BLEEDING DISORDERS IN NEWBORNS: PREVALENCE, RISK FACTORS AND COAGULATION INDICES AT MUHIMBILI MEDICAL CENTRE, DAR ES SALAAM, TANZANIA.

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IBRAHIM Z.K. MADUHU, MD (DAR)

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ABSTRACT

Neonatal bleeding disorders (NBD) are common causes of morbidity and mortality at the neonatal unit, Muhimbili medical centre, Dar Es Salaam. However, no study has yet been done to determine the magnitude or the associated risk factors. Therefore a descriptive cross-sectional and case control study was carried out from January 22nd to April 25th, 1998 to determine the prevalence and risk factors associated with NBD. During this period a total of 1628 newborn infants were admitted to the neonatal ward and out of these 589 babies was recruited into the study. One hundred seventy five infants (29.9%) with bleeding disorders were recruited as cases and 414 (70.2%) without evidence of bleeding as controls. Only infants where parental consent was obtained were included into the study. For each infant a thorough history was taken, physical examination done and a full blood picture together with coagulation profile determined.

Twin babies were 18 (10.3%) of the study cases as compared to 46 (11.1%) in the controls. Singleton babies were 157 (89.7%) of the cases and 368 (88.9%) of the controls. The difference in bleeding status between twin and singleton infants was found not to be statistically significant (X^2 =0.09, P = 0.76). Seventy nine (45.1%) of the study cases and 148 (35.7%) of the control cases were born with Low Birth Weight (LBW). The difference was statistically significant (X^2 = 4.58, P = 0.03). Furthermore 54 (30.9%) of the study cases and 95 (22.9%) of the controls were born prematurely and the difference was statistically significant (X^2 = 4.07, p = 0 .04). One hundred and eight (61.7%) of the cases and 199 (48.1%) of the controls were asphyxiated and the difference was highly significant (X^2 = 9.18, P = 0.002). In 27 (31.4%) of the cases and 121 (54.8%) of the

controls, the mother was given general anaesthesia during delivery. The difference was highly significant (X^2 = 13.53, p = 0.0002). The APGAR score was less than 7 in 86 (49.1%) of the cases and 124 (30%) of the controls. The difference was highly significant (X^2 = 20.43 p = 0.00003). Fifty six (32%) of the study group and 166 (40.1%) of the controls cases were delivered by a mode other than Spontaneous Vertex Delivery (SVD) and the difference was also highly significant (X^2 = 30.97, p = 0.0000008).

The Prothrombin Time (PT) was prolonged in 5 (2.9%) of the cases and 13 (3.4%) of the controls, but the difference was not statistically significant (X^2 = 0.56, p = 0.7). The Activated Partial Thromboplastin Time (APTT) was also prolonged in 14 (8.1%) of the cases and 33 (8.8%) of the controls. However, the difference was also not statistically significant (X^2 =1.52, P = 0.46). The proportion of infants with vitamin-K deficiency (crude prevalence) as judged by abnormal PT and APTT in the study population ranged between 2.9% to 8.8%, but the majority of them did not have any obvious bleeding clinically.

The prevalence of NBD was found to be 10.7% and that vitamin-K deficiency related bleeding was not a significant problem at the neonatal unit. However, large scale studies are needed to confirm this finding. Asphyxia neonatorum, LBW, mode of delivery other than SVD and general anaesthesia were the only risk factors significantly associated with bleeding.

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DECLARATION

I declare that this dissertation is my original work and has never been submitted for a degree in any University.

..... Date: 14 Oct. 1999

I.Z.K. Maduhu

DEDICATION

This dissertation is dedicated to my parents.

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ABBREVIATIONS

ABD	-	Assisted Breech Delivery.
APTT	-	Activated Partial Thromboplastin Time
CA: Ag	-	Coagulant Antigen ratio
DIC	-	Disseminated Intravascular Coagulation
dl	-	decilitre
EDTA	-	EthylenyleDiaminotetraAcetic acid
FBP	-	Full Blood Picture
g	-	grammes
Hb	-	Haemoglobin
Hct	-	Haematocrit
HDN	-	Haemorrhagic Disease of the Newborn
LBW	-	Low Birth Weight.
LCVE	-	Low Cavity Vacuum Extraction.
LSCS	-	Lower Segment Caesarean Section.
MMC	-	Muhimbili Medical Centre
MUCHS	-	Muhimbili University College of Health Sciences
NBD	-	Neonatal Bleeding Disorders
PIVKA-II	-	Protein Induced by Vitamin K absence Analogue to Factor II
PT	-	Prothrombin Time
PTT	-	Partial Thromboplastin Time
RBC	-	Red Blood Cells
RDS	-	Respiratory Distress Syndrome

SPL	-	Specialised Paediatric Laboratory
SVD	-	Spontaneous Vertex Delivery.
µl	-	Microlitre
VMC	-	Vasomotor Centre
WBC	-	White Blood Cells
WHO	-	World Health Organisation

INTRODUCTION AND REVIEW OF LITERATURE

1. Introduction

Neonatal bleeding disorders (NBD) are common causes of morbidity and mortality at the neonatal unit of Muhimbili Medical Centre (MMC). Hospital records show that NBD constitute 10% of all the admissions and 10% of the overall mortality (Manji K P. personal communication). During the first two weeks of September 1997 alone, there were 322 admissions to the Neonatal unit, and 31 (9.6%) of them had bleeding disorder as a reason for admission. The overall mortality during this time was 24.8%. Ten percent of the deaths were associated with NBD, which included cord bleeding, cephalohaematoma, subaponeurotic haemorrhage and prolonged bleeding from an injection site. Other manifestations of NBD were internal bleeding such as intracranial, pulmonary and gastrointestinal haemorrhages, and haemathrosis. Echymoses, patechiae and bruises were not uncommon manifestations.

About half of the NBD at the neonatal unit were accidental in nature like cord bleeding and traumatic such as bruising of the presenting part, cephalohaematoma, lacerations and cut wounds. The remaining half had no established cause. Most of the infants with NBD recovered after vitamin K administration and blood transfusion, but a few died due to excessive bleeding.

2. Haemostasis.

Haemostasis is a phenomenon that leads to clot formation on a damaged blood vessel. The haemostatic mechanism involves a cascade of chemical reactions leading to the activation of a proenzyme prothrombin to thrombin, which in turn converts a protein

fibrinogen to fibrin threads that enmesh platelets, blood cells and plasma to form a clot. Activation of prothrombin to thrombin involves more than a dozen soluble proteins, which interact in a sequential manner following endothelial damage (intrinsic pathway) or tissue injury (extrinsic pathway) to result in formation of an insoluble clot. Figure 1 below illustrates the haemostatic process.

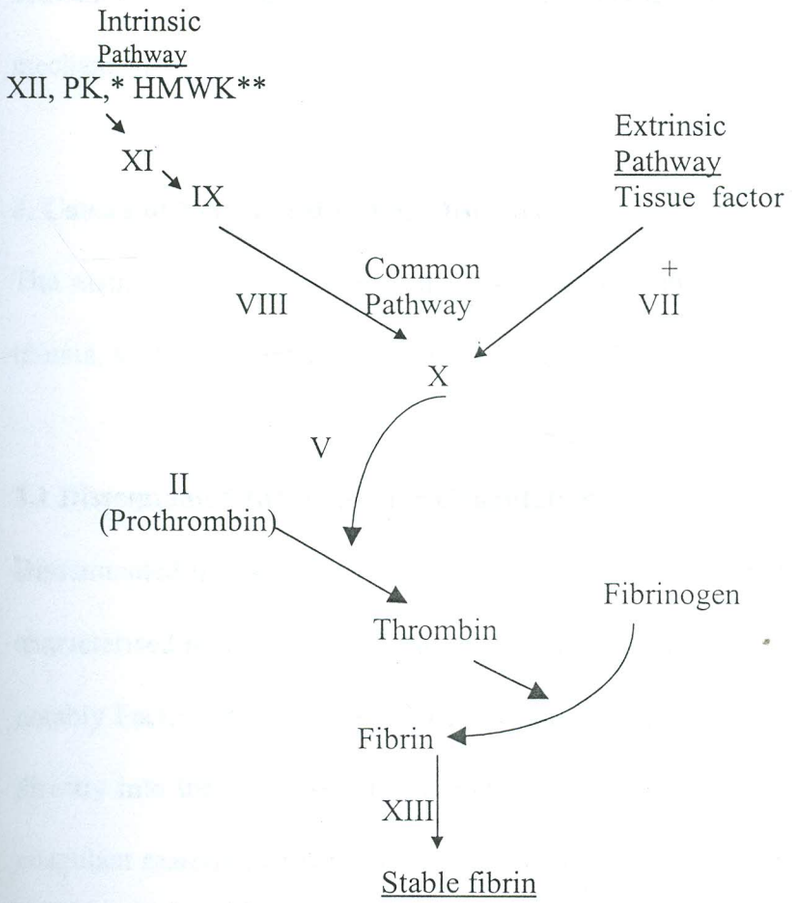

Figure 1. A simplified schematic illustration of the coagulation mechanism.
* PK = prekallikrein.

