IMPACT OF RED BLOOD CELLS TRANSFUSION ON CLINICAL OUTCOMES OF EXTREMELY PRETERM INFANTS AT HOSPITAL USM

BY

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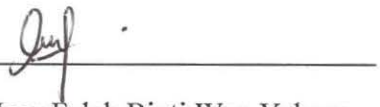
DECLARATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Masters of Medicine in Transfusion Medicine. It is also not to be sent to any other universities. With that, this research might be used for consultation and will be photocopied as reference.



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LIST OF SYMBOLS

%	Percent
=	Equal to
≤	More than and equal to
≤	Less than and equal to
>	More than
±	Plus minus
&	And

LIST OF ABBREVIATIONS

CI	Confidence Interval
CLD	Chronic Lung Disease
dL	Deciliter
ELBW	Extreme Low Birth Weight
g	Gram
Hospital USM	Hospital Universiti Sains Malaysia
Hb	Haemoglobin
HCT	Haematocrit
IVH	Intraventricular haemorrhage
LOS	Length of stay
ml	Mililitre
NEC	Necrotizing enterocolitis
NICU	Neonatal Intensive Care Unit
O & G	Obstetric and Gynaecology
OR	Odd Ratio
PPROM	Prelabour premature rupture of membrane
RBC	Red Blood Cell
ROP	Retinopathy of Prematurity
SVD	Spontaneous Vaginal Delivery
VLBW	Very Low Birth Weight
WHO	World Health Organization

ABSTRAK

PENGENALAN: Bayi yang sangat pramatang adalah antara pesakit yang banyak menerima pemindahan darah. Walau bagaimanapun, terdapat pelbagai kontroversi yang berkaitan transfusi sel darah merah dalam kalangan bayi pramatang termasuk garis panduan transfusi untuk bayi pramatang, indikasi transfusi dan kesan-kesan transfusi darah. Kajian dalam kalangan populasi bayi pramatang menunjukkan perkaitan dengan morbiditi dan kematian. Kajian ini dilakukan untuk menentukan impak transfusi sel darah merah dan kesannya dalam kalangan bayi yang sangat pramatang.

KAEDAH KAJIAN: 97 rekod bayi yang sangat pramatang dari Januari 2014 hingga Jun 2017 diperolehi. Data tentang sejarah transfusi sel darah merah dan kesan-kesannya kepada bayi tersebut direkodkan dan perkaitan di antaranya dibuat.

KEPUTUSAN: Kadar kematian bayi yang sangat pramatang di Hospital USM adalah 17.5%. Regresi logistik berganda menunjukkan perkaitan antara kematian dalam tempoh perawatan di hospital dengan bilangan kekerapan pemindahan darah dalam tempoh 60 hari (*OR*: 0.993; 95% *Confidence Interval:* 0.990-0.997; *p-value* : 0.005). Berat lahir juga menunjukkan perkaitan dengan kematian dalam tempoh perawatan di hospital (*OR*: 0.995; 95% *Confidence Interval:* 0.991-0.999; *p-value:* 0.001).

KESIMPULAN: Bilangan kekerapan pemindahan darah dalam tempoh 60 hari dan berat lahir adalah berkait rapat dengan kematian dalam tempoh perawatan di hospital dalam kalangan bayi yang sangat pramatang di Hospital USM.

ABSTRACT

INTRODUCTION: Extremely preterm infants were among heavily transfused patients. However, lots of controversies involved in neonatal transfusion which include guidelines for transfusion in prematurity, indications of transfusion, risks and benefits of red blood cells transfusion. Studies on neonatal populations showed that there were associations between red blood cells (RBC) transfusion and increased risk of morbidities and mortality. This study was to determine the impact of RBC transfusion with clinical outcomes in extremely preterm infants.

METHODOLOGY: A total of 97 neonates medical record between January 2014 to June 2017 had been retrieved. Data on transfusion history and clinical outcomes of these neonates following RBC transfusion were recorded and their associations were determined.

RESULTS: The mortality rate of extremely preterm infants in Hospital USM was 17.5%. Multiple logistic regression showed association between in-hospital mortality and frequency of transfusion within 60 days (OR: 0.993; 95% Confidence Interval: 0.990-0.997; p-value: 0.005) and birth weight (OR: 0.995; 95% Confidence Interval: 0.991-0.999; p- value: 0.005).

