umbilical cord and removal of the placenta increases the SVR in infants and the PVR abruptly falls due to the increase in oxygen tension, levels of vasodilators such as NO and PGI_2, decrease of vasoconstrictors such as ET-1 and TXA_2 and lung expansion. Pulmonary vasodilatation results in increased pulmonary blood flow and the lungs take over gas exchange function; the increased systemic pressures induce closure of the foramen ovale and improved oxygenation (13). The main determinants of perinatal circulation transition are NO- cyclic guanosine monophosphate (cGMP) pathway (regulated by endothelial nitric oxide synthase and soluble guanylyl cyclase), and arachidonic acid – prostacyclin pathway (regulated by cyclooxygenase enzyme and prostacyclin synthase) (1, 10, 27).

1.1.3. Pathophysiological mechanisms and risk factors of PPHN

PPHN is a clinical syndrome characterised by severe respiratory failure and hypoxemia due to failure of pulmonary circulation adaptation after birth. Usually PPHN is secondary to an underlying parenchymal lung disease (MAS, RDS, and pneumonia/sepsis) or a primary disease without an identifiable cause (1, 10, 13). The potential mechanisms involved in the pathogenesis of PPHN include: abnormal vasoconstriction of pulmonary vasculature following parenchymal lung diseases, abnormal pulmonary vasoreactivity and structural remodelling, and hypoplasia or underdevelopment of pulmonary vessels due to congenital diaphragmatic hernia or lung hypoplasia (1, 13, 27).

MAS is the far most common cause of secondary PPHN followed by pneumonia and RDS (11, 25). Meconium aspiration may occur in utero or in the perinatal period and results in airway obstruction which causes ventilation perfusion mismatch, surfactant dysfunction, and release of inflammatory mediators such as interleukins (IL-1β, IL-8), tumour necrosis factor alpha (TNF-α), and platelet activating factor(PAF) (25). The resulting hypoxia stimulates the release of vasoconstrictors (ET-1, TXA2 and PGE2) with consequent maladaption of pulmonary circulation and extra pulmonary right –to- left shunting blood (1, 13, 27).

Abnormal pulmonary vasoreactivity or vascular remodelling may results from excessive intra-uterine pulmonary artery pressure (PAP), chronic hypoxia or from exposure to drugs such as non-steroidal anti-inflammatory drugs (NSAID) (10, 14, 28), and selective serotonin reuptake inhibitors (SSRI) especially fluoxetine during late pregnancy (29-32). The exposure to NSAID increases the risk of PPHN by 6-fold by inhibiting PGI_2 and TXA_2 synthesis, resulting in premature closure of the ductus arteriosus. The exposure to SSRI after 20 weeks gestation results in increased levels of foetal serotonin, a potent vasoconstrictor, which induces vascular remodelling or proliferation of vascular smooth muscle (33).

Other factors which are reported to increase the risk of PPHN include perinatal asphyxia, being large for gestational age(LGA), maternal tobacco smoke exposure (34), high maternal BMI, diabetes mellitus, pregnancy induced-hypertension and preeclampsia (35, 36), Black or Asian race, caesarean section and maternal asthma (36).

1.1.4. Clinical presentation and diagnosis

PPHN is usually a disease of term and post-term newborn although premature babies can also be affected (1). PPHN is suspected in term or near-term infants unstable shortly after birth, with evidence of significant differential oxygen saturation (difference of ≥ 10% between pre- and post-ductal saturation) or difference in arterial oxygen tension (PaO_2) of ≥ 20 mm Hg

associated with severe hypoxemia that does not improve when the infant is put on 100% supplemental oxygen (hyperoxia test) and which is not explained by the chest x-ray findings (1, 37). However PPHN is not excluded by the absence of differential saturation because the differential saturation does not happen when the right –to- left-shunting of blood occurs through the foramen ovale (1). Thus echocardiography is the gold standard diagnostic test for PPHN since it demonstrates the level and direction of shunting, measures the PAP and excludes congenital cardiac defect (1, 37).

