# A STUDY ON THE RELIABILITY OF BILISPECT TO MEASURE BILIRUBIN LEVELS IN TERM NEONATES

BY:

DR TENGKU HASNITA TENGKU HUSSAIN

Dissertation submitted in partial fulfillment of the requirements for Degree of Master of Medicine (Paediatrics)


May 2015

## ACKNOWLEDGEMENT

In the name of Allah, The Most Gracious and The Most Merciful, many thanks to Him for granted me His grace in completing this thesis. Alhamdulillah, praise be upon Him.

Secondly my big thank you goes to my beloved family especially my better half and my mother for endless love, support and courage that I received throughout the program. Without all those, probably I would not reach this state.

I would like to express my special thanks and deepest appreciation to my supervisor, Professor Dr Hans Van Rosternberghe. His positive guidance smooth my way through this journey. Special thanks also to Ass Prof Dr Noraida as the neonatologist incharge of neonatal wards. I would like to extend my appreciation to post-graduate students in Biostatistic Department who helped me with the statistical part.

To all the junior doctors, colleagues and nurses mainly in neonatal wards, many thanks for all your cooperation in helping me to complete this study. I might not be able to return all the good deeds, but I am sure it is counted.

**TABLES OF CONTENTS** **PAGE**

LIST OF TABLES

LIST OF FIGURES

ABREVIATIONS

| | |
|---|---|
| HO | House-officer |
| HUSM | Hospital University Sains Malaysia |
| ICC | Intra Class Correlation |
| NICU | Neonatal Intensive Care Unit |
| SCN | Special Care Nursery |
| TSB | Total Serum Bilirubin |
| TcB | Transcutaneous Bilirubin |

ABSTRAK

**PENGENALAN:**

Jaundis menrupakan masalah yang biasa dihadapi oleh bayi di awal usia kelahiran. Pemantauan bagi masalah ini adalah penting untuk mengelakkan berlakunya komplikasi. Bilispect merupakan satu alat yang boleh memberi bacaan jaundis melalui kulit. Alat ini boleh membantu pengurangan sampel darah untuk bayi.

**OBJEKTIF:**

Untuk menilai kebolehpercayaan Bilispect sebagai alat untuk mengukur tahap jaundis bayi, sebelum dan selepas rawatan ‘phototherapy’.

**KAEDAH:**

Kajian ini merupakan kajian ‘cross sectional’ di HUSM untuk bayi yang mengalami masalah jaundis dan memenuhi kriteria yang ditetapkan.

**KEPUTUSAN:**

Sejumlah 74 bayi telah menyertai kajian ini. Seramai 44 bayi menyertai kedua-dua bahagian, sebelum ‘phototherapy’ dan selepas ‘phototherapy’. Penambahan seramai 15 orang bayi samada untuk bacaan sebelum ‘phototherapy’ atau selepas ‘phototherapy’. Ini menjadikan 59 sampel untuk kedua-dua bahagian. Keputusan menunjukkan tiada perbezaan dalam distribusi jantina di kedua-dua bahagian. Purata umur adalah lingkungan 3 hari selepas kelahiran dan purata usia kandungan ialah 39 minggu. Perbezaan bacaan keputusan di antara dua kaedah bagi sebelum ‘phototherapy’ ialah -132.6 ke 190.8. Manakala bagi keputusan selepas ‘phototherapy’ ialah -45.2 ke 164.0. Penilaian Pearson correlation ialah lemah iaitu nilai r 0.466 dan pertengahan iaitu nilai r

0.566 bagi kumpulan sebelum dan selepas ‘phototherapy’. Peratusan persamaan di antara kumpulan ujian darah bilirubin dan bacaan Bilispect ialah tinggi dengan nilai 93.22% bagi sampel pre-‘phototherapy’ dan 96.6% bagi sampel pasca-‘phototherapy’.

**KESIMPULAN:**

Keputusan menunjukkan bacaan Bilispect berkadaran dengan ujian darah bilirubin dari segi statistik namun berikutan terlalu banyak sampel yang mempunyai perbezaan yang besar, penggunaan Bilispect dalam klinikal adalah terbatas.