CONCLUSION: The frequency of transfusion within 60 days and birth weight were significantly associated with in-hospital mortality among extremely preterm infant in Hospital USM.

CHAPTER 1

INTRODUCTION

1.1 Title

Impact of red blood cell transfusions on clinical outcomes of extremely preterm infants at Hospital Universiti Sains Malaysia.

1.2 Overview

Every year, around 15 million babies are born preterm and this number is increasing in trend. It ranges from 5% to 18% of total delivery annually (World Health Organization, 2018). On top of that, half of the neonatal deaths occurred among the preterm infants were due to direct complications of prematurity (World Health Organization, 2018). The incidence and severity of the complications increased with decreasing gestational age (Park *et al.*, 2018). The preterm infants require close collaboration between antenatal care and neonatal care in order to improve the survival and reduce the morbidities among this age group (Blencowe *et al.*, 2013).

Blood transfusion is one of the treatment options to improve the condition of the preterm infants (Bowen *et al.*, 2015). The preterm infants are often been transfused due to relatively small blood volume and immature hematopoietic system (Portugal *et al.*, 2014). Consequently, most of the preterm infants less than 32 weeks gestational age required transfusions during the admission and some of them may need the transfusions up to 28 days of life (National Comparative Audit, 2010). Most of the extremely

preterm infants have been transfused at least once during their hospital stay (New *et al.*, 2016). Besides, Whyte (2009) reported up to 95% of extremely low birth weight (ELBW) preterm infants received at least one transfusion during their hospital stays (Whyte *et al.*, 2009).

Despite that, there were lots of controversies and dilemma in neonatal transfusions (Howarth *et al.*, 2018). The benefits need to be weighed against possible adverse outcomes (Christensen and Ilstrup, 2013). Besides, multiple factors need to be considered before the transfusion in the neonates was commenced (Bell, 2008).

In addition, most of the guidelines for neonatal transfusion were based on the expert opinion due to limited high-grade scientific evidence in this vulnerable group (Girelli *et al.*, 2015). Hence, the decision to transfuse was depend on the clinicians, the condition of the patient and guideline adopts by the institution (Strauss, 2010).

The most common indications for red blood cell (RBC) transfusion in preterm infants are to increase the circulating blood volume, cardiorespiratory compromise, anaemia of prematurity, growth failure, apnoea, low haemoglobin and haematocrit level (Josephson *et al.*, 2010). The commonest indication was neonatal anaemia with the volume of transfusion of 10-20 ml/kg over 4 hours (New *et al.*, 2016). Otherwise, Kaur (2015) reported sepsis as the leading cause of multiply transfused neonates (Kaur *et al.*, 2015).

However, since 20 years ago, there have been increasing concerns over possible adverse effects related to RBC transfusion in patients under critical care (Wang *et al.*, 2016). Furthermore, there were growing evidence comparing restrictive and liberal transfusion practices in preterm infants to reduce the frequency of transfusions in order

to reduce the donor exposure and adverse events (Bell, 2008). Many studies showed an association between transfusion and poor outcome, including the risk of mortality, morbidities and increased length of stays in adult and children population (Clevenger and Kelleher, 2014; Corwin *et al.*, 2004).

Different from adult populations, studies on neonatal populations have suggested associations between RBC transfusion and increased risk of specific complications such as chronic lung disease (CLD), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), and extension of intraventricular haemorrhage (IVH) (Wang *et al.*, 2016). Reported mortality risk after 28-days of life was higher in preterm infants who received more than two transfusions compared with infants who received two transfusions or less during the admission (dos Santos *et al.*, 2011). Late-onset sepsis also reported being associated with RBC transfusions which ultimately lead to prolonged hospital length of stay (Pessoa-Silva *et al.*, 2001).

Nonetheless, there were contradictory reports from the literature regarding the association between RBC transfusion and NEC, ROP, or CLD has been disproved individually (Keir *et al.*, 2016). One report discussed the advantageous effect of RBC transfusion on long-term outcome in preterm infants (Whyte *et al.*, 2009). There was very limited evidence to define optimal volumes for neonatal red cell transfusions, in particular relating to long term outcomes (New *et al.*, 2016).