1.1.5. Treatment of PPHN

The treatment goal of PPHN consists of decreasing pulmonary artery pressure and increasing systemic pressures to overcome the extra pulmonary right - to- left shunt and hence improve pulmonary blood flow and tissue oxygenation (37). The strategies that are used to achieve optimal oxygenation, pulmonary vascular relaxation, and to maintain adequate cardiac output include general supportive measures for sick infants, mechanical ventilation, surfactant replacement, pulmonary vasodilators and hemodynamic support by volume expansion and/or inotropes (dopamine, dobutamine, and adrenaline). ECMO is the last resort treatment for those who fail to respond to these therapies (11). General supportive measures of PPHN treatment include correcting the metabolic disturbances as they arise, nursing in a quiet environment, ensuring adequate sedation and minimal handling as these infants are sensitive to all kinds of stimulation.

1.1.5.1. Mechanical ventilation

Mechanical ventilation improves ventilation- perfusion (V/Q) mismatch by providing alveolar recruitment and adequate lung expansion. However people have moved away from hyperventilation approach due to lung injury and neurological complications (10). HFOV is the preferable mode of ventilation because it helps to improve oxygenation without or with

minimal lung injury compared to conventional ventilation although neither is more effective in preventing ECMO (38) . HFOV in combination with iNO and surfactant replacement have shown to reduce the need for ECMO (39-41).

1.1.5.2. Pulmonary Vasodilators

Selective pulmonary vasodilators such as iNO, phosphodiesterase inhibitors (sildenafil and milrinone) and magnesium sulphate play an essential role in treatment of PPHN.

i) Inhaled nitric oxide

The iNO is a fast, potent selective pulmonary vasodilator and constitutes the standard therapy of PPHN. The iNO is indicated in term or near-term infants (≥34 weeks of gestation). Before starting this treatment the diagnosis of PPHN must be confirmed with echocardiogram and a right-to- left shunt ductal- dependent congenital heart defect must be excluded (14, 18). Although iNO reduces the need for ECMO, studies have shown that 40% of infants with PPHN do not respond to iNO and it does not reduce mortality or length of hospitalisation (18, 42, 43).The iNO has dose- dependent adverse effects such as methemoglobinaemia, pulmonary oedema, platelets dysfunction and production of toxic metabolites. Thus, close monitoring of methemoglobinaemia and metabolites (NO and NO_2) is required when using iNO (10).

ii) Phosphodiesterase inhibitors

Phosphodiesterase inhibitors such as Sildenafil and milrinone, used alone or in combination with iNO, have shown to improve oxygenation in infants with PPHN (44).

Sildenafil is a phosphodiesterase inhibitor type 5 (PDE5) with selective pulmonary vasodilator activity: it improves oxygenation by increasing cGMP levels. Oral Sildenafil has

been shown to be a safe and cost- effective treatment of PPHN (18, 19), largely used as an alternative treatment to iNO in resource-limited settings (45, 46). A potential adverse effect of Sildenafil is hypotension following systemic administration (10, 18) but is rare after oral administration.

Milrinone is a phosphodiesterase inhibitor type 3 (PDE3) with an inotropic and vasodilator activity which increases cyclic adenosine monophosphate (cAMP) levels. A study by McNamara et al.(47) has shown that milrinone, when intravenously administered, improves oxygenation in patients with PPHN unresponsive to iNO.

iii) Magnesium sulphate

Magnesium sulphate is a calcium channel blocker with a non-selective vasodilator activity. It has been shown that magnesium sulphate improves oxygenation in infants with severe PPHN(48, 49), and is a safe and cost-effective alternative treatment, especially in resource-limited settings where iNO is not available (16, 17, 50). However randomized controlled trials are needed to evaluate its efficacy and safety in PPHN treatment (51).

1.1.5.3. Extracorporeal membrane oxygenation (ECMO)

ECMO refers to a cardiopulmonary bypass circuit which is used to perform gas exchange by using a machine in patients with severe cardiorespiratory failure. The temporary diversion of blood circulation by cannulation of great vessels (usually jugular vein and carotid artery in neonates) permits the lungs' rest and recovery from the injury (52, 53). The overall survival rate of infants treated with ECMO was estimated at 85% (range 98% in MAS to 55% in diaphragmatic hernia) in a review study by Bartlett ("Extracorporeal life support : history and new directions, 2005") (52). ECMO is used as a rescue therapy for infants with respiratory failure and who are unresponsive to other therapies (20, 21). Its introduction has

remarkably improved the survival rate of infants suffering from PPHN and it constitutes the standard therapy of PPHN in well-equipped centres (22, 23). However the use of ECMO has declined since the introduction of new therapies such as HFOV, surfactant and iNO. ECMO therapy is not readily available in developing countries due to financial and staff constraints as it is very expensive and labour intensive (23).