**HMWK =high-molecular-weight kinninogen.

(Illustration from Buchanan, GR. Coagulation disorders. The Pediatric Clinics of North America, 1986; 33: 205)

Among the clotting factors that are involved in this cascade, four of them, namely prothrombin (factor II), proconvertin (factor VII), plasma thromboplastin component (factor IX) and Stuart factor (factor X) are known to be vitamin K dependent for their biosynthesis and activation [1]. The clotting factors are basically plasma proteins, mostly globulin. They are inactive forms of proteolytic enzymes or proenzymes, which upon activation to their enzymatic potential cause successive cascading reactions in the clotting mechanism.

3. Causes of Neonatal Bleeding Disorders

The main causes of NBD include Disseminated Intravascular Coagulation (DIC), birth trauma, accidental bleeding and Haemorrhagic Disease of the Newborn (HDN) [2].

3.1 Disseminated Intravascular Coagulation

Disseminated Intravascular Coagulation (DIC) is an acquired pathophysiological process characterised by the intravascular consumption of platelets, plasma clotting factors, most notably Factors II, V, VII and XIII, and fibrinogen [3]. Thrombotic activity is introduced directly into the blood stream through activation of the extrinsic pathway by release of coagulant material, activation of the intrinsic pathway by diffuse endothelial damage, or by generalised platelet aggregation. The activation of the coagulation cascade leads to fibrin deposition and fibrinolysis, which in turn leads to production of Fibrin Degradation Products (FDP) and depletion of coagulation factors, fibrinogen and platelets. In turn, the depletion results in bleeding when the levels of platelets and clotting factors drop to values that are insufficient to maintain haemostasis [3, 4].

In the newborn, a number of pathological processes may incite episodes of DIC. These include hypoxia, acidosis, tissue necrosis, shock, hypothermia, endothelial damage and obstetric accidents such as abruptio placentae and retention of a dead twin foetus [3, 5]. Thus, it is not surprising that a large number of clinical conditions, such as septicaemia, asphyxia neonatorum and respiratory distress syndrome (RDS) have been reported to be associated with DIC. The cause of DIC in birth trauma is secondary to neurogenic shock. The shock is due to increased vascular capacity following widespread loss of vasomotor tone. The resulting massive vasodilatation, especially of the veins causes pooling of blood in the veins. The reduction in flow through the microcirculation, combined with the increase in viscosity, makes the blood highly coagulable. Moreover, DIC results from the presence of large amounts of traumatised or dying tissue in the body that release tissue thromboplastin into the blood. The activation of coagulation cascade and platelet aggregation results further in microclot formation within the microcirculation. The cells that receive blood from the blocked capillaries become hypoxic and eventually die and release more tissue thromboplastin [2]. In the newborn baby neurogenic shock also occurs following deep maternal general anaesthesia, birth asphyxia and Respiratory Distress Syndrome (RDS). The actual pathology is ischaemic depression or damage of the vasomotor centre (VMC).

In septicaemia vasodilatation is due to a number of factors, the most important one being a toxin that is liberated by the bacteria. The bacterial toxin also activates the clotting factors, thus leading to a wide spread formation of intravascular microclots.

3.2 Birth Trauma:

Birth injuries are those sustained during the birth process. Factors predisposing the infant to birth injury include macrosomia, prematurity, cephalopelvic disproportion (CPD), uterine dystocia, prolonged labour and abnormal presentation [2].

Birth trauma may result in direct bleeding into tissues and organs. The bleeding may lead to only minor manifestations such as bruises, echymoses and lacerations or it may result into very serious conditions such as intracranial haemorrhage, rupture of an abdominal viscus such as the liver, bone fracture, cephalohaematoma and subaponeurotic haemorrhage.

3.2.1 Cephalohaematoma

Cephalohaematoma is a subperiosteal collection of blood overlying a cranial bone. Cephalohaematoma is caused during labour or delivery by a rupture of blood vessels that traverse from skull to periosteum. Repeated buffering of the foetal skull against the maternal pelvis during a prolonged or difficult labour and mechanical trauma caused by use of forceps or vacuum extractor in the delivery of the baby have been implicated. In a few cases cephalohaematoma may be associated with skull fracture, coagulopathy and intracranial haemorrhage. A massive cephalohaematoma may result in blood loss severe enough to endanger life.

3.2.2 Subaponeurotic haemorrhage

Subaponeurotic haemorrhage is a collection of blood in the soft tissue between the galea aponeurotica and the periosteum of the skull. The commonest cause of this condition is difficult instrumental delivery, particularly forceps or vacuum extractor both of which may lead to ripping of the pericranium and its processes between individual skull bones [(2)]. Other factors include coagulopathies, macrosomia, foetal dystocia and precipitate labour. Subaponeurotic haemorrhage may result from an associated skull fracture or rupture of interosseous synchondrosis, which in turn cause injury to major veins and sinuses. Another possible mechanism is distortion or traction on emissary veins bridging the subdural and subaponeurotic spaces.

3.2.3 Intracranial Haemorrhage

Intracranial Haemorrhage may result from trauma or asphyxia, and rarely, from anomaly of vessels. Traumatic epidural, subdural, intraventricular, or subarachnoid haemorrhage is especially likely when the foetal head is large in proportion to the size of the maternal pelvic outlet; when for any reason the labour is prolonged; when there are breech or precipitate deliveries; or when there is injudicious mechanical interference with delivery. Due to birth trauma changes occur in cerebral blood flow which in turn is transmitted to the capillaries causing their rupture and bleeding into brain parenchyma, ventricles and intrameningeal spaces. Intracranial haemorrhage often involves ventricles of premature infants delivered spontaneously without apparent trauma. Intracranial haemorrhage may be associated with DIC and idiopathic thrombocytopenic purpura.

3.2.4 Fractures

Fractures particularly of the clavicle, humerus, femur and skull bones may be associated with severing of blood vessels leading to bleeding into the surrounding tissues. Major causes of clavicular fractures are difficult delivery of the shoulders in vertex presentation and extended arms in breech delivery. The mechanisms responsible for fracture of the humerus are difficult delivery of extended arms in breech presentations and of shoulders in vertex presentations. Skull fractures on the other hand follow delivery of prolonged difficult labour with repeated forceful contact of foetal skull against maternal symphysis pubis, sacropromontory or ischial spine. Fracture of the femur follows a breech delivery when the leg pulled down after the breech is already in the pelvic inlet.

3.2.5 Injuries to soft tissue

Soft tissue injuries are in the form of abrasions, petechiae, echymoses, lacerations and genital traumas. Bleeding from these sites may lead to blood loss, particularly if not attended to promptly. The causes of abrasions are mechanical such as those precipitated by instrumental delivery, CPD and dystocia. The cause of petechial haemorrhage is probably sudden increase in intrathoracic and venous pressure during passage of the chest through the birth canal, or cord tie around the neck. Petechial haemorrhage can also be a manifestation of a haemorrhagic disorder. Echymoses may occur after traumatic or breech delivery. When extensive, echymoses may cause blood loss severe enough to cause shock, DIC and anaemia. Accidental lacerations may be afflicted with a surgical blade during caesarean section. Soft tissue injuries involving the genitalia sometimes occur, especially after breech deliveries and in large babies. Echymoses and haematoma

of the scrotum and labia majora are the commonest, especially when they are the presenting parts in a breech presentation.

3.3 Haemorrhagic disease of the newborn.

Haemorrhagic disease of the newborn (HDN) is a term reserved for bleeding disorders in the neonatal period resulting from vitamin K deficiency. HDN is defined as neonatal bleeding disorder caused by vitamin K deficiency. It is characterised by deficiency of prothrombin, proconvertin, factor IX and factor X [6]. The clinical definition of HDN is bleeding in the first four weeks of life of a newborn with otherwise normal platelet count, normal peripheral blood smear and complete recovery after parenteral administration of vitamin K [7].

4.1. The role of vitamin K in the coagulation system.

The specific action of vitamin K is post-translational carboxylation of glutamic acid residues on vitamin K- dependent coagulation factors [1, 8]. The conversion of glutamic acid to γ-carboxyglutamic acid creates effective calcium binding sites on these proteins, thereby enhancing the coagulation mechanism. The vitamin K-dependent carboxylation of coagulation proteins occurs in the rough endoplasmic reticulum of hepatocytes. Non-carboxylated proteins are functionally defective because they cannot bind calcium, which is an important catalyst in the coagulation process.

In the absence of vitamin K, synthesised prothrombin and other vitamin K– dependent proteins circulate in their non-carboxylated functionally inactive forms. Other authors [8]

describe non-carboxylated prothrombin as protein induced by vitamin K absence analogue to factor II (PIVKA – II) which can be assayed. Abnormal prothrombin (PIVKA–II), a precursor to normal prothrombin, which is antigenically intact but functionally inactive, forms the basis for vitamin K deficiency assay [8, 9] and its normalisation following vitamin K administration is diagnostic of vitamin K deficiency.

4.2. Discovery and nature of vitamin K.

While the term HDN was used as long ago as 1894, it was not until 1929 when vitamin K was suspected to be related to the disease. Later on, a correlation was documented between vitamin K deficiency and low prothrombin [10]. Further studies indicated that low prothrombin levels could be corrected by administration of vitamin K. It has now been established that vitamin K is effective in the prevention and treatment of HDN [11].

The chemical formula for the naturally occurring vitamin K is composed of two benzene rings with an aliphatic chain of hydrocarbons. Vitamin K_1 is chemically known as 2-Methyl-3 phytyl -1-4- naphthaquinone, as shown in figure 2.

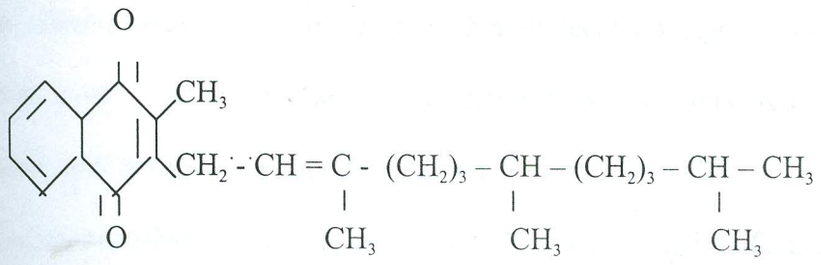

Figure 2. Chemical formula of vitamin-K_1 [13]

There are a large number of related compounds, both naturally occurring and synthetic, which have similar biological activity. These compounds are numbered in the sequence in which they were discovered. They include vitamin K_1 K_2 K_3 etc.

4.3. Sources and storage of vitamin K.

Vitamin K is mainly synthesised in the colon by the action of intestinal flora, particularly *Bacteroides fragilis* and some strains of *Escherichia coli* [12]. Naturally rich dietary sources of vitamin K are green leafy vegetables [13]. Milk formula and other commercially available foods and vegetable oils are normally fortified with vitamin K [14]. Human milk, as compared to cow's milk, is a poorer source of vitamin K [14]. Regarding vitamin K biosynthesis, the intestinal flora of breast– fed infants produce less vitamin K than the intestinal flora of the formula–fed infants [15]. Hence a relative insufficiency of endogenous vitamin K may be partially responsible for the increased incidence of vitamin K deficiency induced haemorrhage in breast–fed infants [16].

The commonly held assumption is that vitamin K is not stored in the body in any significant amount [14]. However, higher molecular weight storage forms of vitamin K may exist [17]. In two studies, when vitamin K was administered, a significant increase in vitamin K levels in the blood was noted for 3 to 5 days [18, 19].

Newborn infants do not have vitamin K reserves and, infact, some of them may be deficient at birth. Shearer et al [20] demonstrated undetectable vitamin K_1 in cord blood in a significant number of neonates. This is because vitamin K does not cross the placental barrier readily or foetal uptake is low probably because of low levels of a binding protein

[20]. This fact underscores the importance of administration of exogenous vitamin K_1 to the newborn infants at risk. On this basis the American Academy of Paediatrics recommends vitamin K prophylaxis to infants with high risk of vitamin K deficiency [6].

4.4. Clinical manifestations of vitamin K deficiency.

Three patterns of vitamin K deficiency haemorrhage occur in infancy. These are early HDN, classic HDN and late haemorrhagic disease. Their descriptions are summarised in table 1 below [21].

Table I. Vitamin K-deficiency haemorrhage in infancy.

	Age	Common bleeding sites	Cause	Prevention by vitamin K administration at birth	Comments
Early HDN	0 to 24 Hours	Cephalohaematoma scalp monitor Intracranial Intrathoracic Intra-abdominal	Maternal drugs Warfarin Anticonvulsant Antituberculous chemotherapy Idiopathic	No	Frequently life-threatening Guidelines for safe management of high-risk pregnancies needed
Classic HDN	1 to 7 Days	Gastrointestinal Skin, Nasal Circumcision	Idiopathic Maternal Drugs	Yes	Incidence increased in breast-fed neonates and reduced by early formula feedings
Late hemorrhagic disease	1 to 12 months	Intracranial Skin Gastrointestinal	Idiopathic Secondary: Diarrhoea Malabsorption Prolonged warfarin exposure	?	Common cause of intracranial haemorrhage in breast-fed infants 1 to 3 months of age, may be aggravated by antibiotic administration

4.4.1 Early haemorrhagic disease of the newborn.

Infants in this category have severe life threatening haemorrhage at birth or during the first 24 hours of life. The causes of early HDN is related to drugs such as anticoagulants, anticonvulsant and antituberculosis taken by the expectant mother that affect vitamin K metabolism [22-29]. Anticoagulants such as warfarin block the action of epoxide reductase, an enzyme that catalyses the conversion of vitamin K epoxide to vitamin K in the vitamin K cycle. Figure 3 below illustrates the blockade by warfarin.

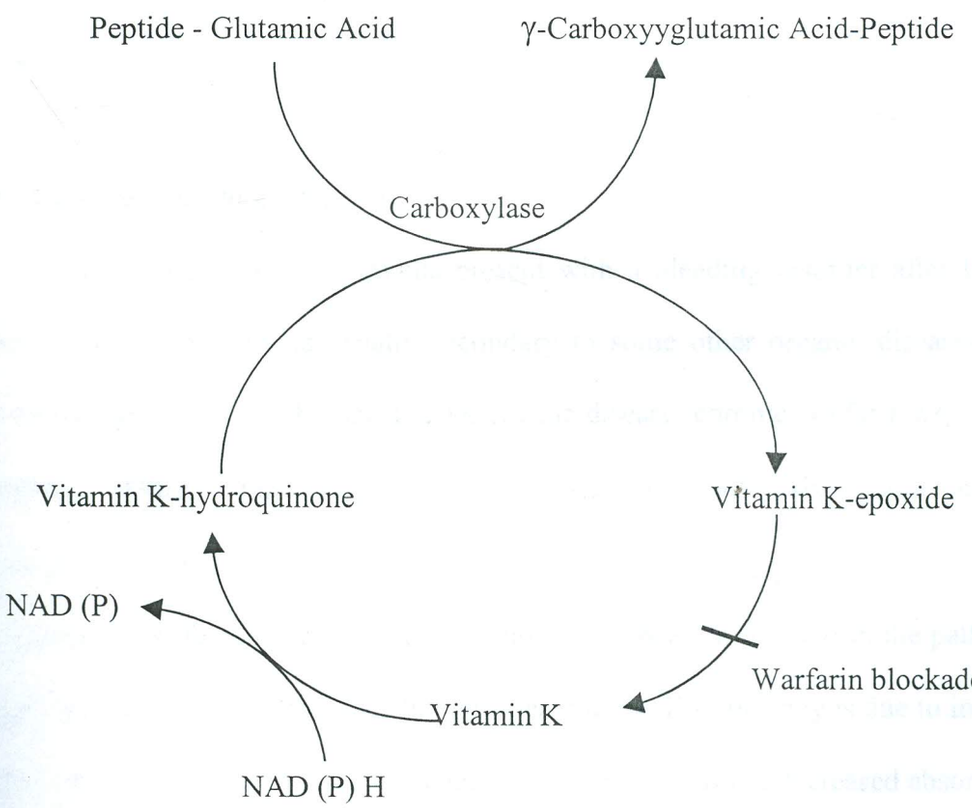

Figure 3. Vitamin K cycle and warfarin blockade. [21]

On the other hand anticonvulsants such as barbiturates and phenytoin are powerful microsomal enzyme inducers and increase the rate of vitamin K degradation. Therefore, actions of both groups of drugs lead to a deficient state.

Rarely, cases of early HDN have occurred without any apparent explanation [28]. The role of other risk factors such as maternal illnesses for which the implicated drugs were taken has not been studied conclusively.

4.4.2 Classic haemorrhagic disease of the newborn.

In this category the newborn infant presents with bleeding diathesis on the second day of life [24, 29]. Most cases of the vitamin K deficiency occurring in this group are idiopathic, although several studies implicate exclusive breast feeding to the pathogenesis of the disease [15,29,30].

4.4.3 Late haemorrhagic disease.

In late haemorrhagic disease, infants present with a bleeding disorder after the fourth week of life. The cause is usually secondary to some other organic diseases such as diarrhoea, cystic fibrosis, biliary atresia, coeliac disease, chronic warfarin exposure, and hepatitis. Other rare conditions include α1-antitrypsin deficiency and abetalipoproteinaemia.

Exclusive breast-feeding and chronic diarrhoea have been implicated in the pathogenesis of late haemorrhagic disease [34-36]. In diarrhoea vitamin K deficiency is due to insufficient dietary intake, decreased vitamin K synthesis by intestinal flora, decreased absorption due to diarrhoea or the use of antibiotics. However, in the majority of patients with late haemorhagic disease no underlying cause could be identified [21,37].

5. Other causes of bleeding disorders in the newborn.

Bleeding disorders other than those due to vitamin K must be excluded from HDN. These are subdivided into those with low platelet count and those with normal platelet count.

5.1. Bleeding disorders with low platelet count

The bleeding disorders associated with low platelet count include idiopathic thrombocytopenic purpura (ITP), aplastic anaemia and DIC. In ITP and aplastic anaemia the coagulation indices are normal, whereas in DIC the coagulation indices are prolonged.

5.2. Bleeding disorders with normal platelet count.

The bleeding disorders associated with normal platelet count include congenital platelet function disorders and plasminogen activator inhibitor deficiency. In both conditions, coagulation indices are normal. In Von Willebrand disease and haemophilia, the PT is normal, but the PTT is prolonged. In liver disease, congenital afibrogenaemia and congenital deficiency of coagulation factors II, V, VII and X, the PT and PTT are prolonged.

6.0 RISK FACTORS FOR BLEEDING IN THE NEWBORN

Several factors have been documented to cause NBD in the western world [9, 32-33]. These are:- prolonged labour, traumatic delivery, instrumental delivery, general anaesthesia, precipitate labour, accidental haemorrhage, neonatal infection and inherited disorders of clotting factors.

6.1 Prolonged labour.

Prolonged labour particularly during the second stage is an important cause of birth asphyxia that leads to ischaemia of the medulla. Medullary ischaemia in turn leads to loss of vasomotor tone with consequent pooling of blood. This results in the DIC cascade.

6.2 Traumatic Delivery

Delivery may be traumatic if the obstetric risk factors are not managed properly. Known obstetric risk factors for traumatic delivery include:- young age of the mothers (below 18 years of age), short stature (height less than 150cm), cephalopelvic disproportional and malpresentation. These are risk factors also for both prolonged and obstructed labour. Mismanagement of breach delivery often ends up in fractures of long bones. Bleeding disorders due to traumatic delivery include bruises of the presenting part, cephalohaematoma, subaponeurotic haemorrhage and bone fractures.

6.3 Instrumental delivery.

Use of vacuum extractor is often associated with bruising or lacerations. Besides, unskilful use of the obstetric instruments may cause ripping of the pericranium and its processes from skull bones resulting in diffuse subaponeurotic haemorrhage.

6.4 General Anaesthesia

Deep general anaesthesia to the mother during delivery may cause a neurogenic shock to the baby due to depression of the vasomotor centre and consequent loss of the vasomotor tone. This in turn leads to DIC cascade.

6.5 Precipitate Labour

This is an abnormally short duration of labour lasting for not more than 6 hours. This duration of labour does not allow the normal moulding of the head to take place; instead there is rapid moulding and a rebound re-expansion of the cranium and its contents after delivery. This may lead to severing of intracranial structures and rapid over filling of capillaries with consequent rupture and bleeding because of the pressure passiveness in the newborn baby.

6.6 Accidental Haemorrhage

Cut wounds and lacerations caused during surgical procedures or a poorly ligated umbilical cord are other common causes of bleeding in the newborn period.

6.7. Infections

Septicaemia, particularly that due to gram negative organisms causes DIC cascade and bleeding disorder.

6.8 Inherited disorders of clotting factors

The vast majority of genetic coagulation disorders are due to factor VIII deficiency (haemophilia A also called classic haemophilia) or factor IX deficiency (haemophilia B or Christmas disease). Other hereditary coagulation factor deficiencies associated with bleeding disorders are rare and they include Von Willebrand disease (autosomal dorminant trait), factor XI deficiency or haemophilia C (autosomal recessive trait) and factor XIII (fibrin stabilising factor) deficiency [4, 5].

7.0 Assay of vitamin K dependent clotting factors.

There are two methods of assaying vitamin K dependent clotting factors, namely, direct and indirect methods.

7.1 Direct methods

These are specific tests that measure the clotting factors or specific markers of vitamin K. Non-carboxylated prothrombin (PIVKA-II), which is functionally inactive but antigenically intact, circulates in the blood during vitamin K deficiency and can be assayed. One method [41] compares the levels of prothrombin measured functionally (factor II coagulant) with that measured antigenically (factor II antigen) to get a factor II coagulant (CA) to factor II antigen (Ag) ratio.

$$\frac{\text{Active prothrombin (Coagulant)}}{\text{Non-carboxylated Prothrombin (Antigen)}} = \text{Ratio (or CA: Ag)}$$

A decreased ratio indicates vitamin K deficiency. Other methods [38] measure abnormal prothrombin or PIVKA - II directly. The method that measures non-carboxylated prothrombin by radioimmunoassay is very sensitive [39]. Direct estimation of vitamin K in blood by high performance liquid chromatography is a most recent and most sophisticated method [19, 21] but is not yet available in Tanzania.

7.2 Indirect methods

These tests reflect the physiological function of vitamin K. In this case, prothrombin time (PT) and partial thromboplastin time (PTT), which reflect the level of vitamin K - dependent factors in blood, are determined [40]. Prothrombin time measures the extrinsic activation of factor X as well as the remainder of the coagulation scheme (V, II, and fibrinogen). On the other hand PTT measures the intrinsic pathway, that is, activation of factor X by factor XII, XI, IX and VII, as well as coagulation reactions (V, II and fibrinogen). Activated partial thromboplastin time (APTT) is similar to PTT except that kaolin is added to the former to hasten the activation of factor XII and thereby speed the overall reaction. These parameters are prolonged relative to controls for age in deficient states, but return to normal when vitamin K is administered. For determination of PT and PTT appropriate sample collection and handling are imperative if valid interpretation of the results is to be expected. Blood must be collected in an appropriate anticoagulant, such as 3.31% sodium citrate solution in a ratio of 1 part of citrate to 9 parts of blood. The blood must be kept at 4°C before and during centrifugation [41].

The tests are easy to perform and reasonably accurate but not specific. Besides the congenital abnormalities of the intrinsic and common pathway, other rare conditions, can cause prolongation of PT and PTT. They include:- heparin, oral anticoagulants, low fibrinogen levels, elevated fibrin or fibrinogen split products, acquired clotting factor deficiencies, immune antibodies, lupus anticoagulants and paraproteinaemia.

8.0 Laboratory findings in vitamin K deficiency [21].

The laboratory findings of coagulation abnormalities that are typically present in vitamin K deficiency are listed in table 2.

Table 2. Laboratory findings in vitamin K deficiency

Abnormal findings
Increased PT (measures factors II, VII, X)
Increased PTT (measures factors II, IX, X)
Increased thrombotest, normotest (measurers factors II, VII, X)
Decreased factors II, VII, IX and X coagulant activity/factor II antigen
Positive non-carboxylated prothrombin (PIVKA II)
Normal findings
Thrombin time (measures conversion of fibrinogen to fibrin)
Fibrinogen
Platelets count
Factors V, VIII, XI, XII coagulant activity
Factors II, VII, IX, X Antigen.

9.0 Controversies regarding vitamin K prophylaxis.

Vitamin K prophylaxis is given routinely to all newborn infants in many industrialised as well as in some developing countries [24, 42, 43]. However, in Tanzania, vitamin K prophylaxis is given only to newborn infants with "high risk" for HDN [44, 45], and this is done only in a few hospitals, particularly referral hospitals. The big question is whether all newborn infants should be given vitamin K prophylaxis routinely.

Another controversy concerns the implication of exclusive breast-feeding in the pathogenesis of classic HDN.

9.1 Do all newborn infants require vitamin K prophylaxis?

Many studies done reported decreased incidence of HDN with the introduction of vitamin K prophylaxis [24]. This epidemiological evidence and the dramatic response observed [42, 43] have been assumed to be the proof of vitamin K deficiency. However the findings noted by different authors beyond the first day of life are extremely diverse. The diversity is mainly attributed to the lack of a standardised way of vitamin K assay. In one of these studies [9], hypoprothrombinaemia was a highly significant finding in cord blood, especially in preterm infants. The normal CA: Ag ratio in normal infants of the same study suggested that hypoprothrombinaemia was the result of reduced production of the protein rather than of vitamin K deficiency. In this study, the classic HDN occurred irrespective of vitamin K administration at birth. It appeared that the chances of an infant developing HDN depended upon a number of risk factors such as exclusive beast feeding, maternal drug ingestion, stressful pregnancy and delivery. Yet another study [8] failed to demonstrate the presence of PIVKA - II in plasma of normal newborn infants, concluding that healthy infants are not likely to be vitamin K deficient. In one study involving the assay of clotting factors II and VII, Gobel and his colleagues [46] found no difference between vitamin K treated and untreated controls. In their study they suggested that normal infants given their first feeds within 24 hours do not develop vitamin K deficiency. Results from research at the Centre for the Study of Haemophilia and Haemorrhagic Disorders in Italy had a similar conclusion [47]. They found no increment of factors II, VII and X in either treated infants or untreated controls. An editorial review in the Lancet [48] suggested that vitamin K prophylaxis might be safely withheld from term infants without risk factors.

The above arguments against vitamin K prophylaxis have been countered strongly by various researchers [20, 42, 43]. Shearer et al [20] did direct vitamin K assays in mothers and their infants and found undetectable levels of vitamin K in cord blood suggesting a deficient state. Aballi [42, 43] strongly warned on the use of isolated laboratory data to draw conclusions due to lack of standardisation. In one of his clinical trials [43], he found a significant correction of bleeding indices after vitamin K administration in treated infants compared to untreated controls.

9.2 Breast feeding and vitamin K deficiency

Many authors implicate exclusive breast feeding as the cause of classic HDN [15, 20, 29, 46, 48,]. In one of the studies [15], newborn infants who were exclusively breast fed had significantly prolonged PT compared with those fed cow's milk or those on breast milk but given vitamin K at birth. Sutherland et al [29] observed that the incidence of bleeding among exclusively breast fed infants who did not receive vitamin K at birth was 15 to 20 times greater than that of infants who were fed cow's milk, or those given vitamin K or both. It has also been shown that the concentration of vitamin K in cow's milk is approximately 4 times more than in human milk [49, 50]. Corresponding to this, vitamin K dependent factors are lower in exclusively breast-fed infants compared with those on cow's milk or given vitamin K at birth. However, Jimenez and his colleagues [51] in their study on vitamin K dependent factors in normal breast fed infants showed that, both exclusively breast-fed and cows' milk-fed infants who received vitamin K prophylaxis at birth had their clotting times prolonged beyond adult control levels. This suggested that

prolonged coagulation time in early infancy reflect normal developmental process which is independent of the kind of milk administered.

10. RATIONALE.

Bleeding diathesis is a common problem at the neonatal unit of MMC, but no study has been done to establish its magnitude, the causes and risk factors involved. Furthermore, exclusive breast-feeding has been implicated in the causation HDN, one of the preventable causes of NBD. Tanzania in line with WHO Global Breast Feeding Programme, strongly advocates exclusive breast-feeding and yet vitamin K prophylaxis is not given routinely to all newborn infants. The policy of not giving vitamin K prophylaxis to all newborn infants may be exposing a significant number of newborn babies to the risk of classic HDN. This study will describe the problem.

11.0 OBJECTIVES.

11.1 Broad Objective.

To determine the prevalence, risk factors and coagulation indices associated with NBD at the neonatal unit, MMC, Dar es salaam.

11.2 Specific Objectives.

11.2.1 To determine the prevalence of Neonatal Bleeding Disorders.

11.2.2 To determine risk factors associated with bleeding disorders in the newborn infants.

11.2.3 To determine the prevalence of Vitamin K related bleeding

11.2.4 To determine PT, APTT, FBP and platelet counts of the newborn infants.

12. PATIENTS AND METHODS.

12.1 Study Setting.

The study was conducted at the Neonatal Unit, Muhimbili Medical Centre Dar es Salaam, Tanzania. Muhimbili Medical Centre is a tertiary referral and university teaching hospital in the country. The Neonatal Unit caters for all strata of people in Dar es Salaam serving a population of about 3 million people with a birth rate of 4.7% [52]. The neonatal unit of MMC has a capacity of 80 beds, however the actual bed occupancy range between 80 to 120 at any one particular moment. The Neonatal Unit admits sick babies aged 28 days or less from Dar es salaam and referred patients from distant regions. The average admission rate is 600 babies a month. Healthy babies whose mothers are either sick or dead or unknown are also cared in this unit.

12.2 Study design.

Both descriptive cross section and prospective case control study designs were used. In studying the causes and prevalence, a descriptive design was used while in the study of risk factors, a prospective unmatched case control design was used.

12.3 Sample size.

The sample size was calculated using EPI-info version 6 computer programme. In calculating the sample size, the case control design, was used. The formula [53] used is shown below:

$$n = 2[Z_\alpha \sqrt{2pq} + Z_\beta \sqrt{p_1q_1 + p_0q_0}\,]^2 / (p_1 - p_0)^2$$

Where: n =Sample size.

p_0 = estimated exposure rate among control (the prevalence of vitamin K deficiency) = 13%.

$p_1 = p_0 * R/[1+p_0(R-1)]$.

R =relative risk.

$p = 1/2 (p_1+p_0)$.

q =1-p.

Z_α =alpha risk =5%.

Z_β =power of the study 80%.

In calculation of the sample size the following assumptions were made :-

-Ratio of control per case = 2.

-Odds ratio worth detecting = 2.

- 95% confidence level.

The calculated sample size was 546 infants, 182 as cases and 364 as controls. Sixty more babies were recruited, 20 being cases and 40 as controls, to increase power of the study and cater for the laboratory and blood sampling errors. Out of the expected 606 newborn babies only 589 met the criteria for analysis, 175 of them as cases and 404 as controls. The study cases were defined as those with bleeding disorder, while controls were those without any bleeding disorder.

12.4 Inclusion criteria.

1. Newborn infants who were not more than 4 weeks old and had not received vitamin K nor blood transfusion.
2. Parents informed consent granted.

12.5 Study population.

The study population included all newborn infants who were admitted to the Neonatal Unit for whatever reason. All consecutive admissions of infants who were bleeding were recruited as cases. Two concurrently admitted infants with no clinical evidence of bleeding were recruited as controls.

12.6 Data collection.

Informed verbal parental consent was obtained after counselling and before recruitment of any infant. Information was collected using a formatted questionnaire and a computer module. At the time of recruitment, perinatal information, as well as reasons for admission were obtained and filled into the questionnaire. A thorough physical examination including the assessment of gestational age by Dubowitz method[54] was done by the investigator. Newborn infants who required medical treatment were managed according to the standard protocol at the unit.

12.7 Specimen collection.

Three millilitres of blood were drawn from each newborn infant in the study by femoral venepuncture on admission to the unit, using disposable syringes and needles and after thorough cleaning of the site with a cotton swab soaked in 70% alcohol. The first 1.8mls of blood were collected in glass tubes containing 0.2mls of 3.13% sodium citrate for coagulation indices assay, while the remaining 1.2 mls were collected in Ethylene Diamino Tetra Acetic acid (EDTA) glass containers for full blood and platelet counts.

The collected samples were protected from strong light and heat by storing them in an ordinary ward refrigerator at temperature of about 4°C. The details of laboratory procedures are described below.

12.8 Estimation of PT and APTT .

PT and APTT tests were performed at the department of Haematology and blood transfusion, MMC Dar es salaam, using standard methods as described by Quick [55] and Proctor [56] respectively. All the tests were done by a senior laboratory technician.

12.8.1 Prothrombin time (PT).

The plasma of the cases and controls obtained after centrifugation, was placed in a clean glass tube and either examined at once, or stored at 4°C for not more than 6 hours pending examination. Estimation of PT was done using a preparation of rabbit brain emulsion as a source of thromboplastin as described by Douglas [57]. The emulsion was then mixed with citrated plasma. The mixture was allowed to recalcify and the clotting time estimated. A fibrin clot developing within a second or so marked the end point. The normal range for PT was taken to be 13 to 18 seconds.

12.8.2.Activated partial thromboplastin time (APTT).

The plasma of the cases and the controls was pre-incubated with Kaolin, which activated contact factor and other phospholipids to replace platelet activity. The preincubation hastens the reaction which is usually very slow if allowed to take place when plasma is allowed to clot in a glass tube. 'The activated' test and control plasma were then mixed

with equal volume of chloroform extract of rabbit brain suspension and allowed to recalcify. The visible clots marked the end point. The normal range for APTT was taken to be 35 - 45 seconds.