ABSTRACT

**INTRODUCTION :**

Neonatal jaundice commonly occurred in newborn babies. The monitoring of it becomes very important to prevent complications. Blood taking is an invasive procedure that associated with pain in newborn. Availability of new devices with updated technology leading to non invasive monitoring in newborn. Development of Bilispect as transcutaneous bilirubinometer gave opportunities to reduce blood taking in newborn.

**OBJECTIVES:**

To test reliability of Bilispect in monitoring bilirubin level of term babies, pre and post-phototherapy.

**METHODS:**

This study was a cross sectional study in HUSM for term babies with neonatal jaundice who fulfilled inclusion criteria.

**RESULT:**

There were total of 74 babies involved in this study. Among these, 44 babies involved in both part pre-phototherapy and post-phototherapy reading. Additional of 15 babies only involved in one part of the study, either pre-phototherapy or post-phototherapy. Total of 59 samples were collected in each part. The gender distribution was equal for pre and post-phototherapy group. The mean age was 3.9 days with mean gestation was 39weeks. The differences of serum bilirubin and Bilispect reading for pre-phototherapy result was from -132.6 to 190.8. For the post-phototherapy result, the differences value range from -45.2 to 164.0. Pearson

correlation value derived were weak positive correlation (0.466) and moderate positive correlation (0.566) for pre and post-phototherapy respectively. The Bland Altman plot degree of agreement between these methods were 93.22% for pre-phototherapy and 96.6% for post-phototherapy.

**CONCLUSION:**

The results of this study showed that the Bilispect device had a statistically significant correlation with the serum bilirubin values. However for too many samples the differences between the two were too big to support clinical usage of the Bilispect at this point in time. In conclusion serum bilirubin is still a gold standard for jaundice monitoring in newborn.

# CHAPTER 1: INTRODUCTION

## 1.1 History of the jaundice

The word jaundice comes from the root ‘jeune’, which means yellow in French. The yellow color resulted from the unconjugated, non-polar, lipid soluble bilirubin pigment in the skin. Neonatal jaundice is a common problem. Up to 60% of term babies have jaundice and it is more common in premature babies; around 80%.

Even though neonatal jaundice is in most case benign and self-limiting (William Cashore; 2010), in babies with severe jaundice, the unconjugated bilirubin enters the brain and is neurotoxic. That is why managing neonatal jaundice is very important as early treatment can avoid the progression to severe jaundice. There are several risk factors that can put the baby at higher risk to develop jaundice. By recognizing the risk factors, earlier treatment can be started and the unwanted outcomes from severe jaundice can be avoided.

Mild jaundice is part of a physiological process in newborn babies (Blackburn, 1995). A normal transition in the bilirubin metabolism leads to this problem. In utero, the placenta is the place where bilirubin is excreted for the baby. After the delivery mainly the liver is responsible for the metabolism and excretion of bilirubin. Due to immaturity of the liver in newborns, accumulation of bilirubin occurs. The high number of red blood cells in newborn and the shorter life span of the individual red blood cell carrying foetal haemoglobin also contribute to this problem. In premature babies, the organs are more immature compared to term babies and the incidence of haemolysis is higher, which explains the higher jaundice incidence in premature.

## 1.2 Risk factors for neonatal jaundice

Prematurity is one of the risk factors for neonatal jaundice and sometimes can lead to early onset and severe jaundice. Thus, knowing the correct gestational age for the babies is vital as we might miss early detection of severe jaundice in premature babies. ABO incompatibility and Rhesus incompatibility are also risk factors for neonatal jaundice (William, 2010). It was made compulsory for pregnant ladies to do the blood group and rhesus investigation upon booking. In a way, the health staff should be more alert looking for jaundice in babies of a rhesus negative mother or O blood group mother. As we practice in HUSM, a baby of a rhesus negative mother must be admitted straight away at birth for monitoring of early onset jaundice. The jaundice work up is taken at birth.