1.1.5.4. **New** therapies

PPHN mortality rate remains high despite the current progress in mechanical ventilation and existing pulmonary vasodilators, especially in centres that lack ECMO (4). Thus new therapies target is to prevent smooth muscle cell proliferation and remodelling or stimulate endogenous NO production(11, 13). The new therapies which are undergoing investigation include antioxidants such as recombinant human superoxide dismutase, antenatal steroids (betamethasone) (11, 13), endothelial progenitor cells, Rho kinase inhibitors and vasoactive intestinal peptide (13).

1.1.5.5. Prevention and management of MAS

MAS has significantly declined in developed countries due to improved antenatal and intrapartum obstetric care and neonatal care; while its incidence is still very high in developing countries and it is associated with high morbidity and mortality (54). In the past several practices including amnioinfusion, intrapartum oral and nasopharyngeal suction, gastric lavage, and intubation and suction of all infants born through meconium stained amniotic fluid (MSAF) had been recommended (during different periods of time) in order to prevent MAS. However those practices have been abandoned by obstetricians and neonatologists as it was shown that they did not prevent MAS in randomised clinical trials (54, 55). The current recommendations from the American National Resuscitation Program (NRP), the American Academy of Paediatrics (AAP) and the American College of Obstetrics

and Gynaecology (ACOG) (54), which have been adopted by South African neonatal resuscitation program . With regard to MAS prevention and management of infants born through MSAF, these recommendations include: i) close intrapartum monitoring of foetal heart rate and the presence of a staff with skills in neonatal resuscitation at delivery of at-risk pregnancy, ii) intubation and endotracheal suction of depressed infants born through MSAF, iii) early initiation of assisted ventilation and surfactant replacement therapy for respiratory depressed and hypoxic infants (54, 55).

2. PATIENTS AND METHODS

2.1. Patients and methods

The present study is a retrospective descriptive study of newborn infants with a discharge diagnosis of persistent pulmonary hypertension of the newborn (PPHN). The infants included both inborn and out born neonates admitted at CMJAH neonatal unit from January 2006 to December 2013.

The data for this study was retrieved from a computerised neonatal database of CMJAH neonatal unit. Neonatal data is collected and managed using the REDCap (Research Electronic Data Capture) software hosted by the University of the Witwatersrand. Data is collected on discharge for each infant for the purpose of clinical audit. Infants with a discharge diagnosis of PPHN were identified by reviewing the computer database. Maternal and infant data were retrieved from the computer data base and completed by a review of medical records if necessary. Maternal data consisted of age, parity, gravidity, and disease during pregnancy, NSAID use and mode of delivery while the infant data consisted of gestational age, birth weight, gender, and place of birth, Apgar score, ventilation mode and duration, drug therapy in ICU, echocardiograph findings, hospital stay and outcome on discharge. Intensive care unit (ICU) charts were not available so ventilation parameters were not available and we were unable to calculate the oxygenation index in order to determine the severity of respiratory failure.

The diagnosis of PPHN was made on clinical presentation by attending physician when a patient was unstable immediately after birth; he/she had differential oxygen saturation (difference between pre-ductal and post-ductal) $\geq$ 10 % or difference in arterial $PO_2 \geq 20$ mmHg, hypoxemia disproportionate to the chest x-ray changes and was unresponsive to hyperoxia test. Due to a shortage of paediatric cardiologists at CMJAH, echocardiography to

confirm PPHN was usually performed at a later stage; and not all patients included in the study had echocardiography.

Preterm and term infants, inborn or out born, who fulfilled the criteria were included in this study. Infants with congenital diaphragmatic hernia were also included in the study while those who had a cyanotic congenital heart defect on echocardiography were excluded. We also excluded the patients whose data were not retrievable either from either the computer database or medical records.