The PINT trial compared the outcome between the restrictive and liberal group of extremely preterm infants. However, there were no statistical difference seen in the outcome which includes ROP, CLD, brain injury and death (Kirpalani *et al.*, 2006). The conflicting evidence also reported by Keir (2016) which showed no association between RBC transfusions NEC, ROP and CLD (Keir *et al.*, 2016). Bell (2008)

reported no significant association between transfusions in mortality, yet the post hoc analysis for IVH favor the liberal practices (Bell, 2008). PINT study supported the liberal practices in cognitive function (Kirpalani *et al.*, 2006).

In Malaysia, estimated 12.3 percent of the 500,000 births of premature infants were reported (Perinatal Society of Malaysia, 2014). The incidence of infant mortality in Malaysia was seven deaths per 1000 live births in 2012. The major causes of mortality in 2010 for children below the age of five years were 31 percent for congenital anomalies, 24 percent for prematurity and eight percent for birth asphyxia (Child and Maternal Health, 2014). Generally, the mortality was high in neonates and infants less than 1- year- old and a lot of measures done to improve the management of this group of a patient which include RBC transfusion (Child and Maternal Health, 2014).

Guidelines for the rational use of blood and blood products published by Ministry of Health Malaysia in 2008 provide suggested transfusion thresholds for transfusion in neonates but non-specific for preterm infants (Ministry of Health, 2008). Currently, no local published data regarding the complications arise from the RBC transfusion in this group of patient. The aim of this study was to determine the clinical outcomes in extremely preterm infants who received RBC transfusion and the association between RBC transfusion and in-hospital mortality.

CHAPTER 2

LITERATURE REVIEW

2.1 Pathophysiology of anaemia in preterm infants

Blood transfusion is one modalities of treatment in neonates. The preterm infants are among the heavy user of blood products especially red blood cell (Goel and Josephson, 2018). Most of the preterm infants will receive at least one blood transfusion during the admission (Wang *et al.*, 2016). Nearly all ELBW infants and infants less than 29 weeks will receive blood transfusions in their first weeks of life (Strauss, 2010).

Neonatal anaemia is the commonest cause of red blood cell transfusion in the preterm infants (New *et al.*, 2016). Neonatal anaemia is defined as a haemoglobin level below the lower limit adjusted for age (Venkatesh *et al.*, 2012b). It can be caused either by physiological or non-physiological factors (Colombatti *et al.*, 2016).

2.1.1 Physiological causes of anaemia in preterm infants

Physiological causes play an important role in the development of anaemia of prematurity (Colombatti *et al.*, 2016). The circulating RBC is reducing during the first weeks of life. In extremely preterm infants, the haemoglobin level reaches the nadir haemoglobin earlier and lower which is between 5 to 10 weeks at the level of 8-10 g/dL instead of 9.5-11.5 g/dL at 6-12 weeks in term infants (Colombatti *et al.*, 2016). Unlike

the term infants, the nadir haemoglobin is not well tolerated in preterm infants and may require red blood cell transfusion as it often leads to symptomatic anaemia (Bell, 2008). Besides, the circulating life span of red blood cells in preterm infants is shorter compared to adult which is 35 to 50 days (Colombatti *et al.*, 2016).

Furthermore, low plasma erythropoietin level due to inadequate production and increase catabolism also contribute to anaemia of prematurity (Venkatesh et al., 2012a). The site of production for erythropoietin is in the liver which is less sensitive to tissue hypoxia than the renal. Consequently, it leads to delay compensatory response following phlebotomy loss, bleeding and etc (Strauss, 2010).

2.1.2 Non physiological causes of anaemia in preterm infants

Besides anaemia of prematurity, phlebotomy loss due to frequent monitoring of the preterm infants in the intensive care unit also a major contributor towards neonatal anaemia (Venkatesh et al., 2012a). Co-morbidities such as sepsis, inflammations, acute bleeding and infections may worsen the anaemia in the preterm infants (Venkatesh *et al.*, 2012b).

2.2 Indications for transfusion in preterm infants

Transfusions in preterm infants are controversial and practices vary in different institutions (Santos *et al.*, 2010). Most of the guidelines are based on the expert opinion or local guidelines (Howarth *et al.*, 2018). Different guidelines set different values for target haemoglobin and haematocrit levels depend on multiple factors such as

gestational age, postnatal age, oxygen requirement, cardiopulmonary status and etc (Guillen *et al.*, 2012).

Symptomatic anaemia occurs when the oxygen consumption is greater than the oxygen delivery (Colombatti *et al.*, 2016). Various studies and trials were done to establish evidence-based criteria for transfusions in neonates which compare different tolerable haemoglobin and haematocrit level (Bell, 2008; Chen *et al.*, 2009; Kirpalani *et al.*, 2006). Most of the studies favor restrictive strategies as similar outcomes seen in both restrictive and liberal strategies (Venkatesh *et al.*, 2012b). On top of that, clinical condition of the patients remains as an important factor to decide the need of the transfusion in the preterm infants (Howarth *et al.*, 2018).

Colombatti (2016) classified neonatal anaemia into three different groups which are blood loss, reduced production and increased destruction (Colombatti *et al.*, 2016). Transfusion in neonates is indicated in blood loss which includes acute blood loss from fetomaternal haemorrhage, acute bleeding such as intraventricular haemorrhage or gastrointestinal bleeding and phlebotomy loss (dos Santos *et al.*, 2015). Decreased production can be due to anaemia of prematurity or physiological changes in infants (Colombatti *et al.*, 2016). Besides that, increased destruction can be due to immune and non-immune causes such as sepsis and inflammations may worsen the anaemia (Ali, 2018).

2.3 Frequency and volumes of transfusion in preterm infants

Besides transfusion threshold, transfusion volumes per kilogram body weight and its relation with the frequency of transfusion remain an issue (Khodabux *et al.*, 2009). The recommended volumes for transfusion in preterm infants is 10-20 ml/kg and usually given at a volume of 15 ml/kg over 4 hours in stable cases (New *et al.*, 2016). Volumes more than 20 ml/kg may put the patients at risk of volume overload (New *et al.*, 2016). National Comparative Audit reported 24% of early transfusions are at 20 ml/kg at the standard rate of 5 ml/kg/hour (National Comparative Audit, 2010).

Khodabux (2009) concluded there were no differences in overall complications in a high and low volume of transfusion. Due to study limitation, the author cannot conclude any preferences for the transfusion volumes (Khodabux *et al.*, 2009). However, Colombatti (2016) recommended the volume of 20 ml/kg can produce better haemoglobin and haematocrit level without compromising pulmonary function. It also can reduce the frequency of transfusion and donor exposure (Colombatti *et al.*, 2016).

Most of the extremely preterm infants and ELBW infants required transfusions in the first week of life (Strauss, 2010). Around 90 percent of ELBW infants and 40 percent VLBW infants received an average of five transfusions during their admission (Ali, 2018). The number of transfusions is higher during the first 28 days and then decreasing in trends (Santos *et al.*, 2010).

2.4 Risks of transfusion in preterm infants

Each transfusion carries significant risks to the recipients. There was increasing concern over the risks of transfusion in this frequently transfuse preterm infants (Wang *et al.*, 2016). The disadvantages and the risks of RBC transfusion were not well reported (Keir *et al.*, 2016). Different studies reported different risks of in-hospital mortality, sepsis, CLD, NEC, ROP and IVH in relation to transfusion in preterm infants (Venkatesh *et al.*, 2012a). Meta-analysis by Keir (2016) demonstrates no statistically significant different in the outcomes of the preterm infants exposed to the different dose of transfusion practices (Keir *et al.*, 2016).

A study by dos Santos (2011) concluded that transfusion was associated with mortality with relative risks of 1.49 in VLBW preterm infants who received at least one transfusion within 28 days of life (dos Santos *et al.*, 2011). The frequency of transfusion was associated with increased mortality within 30 days of life in ELBW infants (Wang *et al.*, 2016).

Chen (2009) reported no significant difference in developing chronic lung disease in Very Low Birth Weight (VLBW) infants who received liberal or restrictive transfusion strategies but the incidence was higher in patients who received more than 30 ml packed cell within 30 days of life (Chen *et al.*, 2009). The risks of developing ROP associated with the frequency of transfusion within 30 days of life (Wang *et al.*, 2016). The risk of developing CLD, ROP and NEC are due to immature antioxidant system in premature infants (Iskander *et al.*, 2018). The risk of developing CLD, ROP, NEC and mortality was higher in VLBW preterm infants who received more than three transfusions during the hospital stay (Ghirardello *et al.*, 2017).

Multiple theories postulated regarding transfusion-associated NEC which includes hypoxic-ischaemic gut injury, mechanical factors affecting guts circulation such as increased splanchnic injury following transfusion, oxidative injury due to stored transfused blood and feeding (Iskander *et al.*, 2018). The incidence of developing NEC increased to 25-40% in preterm infants within 24-72 hours post transfusion (Iskander *et al.*, 2018). A retrospective cohort study by Wallenstein (2014) refuted the findings (Wallenstein *et al.*, 2014b). The meta-analysis on NEC supported the findings of increased incidence in NEC especially in extremely preterm and low birth weight infants (Mohamed and Shah, 2012).

Intraventicular haemorrhage in preterm infants is not well understood but postulated to be due to a fragile germinal matrix and oxidative injury (Iskander *et al.*, 2018). VLBW preterm infants who received the transfusion in the first week of life had a higher incidence of IVH by 2% (Christensen *et al.*, 2014). By reducing the transfusion frequency, the incidence of IVH is also reducing in trend (Christensen *et al.*, 2014). Baer (2011) reported that the risk of developing IVH was doubled if the preterm infants were exposed to transfusions in the first week of life (Baer *et al.*, 2011). However, contradicting result reported by Chen (2009) whereby there was no significant difference in IVH incidence in liberal and restrictive groups (Chen *et al.*, 2009).

More than 40% of VLBW preterm infants develop early and late onset of sepsis (dos Santos *et al.*, 2011). Transfusion was postulated to be associated with sepsis in VLBW infants. It was suggested to be related with transfusion related inflammation and immunomodulation (E Hassan, 2015). Nevertheless, there is no reported association between transfusion and sepsis in preterm infants by Keir (Keir *et al.*,

2016). Transfused VLBW preterm infants had lower gestational age and length of stay compared to non-transfused preterm infants (E Hassan, 2015).

2.5 Benefits of transfusion in preterm infants

The advantage of transfusion is to improve the tissue oxygenation in order to prevent organ failure (Fredrickson *et al.*, 2011). In bleeding cases, it is important to replace the blood loss and increase the circulating volumes. Transfusion can also prevent apnoea in preterm infants and promote weight gain (Bell, 2008).

In each transfusion, benefits should outweigh the risks to the recipient. The concept of judicious use of blood should always be applied (Christensen and Ilstrup, 2013; Iskander *et al.*, 2018).

2.6 Research justification and benefits

Extremely preterm infants often need multiple transfusions during their stay in hospital especially red blood cell transfusion. Up to 50−80% of the ELBW preterm infants require at least one packed cell transfusion in Neonatal Intensive Care Unit (Strauss, 2017). However, few studies suggested that red blood cell transfusion leads to mortality and morbidities in preterm infants (Wang *et al.*, 2016).

This retrospective study was done in Hospital Universiti Sains Malaysia (Hospital USM). The purpose of this study was to determine morbidities and an association between red blood cell transfusion and in-hospital mortality in extremely

preterm infants in Hospital USM. The total red cell transfusion frequency and volumes were measured at day 7, day 30 and day 60 of admission. The associated in-hospital mortality and morbidities were based on previous studies and practices in Hospital USM. The morbidities included were the development of ROP, IVH, NEC, CLD, sepsis and length of stay.

In Hospital USM, there were estimated 40 extremely preterm infants admitted annually. This study provided evidence-based data on morbidities among extremely preterm infants and an association between red blood cell transfusion and in-hospital mortality among extremely preterm infants related to red blood cell transfusion.

Currently, there was no local data regarding red blood cell transfusion in extremely preterm infants and its related clinical outcomes. Besides, there was limited study on association between volumes of transfusions and mortality or morbidities in extremely preterm infants. In the other hand, different studies describe different risks and benefits of RBC transfusion among this group of patients.

2.7 Research question

What is the association between frequency and volumes of transfusion with in-hospital mortality in extremely preterm infants?

2.7.1 Research hypothesis

i. **Null hypothesis:**

There is no association between frequency and volumes of RBC transfusion and in-hospital mortality.

ii. **Alternative hypothesis:**

There is association between frequency and volumes of RBC transfusion and in-hospital mortality.