There is higher incidence of jaundice in breastfed babies compared to formula-fed babies (Brown LP et al, 1993). However the recommendation is to continue breastfeeding. This is because; breastfeeding alone will not lead to severe jaundice. Another important risk factor for jaundice is G6PD deficiency and neonatal sepsis. In Malaysia, the screening program is compulsory for G6PD deficiency in all newborn babies using the cord blood. As in HUSM, postnatal ward nurses will trace the result and inform the respective health care teams and the mother. The baby will be admitted for observation and, if jaundice occurrs, early treatment, till day 5 of life. Further education on G6PD deficiency will be given to parents. Neonatal sepsis needs to be recognized and treated early. Delay in treating neonatal sepsis will impair the bilirubin metabolism and leads to severe jaundice. Risk factors for jaundice are listed in table 1.

Table 1: Risk factors/causes for neonatal jaundice.

| | | |
|---|---|---|
| Risk factor for jaundice | Common risk factors | • Prematurity<br>• ABO and Rhesus incompatibility<br>• G6PD deficiency<br>• Cephalohematoma or bruises<br>• Exclusive breastfeeding<br>• Sepsis<br>• Polycythemia<br>• Hypothyroidism<br>• Gilbert Syndrome |
| | Less common risk factors/causes | • Crigler-Najjar syndrome, type II and II<br>• Galactosemia<br>• Tyrosinemia<br>• Hypermethionemia<br>• Hypopituitarism |

## 1.3 Pathogenesis of jaundice

Bilirubin is a product of hemoglobin breakdown. It is produced in the reticuloendothelial system. Hemoglobin will break into heme and globin. Globin will be recycled. Heme will be transformed to biliverdin by the action of heme oxygenase. Next, water soluble biliverdin is reduced to unconjugated bilirubin. Because of the hydrophobic nature, unconjugated bilirubin is transported in the plasma tightly bound to albumin. A minute fraction of unconjugated bilirubin is not bound to albumin, and it is this fraction that has the potential to cross the blood brain barrier, leading to the complication of neurotoxicity. When bilirubin reaches the liver, it is transported into the liver cell and it binds to ligandin. Uptake of bilirubin into the hepatocytes is increased with the increment of ligandin concentration. Phenobarbitone can increase ligandin concentration. Bilirubin is bound to glucuronic acid (conjugated) in the hepatocytic endoplasmic reticulum in a reaction catalyzed by uridine diphosphoglucuronyltransferase (UDPGT). Monoconjugates are formed first and predominate in the newborn. Diconjugates appear to be formed at the cell membrane and may require the presence of the UDPGT tetramer. After being converted to a water soluble molecule, bilirubin is excreted in the bile and reaches the intestines. In the intestines, about 20% will be reabsorbed to the enterohepatic circulation after being converted back to unconjugated by the beta glucoronidase enzyme. The rest will be excreted via urine as urobilinogen or in the feces as stercobilinogen.

## 1.4 Monitoring of jaundice

The most non-invasive method of monitoring jaundice is by clinical observation and estimation. Jaundice is visible when the bilirubin level is around 86-120umol/L (Malaysia CPG –Neonatal jaundice 2014). This method is referring to Kramer's rule as a guideline (Kramer Ll, 1969) (Figure 1). The body area is divided into 5 categories. The severity of jaundice is increasing in trend from area 1 to area 5.

It is very important to train the primary healthcare staff to detect the jaundice in newborn babies. According to Kramer's rule, the assessment is made by putting the baby on the daylight or white light source. It is inaccurate after the phototherapy. This method divides the baby into 5 zones, in a cephalocaudal progression. According to the zone, the estimation of total serum bilirubin is increasing in trend towards caudal. However this method easily underestimates or overestimates the baby bilirubin level, especially in darkly pigmented babies (Demott K, et al; 2006) (Levene MI et al; 2008).

Development of medical equipment, based on clinical needs has become possible thanks to the advancement of technology. The nightmare of taking blood from neonates for laboratory investigations has been a main driver for the development of alternative devices allowing non-invasive monitoring. It started with the transcutaneous bilirubin meters which were measuring basically the colour of the skin. These devices tend to be highly dependent on skin color and on accumulation of bilirubin in the skin, which is heavily affected by phototherapy.

Clinical assessment of jaundice begins with visual assessment as the least invasive monitoring (Michael Kaplan et al; 2008). Then non-invasive devices can be used but the device must be reliable so that there is no under treatment or over treatment of neonatal

jaundice occurs. It is also in important to have a device that can give the fast reading on jaundice so that decision can be made on time.

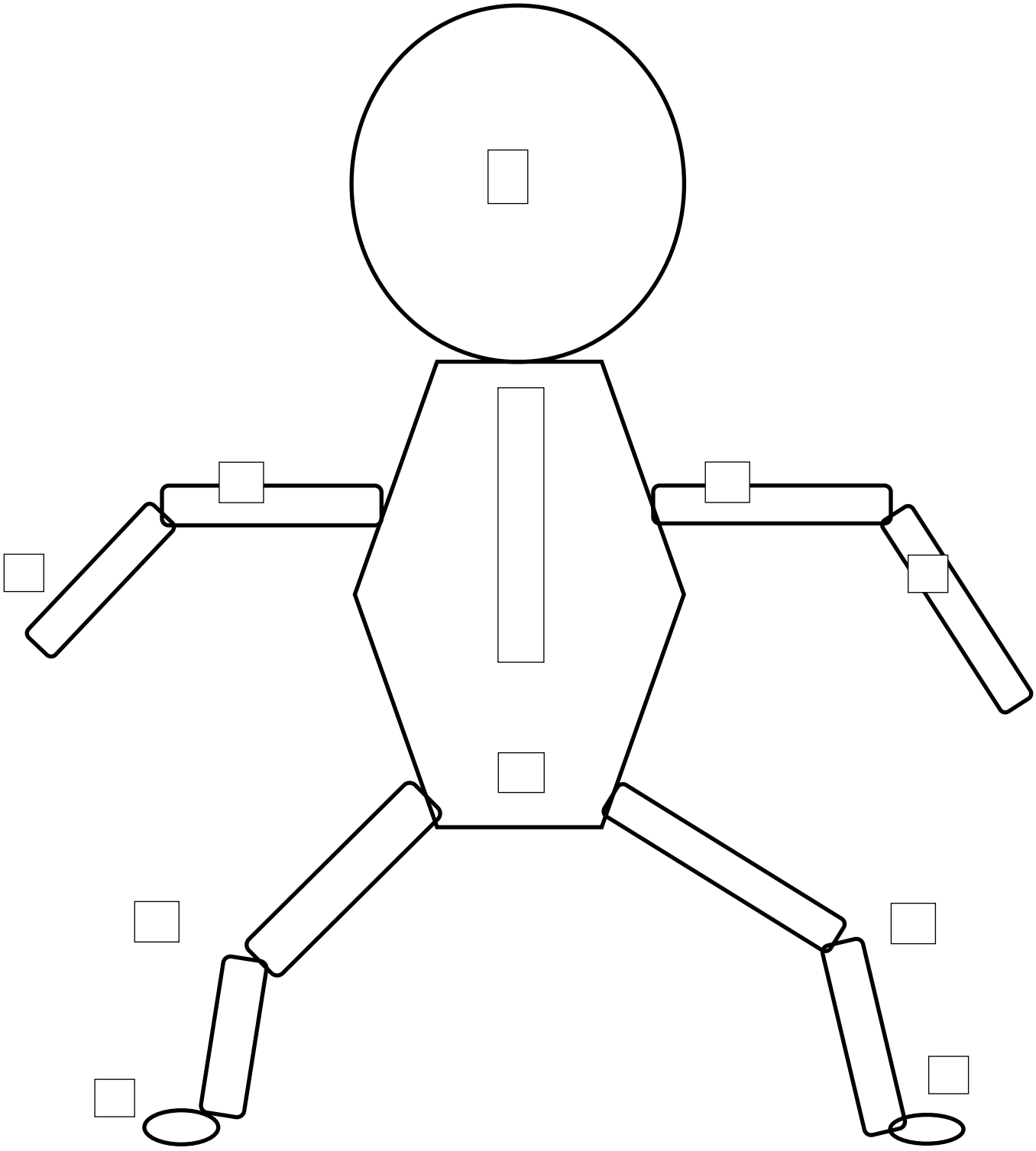

Figure 1: Schematic diagram of Kramer’s rule. Numbers 1-5 referring to the respective areas.

The gold standard for monitoring neonatal jaundice is the total serum bilirubin (TSB) level. Besides TSB, other investigations such as blood grouping with Coomb's test, Rhesus grouping and full blood count are also included in the jaundice work up. In HUSM, TSB sample will then be processed in the laboratory by the colourimetric method using Architect C800 (since 2009) and Olympus AU680 (since 2013) machine. The lacking of TSB measurement is the waiting time is not guaranteed. The optimum timing for the result to be available is within 30 minutes. However, the process of sending the sample from ward to the laboratory itself required additional timing.

The current practice requires the patients to go to the clinics for blood taking. The compliance to this is not always good. Some people may postpone the visit to the clinic and search for alternative methods. That is why there is a need for alternative ways to determine the severity of the neonatal jaundice.

Neonates do experience pain. Thus blood taking procedure as in neonatal jaundice is associated with pain. However, it is a need to do the serum bilirubin in order to give appropriate treatment for the neonatal jaundice. The failure of detecting and monitoring of neonatal jaundice can lead to unwanted complications. Wrong justification of neonatal jaundice can lead to over-treatment and under-treatment of neonatal jaundice.

## 1.5 New modalities in monitoring

The new development in jaundice monitoring is the usage of cutaneous bilirubin meter. The transcutaneous bilirubin (TcB) meter gained the popularity after being introduced. Apart from non-invasive, this device can give immediate result which cut down the waiting time for TSB to be ready. Increasing evidence showed that newer

transcutaneous bilirubin measurement device accurately predict bilirubin levels without blood taking. The great advantage for this non-invasive device are less traumatized to the patient, reduce the risk of infection and potential cost saving as well (KA Jangaard et al; 2006). To balance between the non invasive method and appropriate management of neonatal jaundice, each new device needs evaluation.

Despite having a good prospect on measurement, TcB is not yet acceptable as a gold standard. The device is acceptable as a screening tool mainly because the reading is affected after the phototherapy (Yamauchi Y, 1990). Several TcB devices will be described here, which are Bilicheck, Bilimed and JM-103. Earlier devices give the reading of measurement in index number which then be converted to the actual bilirubin unit. Newer device now can give the result in the actual value.

Bilicheck is a device which used multiple wavelengths by spectral reflectance. It offers better measurement despite different in skin pigmentation by measuring the optical densities of bilirubin, melanin and hemoglobin in the skin. Prior to usage, it needs to be calibrated using Bilical. Bilicheck gives the reading of 5 average measurements. Bilicheck was tested in Hospital Universiti Sains Malaysia (HUSM) in 2006 and the result showed it was a reliable device (Zaidi et al, 2006). Bilimed is a product from Medick, S.A France. The placement of the probe is not directly to the skin, but 2cm apart. It produces single beam generated from 10 light emitting diodes. JM-103 is another device from Draeger Medical System. The measurement using 2 wavelengths and dual optical path system. There was a study by Francesco R et al (2012) comparing those 3 TcB devices on a multiracial populations Francesco R, et al, 2012). It concluded that Bilicheck and JM-103 gave the same weightage of

reliability as a screening tool in neonatal jaundice. However the Bilimed is not suitable.


Figure 2: Bilicheck device

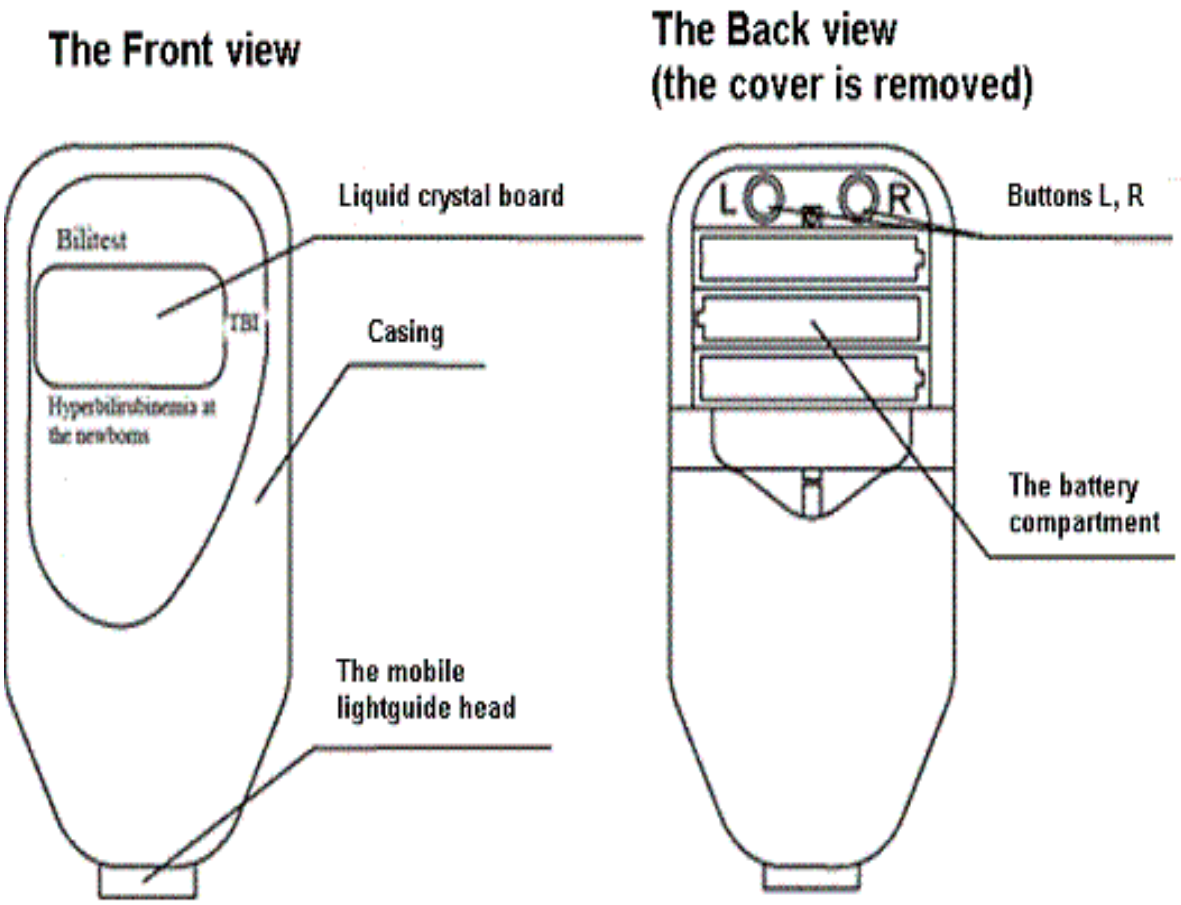


Figure 3: Bilimed device


Figure 4: JM-103 device

## 1.6 Bilispect

Advancement in technology also leads to development of Bilispect device. This is a hand held device for TcB measurement. Bilispect goes beyond the skin (MBR Optical System, 2012). It measures the bilirubin in the skin and the blood. Therefore it can be used before, during or after phototherapy. That is why in this study, the measurement included before and after phototherapy. It also can be used regardless of race different because it is not affected by skin color. It is a handy device that gives TcB measurement within 20 seconds. The button sensor needs to be place on baby's skin and the reading will be ready in seconds. Some of the projected light is absorbed by the various components of tissue and bilirubin, while some of it is reflected.

Another waveguide transmits the light reflected as a result of the physical conditions back to the device. A spectrometer breaks the light down into its separate wavelengths and an electronic evaluation unit analyses it. The resulting data is then processed using an algorithm developed by MBR and the derived rate of total bilirubin value is the resulting total bilirubin on the display of the device.

The device is powered by batteries. A main adapter is included. With fully charged batteries, measurement for five hours is possible.


Figure 5: Bilispect device

## 1.7 Rationale of the study

The emergence of advance technology in medicine needs further statistical evaluation. Thus the need to test the reliability of this transcutaneous bilirubin meter is very important. The transcutaneous device that was introduced before is affected by phototherapy, which leads to limited usage for in patients. Bilispect is proposed as it can be used before and after phototherapy. Therefore, it is very important to do this study for future benefits of the babies with neonatal jaundice. If it is reliable as compared to TSB, it can be the important device in each neonatal ward. Bilispect can reduce the workload of the staff mainly doctors and can minimize blood taking in babies. This will prevent the underestimation of jaundice severity which will lead to severe neurotoxicity complications, as well as overtreatment which warrant unnecessary investigations and treatment for the babies.

## 1.8 Treatment And Complication

Phototherapy remains the mainstay of treatment for neonatal jaundice (Malaysia CPG management of neonatal jaundice, 2014). Newborn will be categorized according to gestational age, chronological age and risk factors. Several types of phototherapy are available; these are fluorescent tube, Light Emitting Diode (LED), fibreoptic and halogen bulbs. Other contributed factors for the efficacy are the blue light range of the phototherapy, the distance from photo to the babies and usage of reflecting curtain (Djokomuljanto et al; 2006). Exchange transfusion (ET) comes into the picture when TSB exceed phototherapy level (Malaysia CPG management of

neonatal jaundice, 2014). ET can reduce the TSB up to 43% (Salaas AA, 2008). The most common method for ET is using the umbilical vein catheter (UVC).

The most unwanted complication of neonatal jaundice is acute bilirubin encephalopathy or kernicterus. There was no local study on the incidence of kernicterus for comparison. In North America and Europe, the estimated incidence of kernicterus ranges from 0.4 to 2.7 cases per 100,000 live births among term and late preterm babies (Maisels MJ et al; 2012). The exact value for TSB level and kernicterus occurrence is unknown (Weng YH, 2011). Long term sequels of bilirubin neurotoxicity are athetoid cerebral palsy, severe deafness and intellectual disability (Bhutani VK, 2011).

# CHAPTER 2: OBJECTIVES

## 2.1 General Objective

To correlate the result of Bilispect in comparison with serum bilirubin in term newborn babies.

## 2.2 Specific Objectives

1. To correlate the result of Bilispect in comparison with serum bilirubin in term babies prior to phototherapy
2. To correlate the result of Bilispect in comparison with serum bilirubin in term babies after starting photo therapy

## 2.3 Research hypothesis

1. Bilispect reading is similar to serum bilirubin in newborn babies prior to phototherapy
2. The reading of Bilispect is not affected even after phototherapy

# CHAPTER 3: METHODOLOGY

## 3.1 STUDY DESIGN

This study was a prospective cross sectional study.

## 3.2 SETTING

This study was carried out in Hospital Universiti Sains Malaysia (HUSM), a tertiary teaching hospital in Kelantan, the most northeastern state of Peninsular Malaysia. HUSM receives referrals from the whole Kelantan and also from the bordering districts of neighbouring states. During the study period, neonatal jaundice patients were admitted either to the neonatal intensive care unit (NICU) or to the special care nursery (SCN), depending on the condition of the baby and the severity of jaundice.

## 3.3 SUBJECTS

The study population was recruited from babies who were admitted to the NICU or to the special care nursery during the study period that fulfilled the following inclusion and exclusion criteria.

## 3.4 INCLUSION CRITERIA

The inclusion criteria were:

1. Neonatal jaundice during the first 7 days of life
2. Delivery at term (completed 37 weeks of gestation)

## 3.5 EXCLUSION CRITERIA

The exclusion criterion was:

1. Terminally ill babies (conservative management and not for any blood taking)

## 3.6 DATA COLLECTION

The main data collected for this study consisted of 59 serum bilirubin readings in jaundiced babies fulfilling the inclusion criteria pre-phototherapy and another 59 post phototherapy. Sampling of subjects and data was based on the convenience of the main investigator since there was only one unit of Bilispect available.

The pre-phototherapy blood samples were collected according to the ward protocol where all babies admitted for jaundice, or admitted for other reasons but detected clinically to have jaundice, were subjected to blood taking for serum bilirubin, blood group with direct Coombs test and full blood count with reticulocytes. The sample was obtained via venous puncture. The reading of Bilispect was taken immediately after the blood taking. If the baby was found to have bilirubin levels below phototherapy, no further data were collected.

The post phototherapy blood samples were collected during blood taking, four hours after start phototherapy or on the next morning, depending on the severity of jaundice and the baby's condition. This next blood taking was

also followed immediately by the Bilispect reading. Also for these post phototherapy data collection, the sampling of the subjects were done based on the convenience of the main investigator and whenever possible it was done for babies included in the study for pre phototherapy data collection too.

Serum bilirubin was sent to the chemical pathology laboratory. The sample was not covered, but was sent to the laboratory following the protocol from the ward which tries to ensure speedy delivery to the laboratory. The time of sample collection and sample being processed was not recorded. The sample was then processed using the colorimetric method, using Architect C8000 (Abbot, California) or Olympus AU680 (Beckman Coulter, California) and the results were obtained in the same units as Bilispect; µmol/L. After the measurements, the samples were destroyed as per laboratory standard protocol. The laboratory staff will do the calibration of the machine daily basis to ensure the result is reliable.

The baseline data of the included subjects and the results of the blood investigations and the Bilispect reading were collected using a standard data collection sheet which is found in the Appendix 2 of this thesis.

## 3.7 BILISPECT READING

Bilispect used in this study was a free loan device from the company (Pall-Thai Medical Sdn Bhd). Only one unit was available. It was operated with the built in battery, which can last for 5 hours. It is a handy device, easily operated with button function. The command language is in English. Every time the Bilispect is turned on, it needs to be calibrated. After the calibration, the reading can be done and the result will be available after 10-20 seconds. The results displayed as µmol/L. One reading is taken on every measurement.

A total of 4 persons were involved as observer, which were the investigator and 3 other house-officers (HO). The investigator was trained by the distributor to use the device during a formal training session of about 30 minutes. Subsequently the main investigator trained the 3 HO prior to starting of this study (for about 30 minutes). There was no inter-observer and intra-observer reliability test done.

For sample collection, the site used was either at right or left forearm. The skin probe was cleaned using the alcohol wipe in between patients to minimize risk of infection. A picture of the Bilispect device during usage is shown in figure 6.

Figure 6: Picture of Bilispect usage

### 3.8 Sample size

The sample size of the study was calculated using the Intra Class Correlation (ICC) from the website of Biostastic Unit, USM (http://www.medic.usm.my/biostat/images/files/Sample_size_calculation_v1_7.xls). ICC was used based on the objectives of this study. Sample size was not required for general objective. For the specific objectives (1) and (2), the sample size calculation is using the intra Class Correlation (ICC) software as follows:

| Sample size calculation – Intra Class Correlation | |
|---|---|
| Observation/Subject (n) | 2 |
| α | 0.050 |
| Power (1-β) | 0.800 |
| Acceptable reliability (p0) | 0.8 |
| Expected reliability (p1) | 0.9 |
| Drop-out | 20% |
| | |
| Sample size | 46 |
| Corrected sample size | 58 |

Table 2: Sample size calculation

As this study was compared the two methods, subject number was put as 2.

α is type 1 error.

The power of the study was 80% with 20% drop out rate.

Corrected calculation: (0.2 x 46) + 46=55.7

For the purpose of this study, all newborns fulfilled the inclusion criteria were enrolled in the study during the study period.

### 3.9 Ethical issues

All parents of the babies involved in this study gave their written informed consent for their babies to be included in this study. The study was approved by The Research Ethical Committee of School of Medical Sciences (PPSP), University Science of Malaysia on 1$^{ST}$ April 2015 (Ref: USM/JEPeM/15020065). The consent form attached in Appendix 2.

### 3.10 Statistical analysis

Collected data were processed and analyzed using IBM SPSS 22. The demographic data were presented in the table as numbers and percentage with mean (SD) according to data distribution.

The mean and difference value of pre-phototherapy group and post-phototherapy group were analyzed using the descriptive analysis. Based on the mean and difference value, the Bland Altman plot were derived. The statistical significant is defined as percentage of agreement. The upper and lower median line for the Bland Altman plot used the formula of (https://www.medcalc.org/manual/blandaltman.php):

Mean +/- 1.96 x SD

The degree of agreement between serum bilirubin and Bilispect was derived from:

100 - (Number of outliers/ number of total subject x 100)