Patients were managed by the attending physician according to the unit protocols. All infants who are clinically suspected to suffer from PPHN were usually ventilated mainly with conventional mechanical ventilation (CMV) and those who failed CMV were changed to HFOV. All infants on assisted ventilation were sedated with venous boluses of morphine/fentanyl with or without a benzodiazepine (midazolam) and most patients were not paralyzed. Sildenafil and magnesium sulphate were the only pulmonary vasodilators used. The unit did not offer iNO or ECMO treatment during the study period. However CMJAH has the capacity to offer iNO and ECMO treatment to some patients with severe respiratory failure in the cardiothoracic surgery unit.

The adjuvant therapy for PPHN included exogenous surfactant for RDS or severe MAS and hemodynamic support by inotropes (dopamine, dobutamine and adrenaline) when indicated. Sodium bicarbonate infusion was frequently used in treatment of severe metabolic acidosis to keep pH $\geq$7.25 if the latter is not corrected by ventilation and improved perfusion. However hyperventilation was not used in our unit. The general measures of treatment that were used include an attitude of ‘minimal’ handling and correction of metabolic disturbances when indicated.

2.2. Statistics

The data was described using standard statistical methods. Categorical variables were described using frequency and percentages, while continuous variables were described using mean and standard deviation or median and range, depending on data distribution. The univariate analysis was done using Pearson Chi square test and Fisher exact test for categorical variables, while Student's t- test or Mann -Whitney tests were used for continuous variables where appropriate in order to compare maternal and infant characteristic between survivors and non-survivors. Statistical significance was accepted at p-value <0.05.

2.3. Ethics

Permission to conduct the study was obtained from the University of Witwatersrand human research ethics committee and from the CMJAH authority (clearance certificate number M130650).

3. RESULTS

During the 8-year period there were estimated 219,514 live births from CMJAH and its referring, surrounding health care clinics. Eighty- one patients were identified with a discharge diagnosis of PPHN. Of the 81 patients, 72 were included in the study and 9 were excluded. Six patients were excluded because they had major congenital heart defect other than PDA or PFO on echocardiography; two were excluded because the relevant data was irretrievable from either the computer database or medical records and 1 was excluded because the discharge diagnosis of PPHN had been allocated to him erroneously. The estimated incidence of PPHN at the CMJAH neonatal unit was 0.33 per 1000 live births.

The patients' mean birth weight was 2.94 kg (SD 0. 69) 95% CI (2.77, 3.10), and the mean gestational age was 38.2 weeks (SD 3.3) 95% CI (37.4, 38.9). The maternal mean age at delivery was 26.2 years (SD 5.8) 95% CI (24.5, 27.9).

Echocardiography to confirm the diagnosis of PPHN by demonstration of right- to- left shunt was only performed in 27 patients (37.5%) of whom 10 patients (37%) had either a PDA and PFO or a PDA only with a right -to- left shunt.

Maternal diseases during pregnancy and demographics are shown in Tables 1 and 2.

Table 1. **Maternal disease during pregnancy**

Maternal disease	**Frequency (%) (n=72)**
PIH	5 (6.9)
Diabetes	1(1.4)
Tuberculosis	1(1.4)
None	59 (81.9)
Asthma	0
NSAID	0

Most of the mothers 59(81.9%) did not have chronic or pregnancy - related disease predisposing their infants to PPHN. However 5 mothers (6.9%) had pregnancy - induced hypertension (PIH). None of the mothers reported non-steroid anti-inflammatory drug (NSAID) use during pregnancy.

Table 2. Demographic characteristics of the patients

Characteristics	**Frequency (%) (n=72)**
Female	37(51.4)
Delivery mode:	
Vaginal	38(52.8)
Caesarean section	31(43.1)
Inborn	44(61.1)
Apgar at 5 min < 7	16(22.2)
Birth weight /GA	
AGA	58(80.6)
SGA	9(12.5)
LGA	4(5.6)
Gestation age groups	
Premature (≤ 34)	10(13.9)
Late premature (35-36)	7(9.7)
Term (≥37)	54(75.0)

The majority of infants were born at term 54(75%) and inborn 44(61.1%); almost half of the patients were female 37(51.4) and born by vaginal delivery 38 (52.8%). Sixteen (22%) had a 5 minute Apgar score below 7. Different pathologies associated with PPHN are shown in Table 3.