2.8 Conceptual framework

The conceptual framework was shown below:

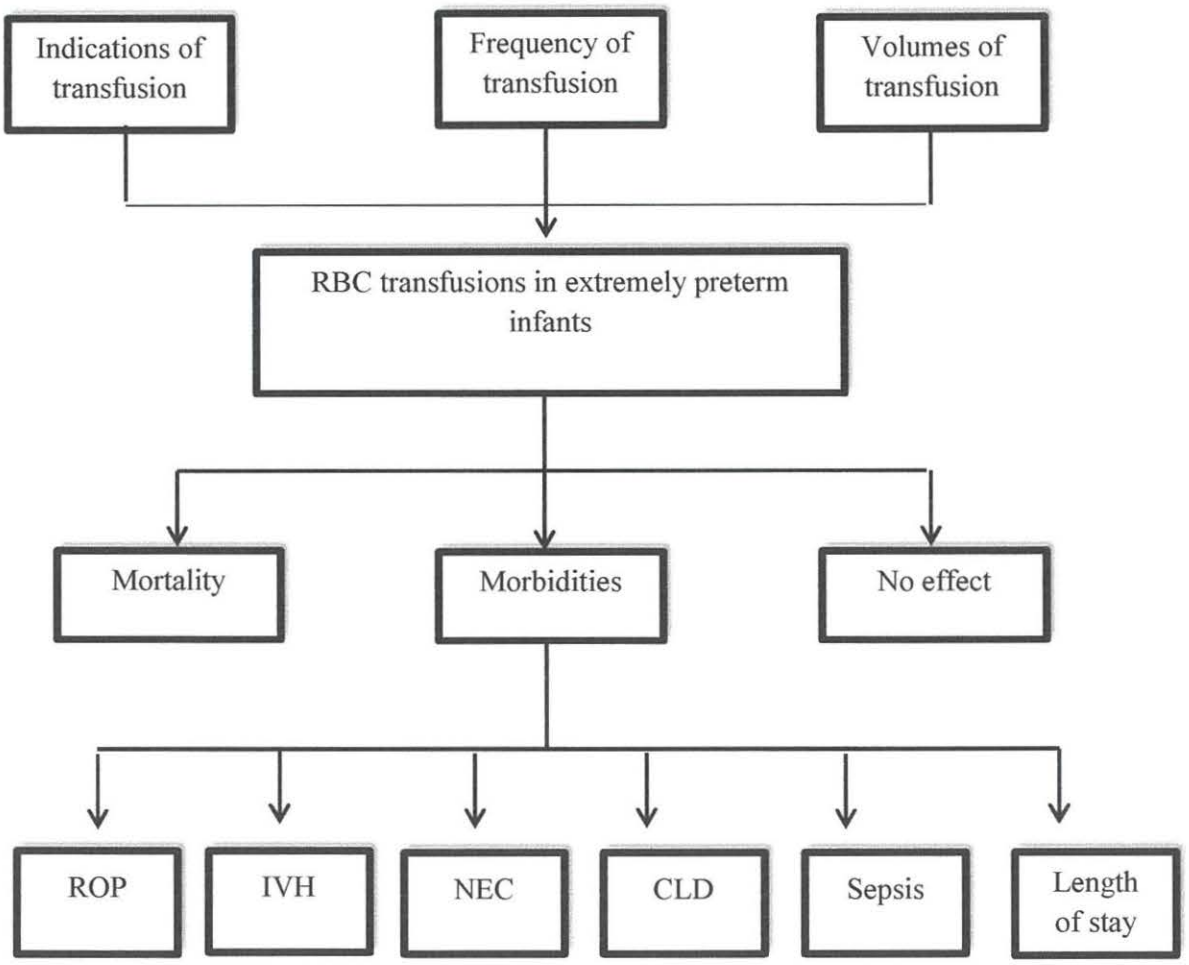

Figure 2.1: Conceptual framework

CHAPTER 3

OBJECTIVES

3.1 General Objective

To determine clinical outcomes in extremely preterm infants who received RBC transfusion at Hospital USM.

3.2 Specific Objectives

i. To determine the frequency, volumes and indications of RBC transfusion within 7 days, 30 days and 60 days of admission in extremely preterm infants.

ii. To determine the clinical outcomes such as in-hospital mortality, retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), necrotizing enterocolitis (NEC), chronic lung disease (CLD), sepsis and length of stay (LOS) in extremely preterm infants who received RBC transfusion.

iii. To determine the association between frequency and volumes of red blood cell transfusion at 7 days, 30 days and 60 days and in-hospital mortality in extremely preterm infants who received RBC transfusion.