12.9 Full blood picture and platelet count.

FBP was done using MICROS *CT16 & CT18* (Cobas micro), an automated high performing electronic counter at the Specialised Paediatric Laboratory (SPL) at the Department of paediatrics and child health, MMC. An experienced Technician performed the tests.

12.9.1 Haematological measurement principle.

The standard normal values for age were used to compare the results. The principle of MICROS*CT* counter is based on an impedance variation generated by the passage of cells through the calibrated micro-aperture. The sample is diluted in a current conductor. The conductivity of the diluent differs considerably from the non-conductivity of the blood cells. The dilution is passed through the calibrated micro-aperture. Two electrodes are placed on each side of the aperture and electricity is allowed to pass through the electrodes continuously. When the cell passes through the aperture, electric resistance (impedance) between the two electrodes increases proportionately with the cell volume. The generated impulses are amplified so that the electronic system can analyse them and eliminate the background noise. Two measuring chambers and detection circuits separately carry out the analysis of WBCs, platelets and RBCs. Each type of cell is

analysed by the *microprocessor,* a high performing electronic device, which uses a very sophisticated impulse sorting system.

12.10 Data Processing and Analysis.

Data entry, cleaning and processing was done using Epi-Info version 6, Excel and dBase IV computer software programmes. Statistical analysis packages of Epi info version 6 and SPSS were employed for the analysis of data. The statistical significance testing of the difference between proportions was done using Chi-square tests at a significance level of 0.05 (p-value <0.05), while odds ratio was used to determine the risk of bleeding for each factor.

The outcome, bleeding or not bleeding was treated as a binary response. The variables were coded as "Normal", "Low" or "High" for all continuous variables, and either "Yes" or "No" for the categorical variables.

Bivariate analysis (cross tabulations) of risk factors against the outcome (bleeding or no bleeding) was made. Each risk factor was treated individually, and then collectively to all risk factors that showed significant difference in a multiple logistic linear regression analysis to assess the independent contribution of each of the confounding factors (risks for bleeding). A computer program SPSS/PC was used to fit the logistic regression model of the form: -

Log odds = B0 + B1X1 +B2 X2 + B3 X3 + B4 X4 + B5 X5+B6X6+B7X7 where,

log odds = odds of the newborn infant to have bleeding tendency given the values of the variables.

B0 = Constant

X1 = General anaesthesia (0= No, 1= yes)

X2 = Pregnancy (0= singleton, 1= multiple gestation)

X3 = Mode of delivery (0=SVD, 1=ABD, 2=LCVE, 3=LSCS)

X4 =Gestation age (0=term, 1=preterm)

X5 = Asphyxia neonatorum (0=no, 1=yes)

X6 = Low birth weight (0= no, 1= yes)

X7 = APGAR Score at 5 minutes (0= <4, 1= 4-7, 2 = >7)

The and the B's are the respective regression coefficients of the above variables.

12.11 Ethical Clearance.

Ethical clearance to carry out this study was granted by the Higher Degrees, Research and Publications Committee of MUCHS.

13.0 RESULTS

13.1 CHARACTERISTICS OF THE STUDY POPULATION:

During the period January 22nd 1998 to April 25th 1998, a total of 1628 newborn infants were admitted to the Neonatal Unit, Muhimbili Medical Centre, Dar es salaam and 589 (36.1%) of them were recruited into the study. The study sample consisted of 307 males and 282 females, thus giving a male: female ratio of 1:1.09. Of the study population 149 (25.3%) were healthy infants admitted for care, 227 (38.5%) were admitted because of low birth weight, 307 (52.1%) were admitted because of asphyxia neonatorum and 175 (29.7%) were newborn infants with bleeding disorders.

The characteristics of the study population by maternal age, birth weight and gestational age are presented in table 1. The maternal age for the cases and the controls ranged from 13 to 42 years. The mean maternal age was 23.7 years (± 5.6) for the cases and 24.8 years (± 6.1) for the controls. There was no statistically significant difference in maternal age between the cases and the controls ($X^2 = 2.76$, $p = 0.09$). The mean gestational age for the cases was found to be 36.3 weeks (± 5.1) while that of the controls was 37.5weeks (± 4.4) and the difference was statistically significant ($X^2 = 7.3$, $p = 0.006$). Birth weight in both the groups ranged from 500 to 6,000gm. The mean birth weight for the cases was 2,505 gm (± 866.7) compared with 2667 gm (± 784.3) for the controls and the difference was also statistically significant ($X^2 = 4.4$, $p = 0.03$).

Table 1: Characteristics of the study population by maternal mean age, birth weight and gestational age by bleeding status.

Characteristic	**Bleeding status**			
	Cases	Controls		
	Mean (SD)	Mean (SD)	X^2	P- value
Maternal age (Years)	23.7 (5.6)	24.8 (6.1)	2.76	0.09
Birth weight (grams)	2504.9 (866.7)	2666.6 (784.3)	4.4	0.03*
Gestational age (weeks)	36.3 (5.1)	37.5 (4.4)	7.3	0.006*

* Significant.

13.2 AGE AT ONSET OF BLEEDING

The age at onset of bleeding ranged from the first hour to the 28th day of life, the mean age being 41.7 hours (± 117.4). Table 2 summarises bleeding tendency by age at onset of bleeding. One hundred fourty six (83.4%) infants started bleeding within the first day of life, 18 (10.3%) started bleeding from the second to the seventh day and the remaining 11 (6.3%) started bleeding after the first week of life. Statistical significance testing could not be done because of the numbers are too small (less than 5) for statistical analysis.

Table 2: Bleeding tendency by age of onset

Age at which Bleeding started	**Bleeding status** Cases	Control	Total
Day 1 or less	142	4	146
Day 2 to one week	16	2	18
Beyond week 1	11	0	11
TOTAL	169	6	175

13.3 TYPE OF BLEEDING DISORDERS IN THE STUDY POPULATION

The distribution of study population by types of bleeding disorders is shown in table 3. There were a total of 175 bleeding infants. Eighty one them had cord bleeding, 77 had bruises or echymoses, 14 bled from injection or venepuncture sites, 11 had cephalohaematoma, 7 had pulmonary haemorrhage, 2 had vaginal bleeding and 1 each had haematemesis, rectal bleeding and unexplained pallor.

Table 3: Distribution of study population by types of bleeding.

Type of bleeding *	Distribution (n=175) Frequency	%
• Cord bleeding	81	46.3
• Bruises and echymosis	77	44.0
• Injection site bleeding	14	8.0
• Cephalohaematoma	11	6.3
• Pulmonary haemorrhage	7	4.0
• Vaginal bleeding	2	1.1
• Haematemesis	1	0.6
• Rectal bleeding	1	0.6
• Unexplained pallor	1	0.6
• Suspected internal bleeding	17	9.9

*Some patients had more than one type of bleeding

Bleeding was suspected in 17(9.9%) of the infants. This included intraventricular haemorrhage being 13, thoracic and abdominal haemorrhage 2 each.

13.4 FULL BLOOD PICTURE AND COAGULATION ASSAY

The FBP and coagulation profile of all infants recruited into the study (study cases and the controls) are presented in table 4. The RBC count was $1.3x10^6$ - $8.2x10^6/\mu l$ with a mean of $4.8x10^6/\mu l$ (± 0.91). The haemoglobin (HB) ranged from 8.5 to 24.0 g/dl with a mean of 15.8g/dl (± 4.6), and the haematocrit (Hct) ranged from 10.6% to 66.2%, with a mean of 45.4% (± 8.3). The WBC was $2.6x10^3$ to $98.5x10^3/\mu l$ with a mean of $12.1x10^3$ per µl (± 7.3). The platelet count ranged from $27x10^3$ to $606x10^3/\mu l$ with a mean of $223.3x10^3/\mu l$ (± 93.7). The PT ranged from 7.6 to 53.9 seconds with a mean of 13.8 (± 3.3 seconds) and the APTT ranged from 30 to 69 seconds with a mean of 37.3 seconds (± 6.0 seconds).

Table 4: Full blood picture and coagulation profile of the study population

Haemograms	Minimum	Maximum	Mean	SD
RBC ($x10^6/\mu l$)	1.3	8.2	4.8	0.91
Hb (gram/dl)	8.5	24.0	15.8	4.6
Hct (%)	10.6	66.2	45.4	8.3
WBC ($x10^3/\mu l$)	2.6	98.5	12.1	7.3
Platelets ($x10^3/\mu l$)	27	606	223.3	93.7
PT (seconds)	7.6	53.9	13.8	3.3
APTT (seconds)	30.0	69.0	37.3	6.0

13.8 CLASSIFICATIONS OF THE STUDY POPULATION ON THE BASIS OF NORMAL, LOW OR HIGH HAEMOGRAM VALUE AND ON THE BASIS OF EVIDENCE OF BLEEDING OR NO BLEEDING STATUS.

Blood specimens from 535 infants met the laboratory criteria for examination of FBP, 171 being cases and 364 as controls. Five hundred and fifty one specimens met the criteria for assay of coagulation indices, 174 being cases and 377 as controls. Table 5 presents a summary of the distribution of haemograms and evidence of bleeding. Standard reference values [58] were used to assign the result value of haemogram assay as normal, low or high (appendix 2).

One hundred sixty four (96%) of the cases had normal RBC count, while 5 (2.9%) and 2 (1.2%) had low and high RBC counts respectively. Among the controls 347 (95.3%) had normal, 9 (2.5%) had low and 8 (2.2%) had a high RBC count. The difference between bleeding and non-bleeding infants was not statistically significant ($X^2 = 0.75$, $P = 0.68$). The haemoglobin was found to be normal in 125 (73.1%), low in 44 (25.7%) and high in 2 (1.2%) of the cases, while it was normal in 279 (76.7%), low in 82 (22.5%) and 3 (0.8%) of the control cases. However, the difference between bleeding and non-bleeding infants was not statistically significant ($X^2 = 0.85$, $P = 0.65$). The platelets count was normal in 157 (91.8%), low in 12 (7%) and high in 2 (1.2%) of the cases, whilst among the controls it was normal in 338 (92.9%), low in 18 (4.9%) and high in 8 (2.2%). The difference was also not statistically significant ($X^2 = 1.56$, $P = 0.45$). The PT was prolonged in 5 (2.9%) of the cases and 13 (3.4%) of the controls. However, there was no significant difference between the cases and the controls ($X^2 = 0.56$, $p = 0.7$). The APTT

was prolonged in 14 (8.1%) of the cases and 33 (8.8%) of the controls but the difference was also not statistically significant (X^2 =1.52, P = 0.46).

Table 5: Distribution of the study population by haemograms and bleeding status.

Haemograms	Disease status: Cases	Control	Total	(%)	X^2	P-value
RBC:						
Normal	164	347	511	(95.5)		
Low	5	9	14	(2.6)		
High	2	8	10	(1.9)	0.75	0.68
Hb:						
Normal	125	279	404	(75.5)		
Low	44	82	126	(23.6)		
High	2	3	5	(0.9)	0.85	0.65
Platelet:						
Normal	157	338	495	(92.5)		
Low	12	18	30	(5.6)		
High	2	8	10	(1.9)	1.56	0.45
PT:						
Normal	109	224	333	(60.4)		
Low	60	140	200	(36.3)		
High	5	13	18	(3.3)	0.56	0.75
APTT:						
Normal	96	188	284	(51.6)		
Low	63	156	219	(39.8)		
High	14	33	47	(8.5)	1.52	0.46

13.5 THE PREVALENCE OF NBD

Out of a total of 1628 newborn infants who were admitted to the neonatal unit during the study period. 175 (10.7%) had bleeding disorders of various types. In this study, infants with vitamin K deficiency as determined by prolonged coagulation indices were found to be 18 (3.3%) on the basis of prolonged PT and 47 (8.5%) on the basis of prolonged APTT of both the cases and the controls. However only 0.9% among those with prolonged PT and 2.5% among those with prolonged APTT had obvious bleeding disorder.

13.6 RISK FACTORS FOR BLEEDING

The bleeding disorders in the study population by risk factors is presented in table 6. Twin babies were 18 (10.3%) of the study cases as compared to 46 (11.1%) in the controls. Singleton babies were 157 (89.7%) of the cases and 368 (88.9%) of the controls. The difference in bleeding status between twin and singleton infants was found not to be statistically significant (X^2 =0.09, P = 0.76). Seventy-nine (45.1%) of the study cases and 148 (35.7%) of the controls were born with LBW. The difference in birth weight between the cases and the controls was significant (X^2 = 4.58, P = 0.03). Fifty four (30.9%) of the cases and 95 (22.9%) of the controls were preterm and the difference between the two groups was statistically significant (X^2 = 4.07, p = 0 04). One hundred and eight (61.7%) of the cases and 199 (48.1%) of the controls were admitted because of asphyxia neonatorum and the difference was highly significant (X^2 = 9.18, P = 0.002). In 59 (68.6%) of the cases and 121 (54.8%) of the controls, the mother was given general anaesthesia during delivery and the difference was highly significant (X^2 = 13.53, p = 0.0002). The APGAR score was less than 7 in 86 (49.1%) of the cases and 124 (30%) of the controls. The difference in low APGAR score between bleeding and non-bleeding infants was highly significant (X^2 = 20.43 p = 0.00003). Babies delivered by modes other than SVD were 56 (32%) of the cases and 166 (40.1%) of the controls and this difference was highly significant (X^2 = 30.97, p = 0.0000008).

Table 6: Bleeding disorders in the study population by risk factors thereof

Risk factor	bleeding status				
	Cases	Control	Total	X^2	P-value
Pregnancy (n =589):					
Singleton	157	368	525		
Multiple	18	46	64	0.09	0.76
Gestation age (n =589):					
Term	121	319	440		
Preterm	54	95	149	4.07	0.04
Low birth weight (n =589):					
No	96	266	362		
Yes	79	148	227	4.58	0.03
Anaesthesia (n =307):					
No	59	100	159		
Yes	27	121	148	13.53	0.0002
Asphyxia (n =589):					
No	67	215	282		
Yes	108	199	307	9.18	0.002
Mode of delivery (n =589):					
SVD	119	248	367		
ABD	12	22	34		
LCVE	14	5	19		
LSCS	30	139	169	4.58	-0.0000008
APGAR score at 5min (n =589):					
>7	89	290	379		
5-7	78	116	194		
<4	8	8	16	20.4	-0.00003

13.7 MULTIVARIATE LINEAR LOGISTIC REGRESSION ANALYSIS

Multiple logistic regression analysis was done to adjust for confounding factors. Only those variables, which were significant in the univariate analysis, were included in the model. Table 7 shows the results of the logistic regression.

Table7: Multiple logistic linear regression analysis for the seven confounding factors.

Variable	β	S.E	df	OR (95% C.I)	p-value
Anesthesia	.2978	.7057	1	1.35 (-1.09, 1.68)	.6730
Multiple preg	.1498	.5075	1	1.16 (-0.84, 1.14)	.7678
Mode of delivery			3		.0156
ABD	-.1000	.6917	1	0.90 (-1.48, 1.09)	.8850
LCVE	-3.3347	1.0594	1	0 .04 (-5.39 -1.25)	.0016*
LSCS	.3595	.7063	1	1.43 (0.16, 1.26)	.6107
Prematurity	.2975	.5452	1	1.35 (-0.77, 1.37)	.5853
Asphyxia	.0083	.3685	1	1.01 (-0.71, 0.73)	.9819
LBW	-.0511	.4173	1	0.95 (-0.87, 0.77)	.9025
APGAR score			2		3640
<4	-.8057	8610	1	0.45 (2.49, 0.88)	.3494
4 –7	-.4659	.3596	1	0.63 (-1.17, 0.24)	.1951
Constant	8238	.5933	1		.1650

- Significant.

Using a backward selection procedure the final model was obtained as: -

Log odds = 0.7553 -0.1957$_{MODE1}$ -3.3201$_{MODE2}$ +0.7111$_{MODE3}$

This means that, the probability of a newborn having a bleeding tendency was high when delivered by LCVE and LSCS. The details of the final model fitting the multiple linear logistic regression are given in table 8.

Table 8: Odds of bleeding tendency by the different modes of delivery.

Variable	β	SE	df	OR	(95%C.I)	p-value
Mode of delivery:			3			.0003
ABD	-.1957	.6562	1	.82	(-1.48, 1.09)	.7656
LCVE	-3.3201	1.0557	1	.04	(-5.39, -1.26)	.0017*
LSCS	.7111	.2806	1	2.04	(0.16, 1.26)	.0113*
Constant	.7553	.1941	1			.0001

* Significant.

These results show that when other variables are controlled, babies delivered by LCVE and LSCS were more likely to have bleeding tendency than those delivered by SVD, [OR= 0.04 (CI = -5.39, -1.25); p = 0.016 and OR= 2.04 (CI =0.16,1.26); p = 0.0113 respectively]. The rest of the confounding factors showed no significant association.

14. DISCUSSION

14.1 PREVALENCE OF NBD

The prevalence of NBD in this study was found to be 10.7% of all newborn infants admitted to the neonatal unit during the three months of the study period, indicating that the NBD is a major problem at the neonatal unit. However as MMC is a tertiary referral hospital, most high-risk deliveries and sick babies tend to be admitted there, hence the prevalence of 10.7% may not reflect the actual prevalence of NBD if all deliveries in MMC were taken into consideration. It is estimated that MMC has 40 – 60 deliveries per day, which would give an average of 4500 deliveries in the 3 months. It is important to note that not all newborn babies delivered at MMC are admitted to the neonatal unit. It is only the sick ones and those whose mothers can not take care of them meet the admission criteria, thus representing a small fraction of a highly selected group. The prevalence would fall to less than 4% if all the deliveries at MMC were considered, or even less so if the whole catchment population was considered. This is also true for HDN. Therefore, to get a clear indication of the prevalence of NBD, a bigger study, which would include all babies delivered in Dar Es salaam would be recommended.

14.2 BIRTH ASPHYXIA AND LOW APGAR SCORE

The risk of bleeding was found to be increased if birth asphyxia was present ($X^2 = 9.18$, OR =1.74, P-value 0.002). This is a well recognised risk factor for periventricular and intraventricular haemorrhage. A study in America [32] observed that autoregulation of cerebral blood flow was impaired in asphyxiated infants. It is proposed that this is due to the fact that these infants are "pressure passive" and respond to systemic pressure changes

by worsening ischaemia (low pressure) or capillary haemorrhage (high blood pressure). APGAR score is used in the clinical assessment of asphyxia neonatorum and in this study the low APGAR score was associated with an increased risk for bleeding ($X^2 = 20.43$ df = 2 P-value = 0.00003). Pathogenesis of bleeding in asphyxial injury is similar to that operating in DIC [59]

14.3 PREMATURE AND LOW BIRTH WEIGHT INFANTS

The risk of bleeding was observed to be increased in both preterm ($X^2 = 4.07$ OR 1.50 P-value = 0.02) and in LBW babies ($X^2 = 4.58$ OR 1.48, P-value = 0.03). The study by Walter et al had similar finding [32]. In these studies it was observed that asphyxia and hypotension at birth were common in premature and LBW infants with haemorrhage. The hypothesis is that the germinal matrix and neighbouring periventricular white matter are in arterial watershed, making them vulnerable to ischaemic injury. Coagulation factors are also deficient in this group of infants. Birth trauma (both physical and asphyxial) is common in this group of infants. Asphyxial birth trauma operates through the DIC phenomenon. Other authors who described asphyxial injury, also found it to be responsible for the bleeding disorders found at autopsy [33]

14.4 GENERAL ANAESTHESIA

In this study general anaesthesia was found to be significantly associated with an increased risk for bleeding ($X^2 = 13.53$ P-value = 0.002). The indication for caesarean section in this study was mostly foetal distress, which was of the order of 259 (73.8%) of the infants, some of whom were subjected to general anaesthesia. Foetal distress is an

important marker of intrauterine asphyxia which when propounded with general anaesthesia, their effects are additive. There are no studies in the literature to compare with the findings in this study.

14.5 PROLONGED LABOUR

Prolonged labour was an important variable, in this study. However it was very difficult to establish the exact duration of labour in most of the mothers. Therefore, the information obtained that there was no significant difference between prolonged and normal labour (X^2 = 0.05, P-value = 0.97) may be artefactual due to recall bias and improper documentation on the perinatal chart. This calls for re-evaluation using a different design.

14.6 MODE OF DELIVERY

This study has established that the risk of bleeding increases if the mode of delivery is other than SVD (X^2 = 30.97, df 3 P-value = 0.0000008). LCVE was associated with bleeding disorders in 73.7%, ABD 35% and LSCS 17.7%. The finding of significant association of mode of delivery and bleeding (X^2 = 30.97 df = 3 P-value = 0.0000008) in this study can be explained by the presence of more than one risk factors, birth asphyxia being one of them. Birth trauma such as bruising of the presenting part was found to be associated with LCVE. Because of the interplay of more than one mechanism, no conclusion can be made by findings in this study.

Apart from the increased risk in general anaesthesia, delivery by LSCS had the lowest proportion (17%) among the other modes, which were associated with bleeding. This can not also be explained by the findings in this study because of the interplay between the indication of LSCS and general anaesthesia.

14.7 MULTIVARIATE ANALYSIS

Results of the multiple logistic linear regression analysis revealed that delivery by LCVE and LSCS were significantly associated with a bleeding tendency as compared to SVD. This finding is supported by the fact that prolonged second stage of labour and instrumental delivery may cause scalp bruising and haematoma of the presenting part. It is a common practice to deliver the high-risk deliveries by LSCS. However, the use of a vacuum extractor in the same is indicated in 'foetal gasping' during the second stage of labour as a salvage procedure. This fact is supported by the findings in this study that most of the infants delivered by LCVE or LSCS had asphyxia neonatorum, which is another important risk factor for bleeding.

The combined effect of the indication for LSCS or LCVE, birth asphyxia, anaesthesia and vacuum extractor to bleeding tendency cannot be concluded by the findings of this study.

14.8 COAGULATION PROFILE

The PT and APTT are coagulation indices that are routinely used to measure indirectly the status of vitamin K. The proportion of infants with vitamin-K deficiency (crude prevalence) as judged by abnormal PT and APTT in the study population ranged between 2.9% and 8.8% but the majority of them did not have any bleeding disorder at all. This

applies to the cases and the controls alike. Strikingly prolonged APTT and PT values were not necessarily associated with bleeding. In this study, only 0.9% and 2.5% of the respective infants with prolonged PT and APTT had bleeding disorders but there was no significant difference between the cases and the controls This finding is supported by other studies done in the USA. [60]

Coagulation indices being physiological markers of vitamin K status are reasonably accurate but not specific because other factors such as DIC may also be responsible for prolonged coagulation indices. Several studies including direct methods came up with similar conclusions [8, 9, 42]. However direct vitamin K assay by other researchers found undetectable levels in most newborn infants. This controversy lies in the different laboratory method used and the lack of standardisation thus making interpretation difficult.

The finding that it is only a small proportion of the study population who have prolonged coagulation indices in this study suggest that vitamin K deficiency is not a major problem in the infants admitted to the neonatal unit. However, this conclusion cannot be generalised for several reasons. First the population used is a high risk group. Secondly, PT and APTT are only indirect (not specific) methods and are subject to other medical conditions of vitamin K assay. Therefore, more specific studies are needed to draw a valid conclusion. The methods include assays of prothrombin, the non-carboxylated prothrombin (PIVKA-II) or vitamin K by high performance liquid chromatography [21].

14.9 VITAMIN K PROPHYLAXIS

The prevalence of vitamin K deficiency as indicated by prolonged PT and APTT is low. Thus vitamin K deficiency is not a major problem in our set up. Even among those infants with prolonged coagulation indices, there was no bleeding in the majority of them. This indicates that there are other factors related to HDN and NBD in general. In this study, asphyxia neonatorum, mode of delivery other than SVD, LBW, prematurity and general anaesthesia were significantly associated with a high risk of bleeding. These findings are supported by another study from the literature[9], which proposed that stressful pregnancy and delivery were responsible for the bleeding disorders rather than vitamin K deficiency. These risk factors may be considered as high risk for bleeding tendency. Although vitamin K deficiency may not be directly related, these risk factors may safely be regarded as indications for administration of vitamin K owing to its potential to reverse HDN in case they may coexist. Even Aballi[42] was unable to demonstrate PIVKA-II in cord blood, but inspite of his findings he still proposed that vitamin K should be administered to high-risk babies. Therefore, the current practice of vitamin K administration to high-risk deliveries at the neonatal unit is justifiable.

14.10 BLEEDING AND BREAST FEEDING

The finding in this study that the prevalence of bleeding tendency is very low in infants with prolonged PT and APTT and the fact that Tanzania is predominantly an exclusively breast feeding society, indirectly indicate that exclusive breast feeding is not a risk factor for HDN. Moreover, the proportion of vitamin K deficiency infants with bleeding is very small. This finding is similar to that reported by Jimenez et al[51] who concluded that

prolonged coagulation indices is a normal developmental process and was independent of the type of milk administered.

15. CONCLUSION

1. The prevalence of NBD at the Neonatal Unit, MMC, Dar es Salaam is 10.7%.
2. Coagulation indices and platelet count were not statistically different between the cases and the controls.
3. Modes of delivery other than SVD are risk factors for NBD.
4. Vitamin K deficiency is not a major cause of NBD at the Neonatal Unit, MMC, Dar Es Salaam.

16. RECOMMENDATIONS

1. Detailed study using direct methods of estimation of vitamin K status may be needed to establish the prevalence of HDN.
2. Although vitamin K related bleeding was not a major problem in this study, more work need to be done on the role of vitamin K prophylaxis to the high risk newborn infants.
3. Because of the interplay of other clinical conditions during delivery, a study to establish the role of mode of delivery other than SVD in NBD is desirable.
4. To establish the true prevalence of NBD, a study design which would include the Neonatal Unit catchment population is recommended.

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