Table 3. Patients' underlying pathologies

Underlying Pathology	**Frequency (%) (n=72)**
Meconium aspiration syndrome (MAS)	43 (59.7)
Congenital pneumonia	9 (12.5)
Respiratory distress syndrome (RDS)	6 (8.3)
Asphyxia*	2(2.8)
MAS/Asphyxia	3(4.2)
Idiopathic PPHN	2(2.8)
Sepsis	1(1.4)
TTN	1 (1.4)
Hypoplastic lung	1 (1.4)
Congenital diaphragmatic hernia(CDH)	2(2.8)
Pulmonary haemorrhage	1 (1.4)
Vein of Galen malformation	1(1.4)

*Asphyxia was defined by low Apgar of ≤ 5 at 10 min, pH ≤7.0 and base excess of ≤ -16, neurological fall out and evidence of multi-organ dysfunction.

MAS was reported in 43 patients (59.7%) and it was the most common underlying pathology seen, followed by congenital pneumonia and respiratory distress syndrome, accounting for 9(12.5%) and 6(8.3%) respectively.

Although CMJAH is a surgical referral centre, especially for level 2 hospitals in Johannesburg, only 7 patients were admitted with congenital diaphragmatic hernia during our study period: only 2 of these patients had PPHN and both of them did not survive.

The management and outcome of patients with PPHN are shown in Table 4.

Table 4. Drug therapy, mechanical ventilation and outcome

Therapy	**Frequency (%) (n=72)**
Mechanical ventilation	
Yes	67 (93.1)
No	5 (6.9)
Mode of ventilation (n=66)	
CMV	67(100)
HFOV	13(18.1)
Surfactant use	
Yes	14(19.4)
No	57(79.2)
Bicarbonate infusion	
Yes	16(22.2)
No	56(77.8)
Inotropic support	
Yes	38(52.8)
No	34(47.2)
Vasodilators use	
Magnesium sulphate	12(16.7)
Sildenafil	9(12.5)
Inhaled nitric oxide	0
Nil vasodilator given	50(69.4)
Number of deaths	25(34.7)

Almost all the patients required mechanical ventilation 67(93.1%), 13 patients (18.1%) failed CMV and required HFOV. The median duration of mechanical ventilation was 4 days (range: 0 - 31). A slight majority of patients 38(52.8%) needed inotropic support while few patients 16 (22.2%) and 14(19.4%) were given sodium bicarbonate infusion or surfactant replacement

therapy respectively. Of the 43 patients who had MAS, only 7(16.3%) were treated by exogenous surfactant. Magnesium sulphate and Sildenafil were used in 12(16.7%) and 9(12.5%) patients respectively. Although the iNO is a standard therapy of PPHN, this was not available in the unit during the study period. Surprisingly 5 patients (6.9%) did not require mechanical ventilation.

The mortality rate was 34.7% (25 patients) and the majority (62.5%) died in the first 24 hours of admission. The median duration of hospital stay was 8 days (range: 0- 42).

The comparison between survivors and non-survivors is shown in Tables 5, 6 and 7.

Table 5. Comparison between Survivors and non survivors

Characteristics (Mean±SD or Median and range)	**Died**	**Discharged**	**p-value**
Birth weight (kg)	2.82 ± 0.77	2.99 ± 0.64	0.30
Agars score at 5min	7.24 ± 1.37	7.26 ±1.46	0.95
Gestation age(weeks)	37.71 ± 3.88	38.43± 2.98	0.39
Duration of ventilation (days)	1(0-26)	6 (.0-31)	0.000*
Duration of hospital stay(days)	1(0-36)	7(1-32)	0 .000*

*median (range), Mann-Whitney test

There was no difference between survivors and non- survivors in regard to birth weight, Apgar score at 5 minutes and gestation age ($p > 0.05$) while the duration of ventilation and hospital stay was longer for the survivors than non- survivors ($p= 0.000$) (Table 5).