CHAPTER 4

METHODOLOGY

4.1 Study Location

This study was conducted at Hospital USM. Hospital USM is located at Kubang Kerian, Kelantan. It has Level III NICU which provides care and support for preterm infants with paediatric sub-specialities including neonatologists. It is one of the referral centre for neonatal care in Kelantan. There are three wards for neonatal care which includes Nilam 1, Nilam 2 and 1 Timur Belakang. Bed occupancy for Nilam 1 and Nilam 2 is 35 beds and 1 Timur Belakang is 35 beds.

4.2 Study Design

This was a retrospective cohort study by medical record review of patients that includes all extremely preterm infants who received red cell transfusion at Hospital USM from 1st January 2014 until 30th June 2017.

4.3 Subject Criteria

Patients that fulfilled the inclusion and exclusion criteria were eligible for the study. The details of the subjects were collected as in research proforma.

4.3.1 Inclusion Criteria

i. Admission between 1st January 2014-30th June 2017
ii. Gestational age less than 28 weeks
iii. Born in Hospital USM
iv. Preterm infants who received RBC transfusion during the hospital stay

4.3.2 Exclusion Criteria

i. Infant with chromosomal anomalies, major structural abnormalities and metabolic syndrome that compromise normal physiological function
ii. Died in the first 24 hours of life
iii. Preterm infants who did not received RBC transfusion

4.4 Calculation of Sample Size

Objective 1: To determine the frequency, volumes and indications of RBC transfusion within 7 days, 30 days and 60 days admission in extremely preterm infants.

Sample size for descriptive study of frequency and volumes of transfusion and indications was based on mean of frequency of transfusion in study by Von Lindern et al., 2011 Calculation is using single mean formula:

Single mean $n = \left(Z \frac{\sigma}{\Delta} \right)^2$

$n = \left(1.96 \frac{2.3}{0.5} \right)^2$ + 10 % drop out

$= 41.5 + 4.2$

$= 45.7$

$= 46$

σ = Standard deviation

Δ = precision

Z = Z statistic for level of confidence =1.96 (95% confidence interval)

The sample size calculated was **46** which added with 10% drop out rate.

ii. To determine the clinical outcomes such as in-hospital mortality, retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), necrotizing enterocolitis (NEC), chronic lung disease (CLD), sepsis and length of stay (LOS) in extremely preterm infants who received RBC transfusion.

Sample size for descriptive study on clinical outcomes of patient including in-hospital mortality, chronic lung disease (CLD), sepsis, necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH) and length of stay (LOS) was calculated based on study by *Wang et al., 2016*. Calculation was using single proportion formula.

Single proportion, $n = \left(\frac{z}{\Delta}\right)^2 p(1-p)$

$n = \left(\frac{1.96}{0.1}\right)^2 0.18\ (1\text{-}0.18) + 10\ \%$ drop out

$= (1536.64 \times 0.18 \times 0.82) + 10\%$ drop out

$= 56.7 + 5.7$

$= 62$

P = expected prevalence or proportion

Δ = precision

Z = Z statistic for level of confidence =1.96 (95% confidence interval)

The sample size calculated is **62** which added with 10% drop out rate.

Objective 3: To determine the association between frequency and volumes of red blood cell transfusion at 7 days, 30 days and 60 days and in-hospital mortality in extremely preterm infants who received RBC transfusion.

Sample size for association between frequency of RBC transfusion and in-hospital mortality was calculated based on study by *MacDorman et al., 2014*. Calculation was using two proportions formula.

Two proportions, $n = \frac{p_1(1-p_1)+p_2(1-p_2)}{(p_1-p_2)^2}(z_\alpha + z_\beta)^2$

$$n = \frac{(0.208\ (\ 1\text{-}0.208) + 0.006\ (1\text{-}0.006)\ )x\ (1.96+ 1.28)^2}{(0.208\text{-}0.006)^2}$$

n= 43.9 x 2 + 10% drop out

n= 87.8 + 8.8

n = **96**

P1 = proportion of the mortality among extremely preterm infant

P2 = proportion of the mortality among general population

Z_α = 1.96 for α= 0.05 (two tailed)

Z_β = 0.84 for 80% power or 1.28 for 90% power

The sample size calculated was **96** which added with 10% drop out rate. Based on the calculations from each objectives, bigger sample size was 96 from the third objective, thus the final sample size for this study was 96.

In this study, total of 97 infants were included which fulfilled the inclusion and exclusion criteria.

4.5 Study Duration

The study duration was from 1st June 2017 until 30th November 2018.

4.6 Development of Research Proforma

A research proforma was develop to collect the data of red blood cell transfusion and clinical outcomes in extremely preterm infants in Hospital USM. It was prepared to ensure the data collection was complete and easy to be reviewed. It was developed based on literature review and previous studies on neonatal transfusion. An expert review of the proforma was done with Dr Nur Arzuar Bin Abdul Rahim and Associate Professor Dr Noraida Binti Ramli before collecting the data. The research data were divided into 5 mains components:

i. Demographic data which includes the data of age, gender and race

ii. Maternal history which includes the relevant maternal antenatal history including underlying illness in the pregnancy such as diabetes, hypertension, anaemia, PPROM, bleeding placenta praevia, cord prolapse and abruptio placenta. Beside, information regarding antenatal dexamethasone administration was also been documented.

iii. Birth history which includes date and time of birth, gestational age, method of delivery, Apgar score and birth weight. Besides, data regarding surfactant administration also documented.

iv. Data on transfusion details and investigations results includes date of transfusion, indications and volumes of each transfusion. Data on hemoglobin level prior to each transfusion was also documented.

Total frequency and volumes of packed cell transfusion at day 7, day 30 and day 60 were calculated and documented.

v. Data on patient's outcomes includes morbidities which are ROP, IVH, NEC, CLD, sepsis and LOS in hospital. If patient passed away, the date, time and causes of death were also documented.

4.7 Data Collection and Sampling Method

Purposive sampling was used in this study. List of patients were traced in the admission book in NICU. All the data involved in this study involving the patients' data were gathered from;

i. Bed Head Ticket (BHT) traced from the Record Unit Hospital USM
ii. Blood Bank Information System used in Hospital USM which was MyTransfusi for transfusion data

4.8 Statistical analysis

Statistical analysis was performed using SPSS version 24.0 for window-software (SPSS, Chicago Illinois, USA) to present the descriptive statistic in objective 1 and 2. Descriptive statistics was used for analysis of the demographic data. The

categorical data were expressed as frequency (percentage) and numerical data as mean (SD), minimum value and maximum value.

The multiple logistic regressions were used to determine the association between frequency and volumes of transfusions at 7 days, 30 days and 60 days and in-hospital mortality in objective 3. Simple linear regression (SLR) was performed to see the association with p value <0.25 was considered for variable selection into the model in multiple linear regression (MLR). P value of < 0.05 was considered as statistically significant.

4.9 Ethical Issue

Ethical approval from Hospital USM was received on 18th May 2017 (Appendix 1). The Human Research Ethics Committee code is: USM/JEPEM/16120603.The data was collected from the patient's medical records and MyTransfusi in Hospital USM. The medical record data was recorded using an index number that the respective patients could not be identified. As consequences the data that been collected was completely anonymous.

4.10 Conflict of Interest

There was no conflict of interest during the duration of this study by investigator.

4.11 Community sensitivities and benefits

Majority of extremely preterm infants receive at least one red cell transfusion as they frequently anaemic. Appropriate transfusion was vital in order to balance transfusion benefits against risks (National Comparative Audit, 2010). However, many study reported the possible adverse outcomes in relations of red blood transfusion although a causal link has not been demonstrated (Wang *et al.*, 2016).

At current, our local data on this issue was not well established. It was hope that this study can fill the knowledge gap and support evidence base medicine in developing red blood cells transfusion guideline for preterm infants in Malaysia.

This study can benefit community by providing evidence base data in relation of current practice of red blood cells transfusion for extremely preterm infants. There was no issue related to community sensitivities and value in this retrospective study.

4.12 Operational definition

Infants	: Babies from birth up to 1 year of age.
Neonates	: Babies less than 28 days of life.
Preterm infants	: Babies who born alive before completed 37 weeks of pregnancy
Extremely preterm infants	: Babies born less than 28 weeks of gestation
Clinical outcomes	: Referred as in-hospital mortality and morbidities measured in the study.
In-hospital mortality	: Refer as death within 60 days of life in this study.