Table 6.Comparison of demographic characteristics and treatment modalities between Survivors and non- survivors

Patients characteristics /treatment	Died (n=24) (%)	Discharged (n=47) (%)	p-value
Mode of delivery (n=68)			0.23
Vaginal	15 (65.2)	23(50)	
Male	15 (60)	27 (57.4)	0.15
Apgar 5- min <7 (n= 63)	8(38.1)	8(19)	0.10
Inborn	12(48)	32(68.1)	0.09
Gestational age (n=70)			0.88
Preterm (<37weks)	6(25)	11(23.4)	
Term (≥ 37weeks)	18(75)	36(76.6)	
Birth weight/GA (n=70)			0.75
AGA	19 (79.2)	39(83)	
LGA or SGA	5(20.8)	8(17)	
Mechanical Ventilation	25(100)	42(89.4)	0.15
CMV/HFOV	18(72)	36(85.7)	0.20
HFOV	7 (28)	6(12.8)	0.12
Inotrope support	18(72)	20(42.6)	0.01
Vasodilators use(n=71)	10(41.7)	11(23.4)	0.11
Type of vasodilator (n=20)			0.38
Magnesium sulphate	7 (66.7)	5(45.5)	
Sildenafil	3(30)	6(54.5)	
Bicarbonate infusion	8(32)	9(19.1)	0.22
Surfactant use	6(25)	8(17)	0.53

The demographic characteristics or treatment modalities were similar between survivors and non-survivors except for the need for inotropic support, where the use of inotropes was associated with poor outcome ($p = 0.01$) (Table 6).

Table 7. Comparison of survivor and non-survivors by underlying pathologies

Underlying pathology	**Died (%)**	**Discharged (%)**	**p-value**
MAS (n=43)	12(27.9)	31(72.1)	
Congenital pneumonia(n=9)	2(22.2)	7(77.8)	
RDS (n=6)	3(50)	3(50)	
MAS and Asphyxia (n=3)	2(66.7)	1(33.3)	
Asphyxia (n=2)	-	2(100)	
Idiopathic PPHN (n=2)	1(50)	1(50)	
Sepsis (n=1)	1(100)	-	
TTN (n=1)	-	1(100)	
Hypoplastic lung (n=1)	1(100)	-	
CDH (n=2)	2(100)	-	
Pulmonary haemorrhage (n=1)	-	1(100)	
Vein of Galen malformation (n=1)	1(100)	-	
Total (N=72)	25(34.7)	47 (65.3)	0.17

There were no statistically significant difference between survivors and non-survivors in regard to the underlying pathologies (p=0.17) though MAS was the most frequent cause of PPHN.

MAS was the most frequent associated disease in this study and the characteristics of patients with MAS are shown in Table 8.

Table 8. Characteristics of patients with MA

Characteristics (n=43)	**Frequency (%)**	**p-value**
Gender :		
Female	26 (60.5)	
Gestational age		
Preterm (<37)	8 (18.6)	
Term (≥ 37)	35 (81.4)	
Mode of delivery		
Vaginal	21(51.2)	
Caesarean section	20(48.8)	
Place of birth		
Inborn	26(60.5)	
Out born	17(39.5)	
Surfactant therapy		
Yes	7(16.7)	
No	35(83.3)	
Mechanical ventilation		
Yes	40(93)	
No	3(7)	
Outcome		0.20
Died	12(27.9)	
Discharged	31(72.1)	

Majority of infants with MAS 35(81.4%) were born after 37 weeks of gestation and almost all of them required mechanical ventilation 40(93%) though few patients 7(16.7%) were treated with surfactant. MAS was more frequent in female and inborn infants 26(60.5%) each and slight majority 21(51.2%) were born by vaginal delivery. The mortality rate in patients with MAS was slightly lower (27.9%) than the overall mortality (34.7%). However there was no statistically significant difference between patients with MAS mortality and those without MAS ($p = 0.20$).

4. DISCUSSION

Meconium aspiration syndrome was the leading cause of PPHN in this study, accounting for 43 cases (59.7%). Approximately 3 to 4 % of infants born through meconium stained amniotic fluid liquor (MSAF) develop MAS. MSAF complicates 7 to 22% of term deliveries and up to 52% of postdate deliveries (≥42 weeks gestation) (56). The incidence of MAS has recently declined due to improved obstetric practices including the reduction of postdate deliveries (>41 weeks gestation), good intrapartum monitoring of foetal heart rate as well as resuscitation of depressed neonates born through MSAF (57).

Both international and South African neonatal resuscitation programs recommend that all infants born through MSAF and who are depressed must be intubated and suctioned in order to clear the meconium substance from the trachea and oropharynx to prevent meconium aspiration (55, 58).

Remarkable progress has been made in pathophysiology understanding and treatment of PPHN (10, 11, 13, 14). However PPHN remains a treatment challenge for neonatologists especially in developing countries and its mortality rate remains high in resource-limited settings (5-9, 59, 60).

Of the 72 patients included in our study 61.1% were inborn, almost half of the patients were female and born by vaginal delivery (51.4% and 52.8% respectively); 80.6% had appropriate birth weight for gestational age. These results differ from those reported in previous studies which reported that PPHN was more associated with male sex, LGA and caesarean section delivery (5, 6, 9, 28, 36, 59, 60). The majority of the patients included in this study (75%) were born after 37 weeks, the mean gestation age being 38.2 weeks (SD 3.3) and the mean birth weight was 2.94 kg (SD 0.69). These results were similar to those reported in the literature and were consistent with the evidence that PPHN mainly affects term and post-term infants (9, 11, 60). The maternal mean age at delivery was 26.32 years (SD 5.8). Only 5

patients (6.9%) had PIH as a known risk factor although it could be underestimated since maternal history was not well documented in infant's medical records. Similar maternal age at delivery was reported in previous studies by Roofthooft in the Netherlands (59), and Hernandez in USA (36). The most frequent underlying disease leading to PPHN was MAS (59.7%) followed by pneumonia and RDS (accounting for 12.5% and 8.3% of the patients respectively). In the literature, MAS has been unanimously reported by many authors as the most frequent lung parenchyma disease resulting in PPHN, followed by idiopathic PPHN and pneumonia or RDS (1, 10, 11, 13, 61) (see Table 9). Our results were similar to those reported in previous studies (2, 6, 7) except that idiopathic PPHN accounted for only 2.8% in our study.

Table 9. Summary of PPHN causes in different studies

Series	**MAS (%)**	**Pneumonia (%)**	**RDS (%)**	**Asphyxia (%)**	**Idiopathic (%)**
Konduri,2009	42	13	17	-	27
Razzaq et al.,2013	35.4	29.1	13.9	40.5	-
Abdel et al.,2013	50	31.25	18.75	43.75	12.4
Smith et al.,1993	34.3	-	-	11.4	-
Hernandez et al.,2007	47.5	30.8	50.4	-	12.8
Rocha et al**.,**2012	24.3	3.8	-	30.7	-
Our study	**59.7**	**13.9**	**8.3**	**2.**8	**2.8**

Of the 72 patients enrolled in our study 93.1% were mechanically ventilated preferentially by CMV; 18.1% of them failed conventional mechanical ventilation and were switched to HFOV while 5 patients (6.9%) did not require assisted ventilation. Fourteen patients (19.7%)

were treated with exogenous surfactant. Similar results were reported in a previous study by Rocha et al. (9) in which 30.7% patients were treated with exogenous surfactant. These results were consistent with the existent knowledge in the literature since it has been stipulated that assisted ventilation constitutes the mainstay of PPHN treatment (1, 13-15, 61). The high proportion of patients treated by mechanical ventilation and surfactant in our study, could explain the severity of their disease though we were unable to calculate the patients' oxygenation index. The oxygenation index is usually used to measure the severity of respiratory failure (1, 25) . None of the patients was treated with iNO or ECMO since our unit did not offer these types treatment.

Magnesium sulphate and Sildenafil were frequently used in this study (16.7% and 12.5% of patients respectively) for pulmonary vasodilatation. Similar findings were reported in previous studies (6, 48, 62) and it has been shown that magnesium sulphate and Sildenafil are valuable, safe and cost-effective alternative vasodilators for the treatment of PPHN in resource- limited settings when iNO is not available (50, 63) .

Although alkali infusion and hyperventilation have nowadays lost the favour of neonatologists in the treatment of PPHN due to associated neurological complications and development of chronic lung disease (1, 2), sodium bicarbonate infusion was used in 22.2% of our patients. Similar findings were reported by Abdel et al. in Egypt (6),where sodium bicarbonate infusion was given to 25% of patients. An alkali infusion overall rate of 75% was reported by Walsh-Sukys *e*t al. (2) in a multicentre study in USA, where 92% of patients received sodium bicarbonate infusion.

Of note, there were no comments about potential complications such as neurosensory deafness and chronic lung disease at discharge found in patients' medical records. Long term follow up data was not available.

Adequate cardiac output and improved perfusion or oxygenation can be achieved either by volume expansion or inotropic support or both (10, 11, 14, 37). However inotropes should be used cautiously since they can also increase the PAP and worsen the right -to- left shunt(10). A slight majority of our patients (52.8%) required inotropic support. The results were comparable to those reported Walsh-Sukys et al. (2) in a multicentre study in which inotropic support overall was 84% (range 46-100%) with variation between centres.

Despite remarkable advances in understanding of physiopathology and managing PPHN, its mortality rate is still high in limited – resource settings or developing countries (4). Of the 72 patients enrolled in this study 25 (34.7%) did not survive. Similar high mortality rates directly or indirectly related to PPHN were reported in previous studies across the world: 48% at Chris Hani Baragwanath Hospital (8) and 31% at Tygerberg Children's Hospital (7) in South Africa, 25% at Al-Minya University Hospital in Egypt (6), 26.6% at Children's Hospital ,Multan in Pakistan (5), 32% at Hospital de Sα̃o Joα̃o EPE in Portugal (9) and 27.6% at Chang Gung Children's Hospital in Taiwan (60) . High PPHN mortality, in limited-resource settings, may be attributed to lack of new therapies such as HFOV, iNO and ECMO, which is the rescue therapy for patients with severe PPHN unresponsive to other treatment modalities. Of note, 40% of neonates with PPHN do not respond to the combination therapy of HFOV and iNO and require ECMO as a last resort treatment (3, 10, 13).

A great concern in this study is that of 25 patients 62.5% died in the first 24 hours of admission. None of the patients was treated with iNO or ECMO; it was not possible to measure the severity of disease /respiratory failure since there was no ventilation parameters available to calculate oxygenation index either before or after the treatment initiation.

On univariate analysis, the patients' characteristics were similar for survivors and non-survivors except the need of inotropic support which was associated with poor outcome

(p = 0.01) and the duration of mechanical ventilation or hospital stay which was longer for survivors than non-survivors (p = .000) (Tables 6 & 7). Theses differences are to be expected as deaths occurred early.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In this study PPHN was more frequent in female infants and associated with vaginal delivery. MAS was the most frequent underlying disease leading to PPHN. Patients' characteristics were similar between survivors and non survivors. Magnesium sulphate and Sildenafil were the only pulmonary vasodilators used .There was a high mortality rate 25(34.7%) and neither treatment strategy influenced the outcome, though the need for inotropic support was associated with poor outcome.

The low incidence of PPHN in this study could be explained by the improved management of infants with MAS in this unit, following the international recommendations (54, 55) mentioned earlier in the text, regarding the "delivery room management" of infants born through MSAF and neonatal resuscitation.

Due to its retrospective design, this study had some limitations: a) there were some missing data regarding patients' characteristics; b) it was not possible to calculate the IO since there were no ICU charts available, therefore it was impossible to measure the severity of respiratory failure; c) only a few patients in this study had an echocardiography and therefore there was a possibility of underestimating PPHN by relying on clinical diagnosis as the incidence in our setting is lower than in other reports.

5.2. Recommendations

ECMO therapy is very expensive and labour intensive, thus currently inaccessible in resource-limited settings. The reduction of MAS incidence by improving antenatal and intrapartum obstetric care, reduction of postdate deliveries (> 41weeks of gestation), good monitoring of at-risk pregnancies , adequate management of infants born through MSAF; and adequate neonatal resuscitation, surfactant replacement therapy and early initiation of assisted ventilation for depressed neonates with MAS could be a cost-effective measures in mitigating PPHN.

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7. APPENDIX

7.1. Ethics clearance certificate

R14/49 Dr I Harerimana

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130650

NAME: (Principal Investigator)	Dr I Harerimana
DEPARTMENT:	Paediatrics and Child Health Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Audit of Neonates with Persistent Pulmonary Hypertension of Newborn at CMJAH
DATE CONSIDERED:	28/06/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Daynia Ballot
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/10/2013

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 Secretary in Room 10004, 10th floor, Senate House, University.
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 resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES