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博 士（医学）学 位 論 文

Fetal growth restriction in Bangladesh

(バングラデシュにおける胎児発育遅延)

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Summary

Introduction:

Fetal growth restrictions (FGR)/intrauterine growth restriction (IUGR) are serious public health problems in the world. It is a global challenge for epidemiologists, nutritionists and clinicians. Important factors leading to high prevalence of IUGR in developing countries are maternal nutritional deficiencies and low birth weight (LBW). LBW is predominantly the result of IUGR. IUGR is a risk factor for miscarriage, perinatal mortality, neonatal mortality and long-term health consequence in the developing countries where malnutrition and low birth weight is prevalent.

Objectives:

The overall purpose of this study was to examine the first trimester fetal growth restriction and occurrence of miscarriage, and to describe fetal growth parameters in Bangladeshi population compared to international growth reference values.

Materials and methods:

The study was conducted within the Maternal and Infant Nutrition Interventions in Matlab (MINIMat study) where icddr,b has been running the Health and Demographic Surveillance System. As a part of the MINIMat study, pregnant women were initially recruited from November, 2001 to October 2003. A total of 4436 women were enrolled in the study by ultrasound examination at 8-10 weeks of gestation. Of them 3058 women successfully measured crown rump length (CRL) was included in analyses to examine growth restriction and miscarriages. A total of 3267 singleton babies were born. Out of these, 2678 pregnant women who completed ultrasound examinations at 14, 19

and 30 weeks of gestation were included for fetal growth analyses. Linear-cubic polynomial model was used to develop fetal growth charts. The z-score was used to examine the deviation of derived model values from the expected international reference values for respective gestational ages. Independent t test was performed to compare each time points, and repeated mixed model was used for the comparison of Bangladeshi growth curve with the international reference curve.

Results:

The occurrence of miscarriages was significantly higher in the smaller categories of CRL(z-score) after adjustments for maternal age, parity, early pregnancy BMI, gestational age at CRL measurement, and socioeconomic status (adjusted relative risk [95% confidence interval (95%CI)] :1.03 [1.02-1.05] for less than -2 z-score). In the present study, advanced maternal age and poor socioeconomic status were also identified as potential risk factors for miscarriage ($P < 0.05$).

The growth of all fetal parameters were significantly smaller than international reference values except for femur length ($P < 0.001$). Biparietal diameter (BPD) was smaller than the expected reference values throughout the pregnancy (significantly smaller than the 50^{th} percentile reference values at 22, 23 and 27-37 weeks of gestation, $P < 0.05$). Occipito-frontal diameter (OFD) started faltering from 17 weeks to onwards (significantly smaller than the reference values at 27-34 weeks of gestation, $P < 0.05$). Abdominal circumference (AC) started faltering from 14 weeks to the end of the pregnancy (significantly smaller than the reference value at 27-37 weeks of gestation, P

<0.05). The deviation of the means for each parameter increased with increased gestational age.

Discussion:

The present population-based study shows that CRL shorter than expected was associated with early miscarriages less than 20 weeks of gestational age. Advanced maternal age and poor socioeconomic status were associated with miscarriage in early pregnancy. The smaller growth was observed at the late pregnancy period compared with international reference values.

Advanced maternal age was an important variable in the prediction of miscarriage. The risk of miscarriage was increased with increasing maternal age. It was documented that the majority of early fetal deaths are due to chromosomal abnormalities and that there is an exponential increase of the risk for fetal trisomy with increasing maternal age. However, in the present study, did not have appropriate data to prove a correlation between chromosomal abnormalities and spontaneous miscarriage. The poor socioeconomic groups of women were deprived from the health care utilization and inadequate caring during pregnancy that could be the possible reasons in early miscarriage. Further community-based studies are required in order to understand clinical and biological phenomenon of spontaneous miscarriages.

Maternal malnutrition might be the possible explanation for the fetal growth restriction in last trimester of pregnancy.

Conclusions:

The smaller than expected CRL for the gestational age was related with the early miscarriages. The fetuses were smaller at the mid-second trimester to last trimester compared with reference values. The growth restriction was started at different gestational age for different parameters. These findings of this study are useful for the reliable assessment of fetal size and growth in Bangladesh.

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ABBREVIATIONS

AC	**Abdominal Circumference**
ARR	**Adjusted Relative Risk**
BDHS	**Bangladesh Demographic Health Survey**
BMI	**Body Mass Index**
BPD	**Biparietal Diameter**
CI	**Confidence Interval**
CRL	**Crown Rump Length**
DNA	**Deoxyribonucleic acid**
FL	**Femur Length**
GA	**Gestational Age**
HC	**Head Circumference**
HDSS	**Health and demographic Surveillance System**
icddr,b	**International Centre for Diarrheal Disease Research, Bangladesh**
IUGR	**Intrauterine Growth Restriction**
LBW	**Low Birth Weight**
LMP	**Last Menstrual Period**
MINIMat	**Maternal Infant Nutrition Intervention at Matlab**

OFD	**Occipito-Frontal Diameter**
SDS	**Standard Deviation Score**
SD	**Standard Deviation**
SGA	**Small for Gestational Age**
WHO	**World Health Organization**

Chapter 1

Background

1.1 Global situation of fetal growth restriction and maternal malnutrition

Fetal growth restrictions (FGR)/intrauterine growth restrictions (IUGR) are serious public health problems in developing countries. It has been reported that IUGR is affected around 3-10% of pregnancies. Approximately 20% of stillbirth occurred in infants who have IUGR. The perinatal mortality rates are 4-8 times higher for infants with IUGR and morbidity is present in 50% of infants who are surviving with compromised intrauterine environment ((1).

Maternal malnutrition is one of the important factors leading to high prevalence of IUGR and low birth weight (LBW) in developing countries. Maternal malnutrition is prevalent in many regions, especially in South Asia, where in some countries more than 10% of women aged 15–49 years are shorter than 145 cm. Pre-pregnancy body-mass index (BMI) of less than 18·5 kg/m², ranges from 10% to 19% in most of the countries. A serious problem of maternal malnutrition is evident in most of the countries in sub-Saharan Africa, south-central and southeastern Asia, where more than 20% of women have a BMI less than 18·5 kg/m². The situation could be considered more critical for Bangladesh and India where the prevalence of low BMI is around 40% in women (2). Low maternal BMI during pregnancy results in intrauterine growth restrictions (IUGR) and increased risk of miscarriage and neonatal morbidity and mortality (3).

Maternal malnutrition has independent adverse effects on pregnancy outcomes particularly in low birth weight (LBW) and prematurity. At least 4 million neonatal death occurred every year in the world were associated with LBW (3). It is reported that the cause of LBW are predominantly the results of IUGR and premature delivery. There is

the evidence that the higher incidence of LBW in a population, the greater proportion of IUGR, with the number of preterm babies relatively high (5).

The incidence of spontaneous miscarriages in pregnancies was 15%, and at least 80% of those occurred in the first trimester of pregnancy (6). Early first trimester growth restriction is the predictor of subsequent miscarriages. Previous hospital-based studies showed an association between the first trimester growth restriction and the increased probability of subsequent miscarriages (7-9). Different studies were clinically used the measurement of crown rump length (CRL) for predicting the miscarriage in early pregnancy (7-8). Limited community based studies were conducted to examine the association between maternal factors and miscarriage but these studies did not address the relationship between ultrasound parameter and miscarriage.

Many risk factors have been involved directly and indirectly for the fetal growth restriction during pregnancy. In fact, 80% of the risk factors of IUGR were non genetic and could be prevented by developing appropriate fetal growth reference charts that allowed timely detection of IUGR (10). The early detection of fetal growth restriction may help to reduce associated morbidity and mortality as well as the early miscarriage.

Therefore, fetal growth charts are important for tracking the fetal size from the early phase of pregnancy up to delivery as well as to examine the early growth restriction and occurrence of miscarriage.

1.2 Situation of fetal growth restriction and maternal nutrition in Bangladesh

Bangladesh is a vibrant developing country. The present territory of Bangladesh was a part of Pakistan. Bangladesh emerged on March 26, 1971, as an independent

country on the world's map following a war of liberation. It is located in the northeastern part of South Asia and covers an area of 147,570 square kilometers. It is almost entirely surrounded by India, except for a short southeastern frontier with Myanmar and a southern coastline on the Bay of Bengal. The population of the country is about 158 million, with a population density of 1,070 persons per square kilometer. Most of Bangladesh is low, flat land that consists of alluvial soil. The most significant feature of the terrain is the extensive network of rivers that is of primary importance to the socioeconomic life of the nation. The tropical climate of Bangladesh is dominated by seasonal monsoons. The country experiences a hot summer season with high humidity from March to June; a somewhat cooler, but still hot and humid, monsoon season from July through early October; and a cool, dry winter from November through the end of February. The fertile delta is subject to frequent natural calamities, such as floods, cyclones, tidal bores, and drought which affect the crops, live-hood and health of people (11).

Background at Matlab and pregnant women

Matlab is a poor rural area located 53 km southeast of Dhaka, the capital city of Bangladesh. It is a low-lying area close to the big Megna river and intersected by the tidal river Gumti and its numerous canals. It is a remote area with road connection built first in 2007–2008, and still there is very little vehicle traffic. In general, people travel by walking, rickshaw, country boat, and, in some cases, by small steamer or motor boats. Small-scale farming is the main income source, with some fishing and trading. Sharecropping and work on others' land on a daily wage basis are main sources of

income for the many landless people. Rice is the main staple food in Matlab and especially in poor families it contributes a major part of the dietary energy intake. Up to 99% of the homes in Matlab have corrugated tin-roof, about 70% have tin walls, and 90% have mudded floors. Cooking is often carried out outdoors using traditional Chulla, a mud-built cylinder, inserted into the ground, on which cooking pots and utensils are placed.

Most of the pregnant women were housewife and involved in household activities such as cooking, washing the utensils, caring the children as well as other family members throughout the pregnancy period (12).

In Bangladesh, maternal malnutrition in women, encompassing both under-nutrition and overweight and fetal growth restriction, is a major problem with important consequences for survival and healthy development of infants (11). Overweight and obese women are also predisposed to a wide range of health problems. Miscarriage is the common pregnancy complication among the pregnant women in Bangladesh. A hospital-based study showed that around 49% women had miscarried in the first trimester of pregnancy (13). The first trimester growth restriction and maternal malnutrition were associated with the miscarriage in early pregnancy (13). The prevalence of maternal malnutrition was high. It was reported that the maternal malnutrition was 40% among pregnant women in Bangladesh. The malnutrition was reported to be 31% among adolescent women (15-19), and obesity has been increasing over the last decade (from 9% to 24%) (11). The incidence of LBW was 21% and premature delivery was 14%, the highest among the South and Southeast Asia. The incidence of LBW is, predominantly the results of IUGR is among the highest in the

world (14). The IUGR was high in Bangladesh. A study in Bangladesh reported that the risk of neonatal death was several-fold higher in preterm infants than in full-term infants whose growth had been restricted in uterus (15).

The fetal growth charts are important for assessing fetal growth and the size during pregnancy but there have not been developed in these charts in the most of the developing countries. As a result the obstetrician and sonographers follows the fetal growth charts that all have been generated by the studies on western population where socio-economic status and nutritional status are different.

The growth charts are used to compare the size of a fetus with reference data for different circumstances and to identify any deviation from normal by plotting the measurements on charts (16).

In Bangladesh, there is no such a standard growth chart of fetal parameters for monitoring the fetal growth pattern during pregnancy. A limited hospital-based study was conducted with a small number of populations to assess the fetal growth charts but these studies neither compared with the international reference value nor mentioned the timing of growth restriction. Moreover, no community-based study was conducted to evaluate the effect of early growth restriction related to miscarriages in developing countries.

Therefore, it is important to understand the magnitude of IUGR among fetuses in rural Bangladesh where the prevalence of malnutrition and LBW are high. A population-based study is required to describe the fetal growth pattern and document the timing of growth restriction and compare to deviation with the international reference values. It

also important to examine early growth restriction related to miscarriage in rural Bangladesh.

1.3 Spontaneous miscarriage/abortion

Definition of miscarriage

Spontaneous abortion, or miscarriage, is defined as a clinically recognized pregnancy loss before the 20th week of gestation. The World Health Organization (WHO) defines it as expulsion or extraction of an embryo or fetus weighing 500 g or less (17).

Global situation of miscarriage/abortions

Spontaneous miscarriage/abortion is the common pregnancy complication in developing countries. The number of induced abortions decreased globally to 43.8 million (28%) in 2008 from 45.6 million (35%) in 1995. The proportion of spontaneous abortions worldwide that take place in the developing world increased between 1995 and 2008 from 78% to 86%. Since 2003, the number of abortions fell by 600,000 in the developed world but increased by 2.8 million in the developing world. The regional data show that in 2008, there were 29 abortions per 1000 women aged 15-44 years in developing countries, compared with 24/1000 in the developed world (18, 19). Nearly half of all abortions worldwide are unsafe, and nearly 98% of the unsafe abortions are occurring in developing countries. In the developing world, 56% of all abortions are unsafe, compared with only 6% in the developed world (18, 19). A study conducted in China revealed that the unsafe/induced abortion was associated with the subsequent

miscarriage in early pregnancy (20). It is indicated that spontaneous miscarriage is increased with increased the unsafe/induced abortion.

Miscarriage in early pregnancy is common. Different studies showed that about 8 to 20 percent of women who know they are pregnant have a miscarriage some time before 20 weeks of pregnancy; around 80 percent of these occur in the first 12 weeks (21). However, the actual rate of miscarriage is even higher since many women have very early miscarriages without ever realizing that they are pregnant. One study that followed women's hormone levels every day to detect very early pregnancy found a total miscarriage rate of 31 percent (22). Several studies reported that early first trimester growth restriction and maternal malnutrition associated with the risk of miscarriage.

Miscarriage in Bangladesh

Miscarriage is the common pregnancy complication in Bangladesh. Different studies showed that the incidence of miscarriage is high among the pregnant women in Bangladesh. A study in Matlab, Bangladesh, showed that the incidence of miscarriage was 56/1000 in treatment area and 66/1000 in comparison area over the period 1982-1991 (23). Another study in three diagnostic centers in Dhaka, Bangladesh, showed that the incidence of abortion among the study population (highly selected) was much higher. It was found that 49% women had aborted within the first trimester. More than 50% abortions took place within the age range from 13-20 years of age (13).

Risk factors of miscarriage

Several risk factors have been identified that increase the rate of miscarriage. In general, the risk factors are categorized as genetic and non genetic factors. Genetic factor are chromosomal abnormalities, early placental failure and fetal trisomy specially trisomy 22 and first trimester growth restriction. Non genetic factor are maternal age, maternal nutrition, previous history of miscarriage, smoking, drug abuse, alcohol drinking, poor socioeconomic status and mother parity. A study in Denmark reported that modification of risk factors acting before and during pregnancy could lead to prevention of 14.7 and 12.5%, respectively, of the miscarriages (24, 25).

Maternal malnutrition and miscarriage

Maternal malnutrition was the risk factor for the miscarriage of the early pregnancy. Women who have a BMI less than 18.5 kg/m^2 before they become pregnant are 72% more likely to suffer a miscarriage in the first three months of pregnancy (26). The higher growth restriction (small CRL) was found among the women those BMI <18.5 kg/m^2. The possible biological mechanisms behind an association between underweight and early fetal loss may be explained by the action of leptin, a hormone which is produced predominantly in the adipose tissue. Leptin and its receptor are expressed in the secretory endometrium in which they may regulate uterine angiogenesis and embryo implantation. Low plasma leptin levels have been hampered the normal process of embryo implantation that caused on early miscarriage which is

lower among the women BMI <18.5kg/m^2 (27). An animal study suggested that the mildly hyperglycaemic and aminoacid-depleted maternal environment generated by under nutrition in rats may act as an early mechanism of programming and initiate conditions of metabolic stress, restricting early embryonic proliferation, and the generation of appropriately sized stem-cell lineages which may lead to fetal demise (28). Under-nutrition and over nutrition both are the risk factors for miscarriage in early pregnancy.

1.4 Fetal growth restriction

Definition of fetal growth restriction

The most common definition of fetal growth restriction is a fetal weight that is below the 10^{th} percentile for gestational age as determined through an ultrasound. This can also be called small-for gestational age (SGA) (29).

Global situation of fetal growth restriction

Fetal growth restriction (FGR) is a condition in which a fetus is unable to achieve its expected size. This functional definition seeks to identify a population of fetuses at risk for modifiable but otherwise poor outcomes. This definition intentionally excludes of fetuses that are small for gestational age (SGA) but are not pathologically small. All fetuses that are SGA are not pathologically growth restriction but they may be

constitutionally small. Of all fetuses at or below 10 percentile for growth, approximately 40% are at high risk of potentially preventable perinatal death. Another 40% of these fetuses are constitutionally small as these diagnoses may be made with certainty in neonate and significant numbers of fetuses are healthy but SGA. The remaining 20% fetuses that are SGA are intrinsically small secondary to chromosomal or environmental etiology. These fetuses are less likely to benefit from prenatal intervention (30).

IUGR is associated with increased the risk of perinatal morbidity and mortality. At least 60% of 4 millions neonatal deaths that occur worldwide in every year are associated with low birth weight. The causes of these LBW are due to IUGR during pregnancy (30). A study in Sweden showed that small fetus was 10 times more risk for fetal death compared with normal fetuses. Another study showed that fetuses with growth restriction were more likely to increase the risk of still birth (30). Fetuses with IUGR who survive the compromised intrauterine environment are at increased risk for neonatal morbidity (31). The prematurity and IUGR also directly and indirectly cause or risk factors for neonatal mortality (32).

Situation in Bangladesh

Gestational age and nutritional status at birth are important determinants of growth patterns in infancy (32). Low birth weight (LBW) of newborns is a challenging problem in developing countries (33). According to UNICEF/FAO report in (2004), the incidence of LBW is 58% in developing countries with highest in south Asia (74%). Of which Bangladesh contributes approximately 30% (34). The incidence of LBW in

Bangladesh, predominantly the results of intrauterine growth restriction is among the highest in the world (35-38). A study in Bangladesh reported that the risk of neonatal death was several-fold higher in preterm infants than in full-term infants whose growth had been restricted in utero (15).

The present study was conducted within the Maternal and Infant Nutrition Interventions Trial in Matlab, Bangladesh (Called MINIMat study). As a part of MINMat study women were recruited from November, 2001 to October, 2003. MINIMat is a continuous study, the infant who were born under this cohort were followed up at 4.5 and 10 years of aged. The 15 years follow up has been scheduled on 2017. Each followed up anthropometrics measurement, nutritional status, lungs functional test were measured of the children.

The prevalence of maternal malnutrition and low birth weight was 40% and 30% respectively. The socioeconomic situation is almost same. In this situation, the fetal growth pattern would not be much changed as the prevalence of maternal malnutrition and low birth weight has not been significantly changed over the period. On the other hand the study has developed the fetal growth equations for five fetal parameters which will be used to develop the fetal growth pattern by using the updated data.

Risk factors of fetal growth

According to the WHO, the growth restriction is a known fact that growth can be affected by several factors. Current knowledge shows that about 60% of the cases of IUGR are associated with certain specific risk factors. These factors can be broken down depending the time at when they are detected: a) Preconception risk factors such as low socioeconomic status of women, older age (<16 >35 years), short stature (height < 145cm), malnutrition, chronic diseases (hypertension, kidney disease, diabetic with vascular disease, chronic lung disease, mesenchymal diseases etc). b) Risk factors detected during pregnancy like multiple pregnancies, weight gain less than 8 kg at term, birth interval less than 12 months, pregnancy-induced hypertension / preeclampsia-eclampsia, anti-phospholipid syndrome, anemia, infection: viral (rubella, cytomegalovirus, valicela, herpes zoster), parasitic: (toxoplasmosis, malaria), congenital malformations, genetic disorder, exposure to teratogens. c) Environmental and behavioral risk factors such as smoking during pregnancy, heavy alcohol consumption, excessive consumption of caffeine, drug addiction, high attitude above sea level, stress, lack of or inadequate antenatal care or excessive physical work (30, 39).

Maternal malnutrition and fetal development

Maternal nutrition is the major non-genetic intrauterine environmental factor as well as the predictor for early miscarriage. Maternal nutrition plays an important role during pregnancy in the regulation of placental-fetal development and affects the

lifelong health and productivity of offspring. The placenta is the organ through which gases, nutrient and waste are exchanged between maternal and fetal circulations. The placenta determines the prenatal growth trajectory of the fetus to influence birth weight depending on its size, morphology and nutrient transfer capacity. During normal pregnancy, the placenta goes a variety of physiological changes, regulated by angiogenic factors, hormones and nutrients related genes, to maximize efficiency for increasing demand for nutrients. Most important, maternal nutritional status may adversely influence placental homeostasis and prevent fetal development (40). Animal studies shows that both maternal under nutrition and over nutrition reduce placental-fetal blood flows and stunt fetal development. Impaired placental syntheses of nitric oxide (a major vasodilator and angiogenesis factors) and polyamines (key regulators of DNA and protein synthesis) may provide a unified explanation for IUGR in response to the two extremes of nutritional problem with the same pregnancy outcome (41).

Low birth weight (LBW)

According to World Health Organization (WHO) low birth weight (LBW) has been defined as a birth weight of a live born infant of less than 2,500 g (5.5 pounds) regardless of gestational age. As per epidemiological observations that infants weighing less than 2,500 g are approximately 20 times more likely to die than heavier babies. More common in developing than developed countries, a birth weight below 2,500 g contributes to a range of poor health outcomes. A baby's low weight at birth is either the result of preterm birth (before 37 weeks of gestation) or due to restricted fetal growth

during pregnancy. More than 20 million infants worldwide, representing 15.5% of all births, are born with LBW, 95.6% of them in developing countries. The level of LBW in developing countries (16.5%) is more than double the level in developed regions (7%). Half of all low birth weight babies are born in South-central Asia, where more than a quarter (27%) of all infants weighs less than 2,500 g at birth. Many factors affect the duration of gestation and fetal growth, and thus, the birth weight. LBW is closely associated with fetal and neonatal mortality and morbidity, inhibited growth and cognitive development, and chronic diseases in later in life (4, 42, 46, 47).

1.5 Fetal biometry and ultrasound in Pregnancy

Fetal and obstetric ultrasound measurements in pregnancy

Fetal body obstetric ultrasound measurements reflect whether the fetus has grown enough or whether the measurements fall outside the normal range. The following measurements can usually be made. Ultrasound is widely used for detection of miscarriage especially in the first trimester of pregnancy. In a pregnant woman who has had a complete miscarriage, no pregnancy sac or embryo will be seen on ultrasound. In other women, a pregnancy sac will be seen but it will be abnormal or an embryo will not be present, indicating that the pregnancy is not viable (43).

Definition of crown rump length (CRL)

Crown-rump length (CRL) is the measurement of the length of human embryos and fetuses from the top of the head (crown) to the bottom of the buttocks (rump). It is typically determined from ultrasound imagery and can be used to estimate gestational age. The crown rump length (CRL) is the predictor for miscarriage. If an embryo is present, the size of CRL is measured and compared to the size that is expected at the woman's stage of pregnancy. The hypothesis is that if the size of CRL is smaller than expected for gestational age or at the women stage of pregnancy that increase the risk of miscarriage (43).

Fetal heart beat

At about 6 weeks after the last menstrual period (LMP), the motion of the fetal heart should be visible on ultrasound. If the pregnancy has progressed to the stage where a heart beat should be present, the failure to detect a heart beat during an ultrasound exam indicates that the pregnancy has likely ended (43).

Gestational sac (GS)

The gestational sac is an intrauterine structure surrounding the pregnancy that can be used to determine if an intrauterine pregnancy (IUP) exists prior to the visualization of the embryo. It can be measured across and the weeks of the pregnancy can be determined with about 5 day accuracy (43).

Biparietal diameter (BPD)

The diameter between the two sides of the head. This is measured after 13 weeks. It increases from about 2.4 cm at 13 weeks to about 9.5 cm at term. Different babies of the same weight can have different head size, therefore dating in the later part of pregnancy is generally considered unreliable. Dating using the BPD should be done as early as feasible (43, 47).

Occipito-frontal diameter (OFD)

The occipitofrontal diameter is measured in the same plane as the BPD and is a measurement of the longitudinal axis, by convention taken from outer skull tables on each side. This measurement can be averaged with the outer-to-outer skull measurement in the transverse plane to provide a basis for estimating head circumference (43).

Head circumference (HC)

Head circumference (HC) is one of the basic biometric parameters used to assess fetal size. HC together with biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) are computed to produce an estimate of fetal weight (43).

Abdominal circumference (AC)

The abdominal circumference is the most single important measurement to make in late pregnancy. It reflects more of fetal size and weight rather than age. Serial measurements are useful in monitoring growth of the fetus. AC measurements should not be used for dating a fetus (43).

Femur length (FL)

Measures the longest bone in the body and reflects the longitudinal growth of the fetus. Its usefulness is similar to the BPD. It increases from about 1.5 cm at 14 weeks to about 7.8 cm at term. Similar to the BPD, dating using the FL should be done as early as is feasible (48).

1.6 References

1. William H. Tooley. Intensive Care Nursery, House Staff Manual; Eight editions, 23rd July, 2003. The University of California 2004, p; 69.

2. http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(07)61690-0/abstract. Maternal and child under-nutrition: global and regional exposure and health consequence. January, 2008. Accessed, 20th July, 2016

3. Belkacemi L, Nelson DM, Desai M, Ross MG. Maternal under-nutrition influences placental-fetal development. Biol Reprod, 2010; 83(3):325-31.

4. http://apps.who.int/iris/bitstream/10665/43184/1/9280638327.pdf: Low birth weight, regional and global estimate, World Health Organization. 2004. Accessed 20th July, 2016.

5. Rondó PH, Abbott R, Rodrigues LC, Tomkins AM. The influence of maternal nutritional factors on intrauterine growth retardation in Brazil. Paediatr Perinat Epidemiol 1997; 11(2), 152-166.

6. Groden J, Gocha AS, Croce CM. Human Basic Genetics and Patterns of Inheritance. In: Creasy RK, Resnik R, Iams JD, Lockwood CJ, Moore TR, Greene MF, eds. Creasy and Resnik's Maternal-Fetal Medicine. China: Elsevier Saunders; 2014:3-36.

7. Papaioannou GI, Syngelaki A, Maiz N, Ross JA, Nicolaides KH. Ultrasonographic prediction of early miscarriage. Hum Reprod 2011;26(7):1685-92.

8. Choong S, Rombauts L, Ugoni A, Meagher S. Ultrasound prediction of risk of spontaneous miscarriage in live embryos from assisted conceptions. Ultrasound Obstet Gynecol 2003;22(6):571-7.

9. Reljic M. The significance of crown-rump length measurement for predicting adverse pregnancy outcome of threatened abortion. Ultrasound Obstet Gynecol 2001;17(6):510-2.

10. Fetal growth restriction, 2015. *http://emedicine.medscape.com/article/261226-overview.* Accessed July, 29, 2016.

11. Bangladesh demographic Health Survey, 2014.

12. Bergkvist C, Kippler M, Hamadani JD, Grandér M, Tofail F, Berglund M, Vahter M. Assessment of early-life lead exposure in rural Bangladesh. Environ Res. 2010 Oct;110(7):718-24.

13. Saha RK, Afroja S, Tanjin S, Chowdhury EA, Roy P and Rashid J. Induced abortion and risk of subsequent miscarriage during pregnancy in perspective of Bangladesh. Journal of Medical and Biological Science Research. 2015. 1 (5), 47-54.

14. Arifeen S, Black RE, Caulfield LE, Antelman G, Baqui AH, Nahar G, Alamgir S, and Mahmud H. Infant growth patterns in the slums of Dhaka in relation to birth weight, intrauterine growth retardation, and prematurity. Am J Clin Nutr 2000; 72:1010-7

15. Lawn JE, Cousens S, Zupan J; Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: when? Where? Why? Lancet. 2005; 5-11;365(9462):891-900.

16. Tinelli A, Bochicchio MA, Vaira L, Malvasi A. Ultrasonographic fetal growth charts: an informatic approach by quantitative analysis of the impact of ethnicity on diagnoses based on a preliminary report on Salentinian population. Biomed Res Int. 2014;2014:386124.

17. Cunningh FG, Lenevo KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL et al. Williams Obstetrics. Stamford, Connecticut, USA: The McGraw-Hill Companies; 2014.

18. https://www.guttmacher.org/fact-sheet/facts-induced-abortion-worldwide. Facts on Induced Abortion Worldwide global incidence and trends, 2012. Accessed 20th July, 2016.

19.http://www.who.int/reproductivehealth/publications/unsafe_abortion/induced_abortion_2012.pdf, World Health Organization. Facts on abortion worldwide. 2008. Accessed 20th July, 2016.

20. Sun Y, Che Y, Gao E, Olsen J, Zhou W. Induced abortion and risk of subsequent miscarriage. Int J Epidemiol. 2003; 32(3):449-54.

21. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000; 14(5):839-54.

22. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC. Incidence of early loss of pregnancy. N Engl J Med. 1988; 319(4):189.

23. Ahmed K, Rahman M, and Gnneken JV. Induced abortion in Matlab Bangladesh. Trends and determinants. International family planning perspective. 1998; 24(3) 328-332.

24. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. Best Pract Res Clin Obstet Gynaecol. 2000; 14(5):855.

25. Feodor Nilsson S, Andersen PK, Strandberg-Larsen K, Nybo Andersen AM. Risk factors for miscarriage from a prevention perspective: a nationwide follow-up study. BJOG 2014;121(11):1375-84.

26. http://www.eufic.org/page/en/show/latest-science-news/fftid/underweight-iscarriage-healthy-diet-pregnancy/. Underweight women at greater risk of miscarriage but having a

healthy diet and reducing stress when pregnant may lower risk. European food information council UFIC), November, 2015, Accessed, 2nd August, 2016.

27. Helgstrand S, Andersen AM. Maternal underweight and the risk of spontaneous abortion. Acta Obstet Gynecol Scand 2005: 84: 1197-1201.

28. Veleva Z, Tiitinen A, Vilska S, Hyden-Granskog C, Tomas C, Martikainen H et al. High and lo w BMI increase the risk of miscarriage after IVF/ICSI and FET. Hum Reprod 2008; 23 (4):878-84.

29. http://americanpregnancy.org/pregnancy-complications/fetal-growth-restriction/, definition of fetal growth restriction, accessed, 29th July, 2016

30. *http://emedicine.medscape.com/article/261226-overview.* fetal growth restriction and different types. Accessed, 20th July, 2016.

31. Marsal K. Intrauterine growth restriction. Curr Opin Obstet Gynecol 2002; 14:127-135.

32. Barker DJP, Clark PM. Fetal under-nutrition and disease in later life. Rev Reprod 1997; 2: 105-112.

33. Shirin F, Mehdi T, Alam MM, Nath RK, Hoque MM**.** Effect of Gestational Homocystein on Fetal Growth in Bangladeshi Women. Ibrahim Med. Coll. J. 2009; (1): 13-16.

34. Peleg D, Kennedy C, Hunter S. 1998. Intrauterine growth restriction: identification and management. www.aa/980800/peleg.html.

35. Goodburn E, Chowdhury M, Gazi R. Low birth weight in rural Bangladesh. J Trop Pediatr 1994; 40:123.

36. Khan M, Curlin GT, Chakraborty J. Growth and development studies: rural Meheran, Comilla. Bangladesh. Med J 1979; 7: 74-90.

37. World Health Organization, Division of Family Health. The incidence of low birth weight: a critical review of available information. World Health Stat Q 1980; 332:197-224.

38. Canosa CA. Intrauterine growth retardation in India and Bangladesh. In: Senterre J, ed. Intrauterine growth retardation. Nestle Nutrition Workshop Series. Vol 18. New York: Raven Press, 1989:183-204.

39. Monitoring fetal growth, self instruction manual. 2nd edition, 2011, pans American Organization.

40. Belkacemi L, Nelson DM, Desai M, Ross MG. Maternal undernutrition influences placental-fetal development. Biol Reprod 2010; 83(3):325-31.

41. Wu G, Bazer FW, Cudd TA, Meininger CJ, SpencerTE. Maternal nutrition and fetal development. J Nutr 2004;134(9):2169-72.

42. Yasmin S, Osrin D, Paul E and Costello A. Neonatal mortality of low-birth-weight infants in Bangladesh. Bulletin of the World Health Organization, 2001, 79: 608-614.

43. *http://www.babymed.com/fetal-and-obstetric-ultrasound-measurements-pregnancy.* Fetal and Obstetric Ultrasound Measurements in Pregnancy. Accessed 15th July, 2016

44. Scott, James R., Ronald S. Gibbins, Beth Y. Karlan, Arthur F. Haney ed. Danforth's Obstetrics and Gynecology 9th Ed. (Philadelphia: Lippincott Williams & Wilkins), 2003.

45. Olds, Sally B, Marcia L. London, and Patricia Wieland Ladewig, eds. Maternal-Newborn Nursing: A Family- Centered Approach 5th Ed. (New York: Addison-Wesley Nursing), 1996.

46 Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. *Bull World Health Organ* 1987; 65**:** 663-737.

47. Kramer MS, Victora C. Low birth weight and perinatal mortality. In: Semba RD, Bloem MW, eds. Nutrition and health in developing countries. Humana Press, 2001.

(Latin American Center for Perinatology - Women and Reproductive Health CLAP/WR

48. Jaiswal P, Masih WF, Jaiswal S, Chowdhary DS. Assessment of fetal gestational age by ultrasonic measurement of bi-parietal diameter in the southern part of Rajasthan. Med J DY Patil Univ 2015; 8:27-30

Chapter 2

First-trimester fetal growth restriction and the occurrence of miscarriage in rural Bangladesh

2.1 Abstract

Introduction: Spontaneous miscarriage is the most common pregnancy complications. It's not only associated with morbidity or mortality of women but also has a significant social and psychological impact on women. Early fetal growth restriction might be a risk factor for occurrence of early miscarriage.

Objectives: This study was aimed to examine the first trimester growth restriction and occurrence of subsequent miscarriage in pregnant women in rural Bangladesh.

Methods: The study was conducted within the Maternal and Infant Nutrition Interventions Trial in Matlab (MINIMat study), Bangladesh. A total of 4436 pregnant women were enrolled in the study when they were at less than 14 gestational weeks. The expected CRL was determined based on an established growth curve of gestational age and CRL, and deviation of CRL from this curve was expressed as a z-score. After identifying related covariates, the multiple Poisson regression model was used to determine the independent contribution from the CRL to miscarriage.

Results: A total of 3058 singleton pregnant women were included in analyses, with 92 miscarriages and 2966 continued pregnancies. The mean z-score of CRL was significantly smaller in miscarriage group of women compared with continued pregnancy (P <0.001). The occurrence of miscarriages was significantly higher in the smaller categories of CRL z-score after adjustments for maternal age, parity, early pregnancy BMI, gestational age at CRL measurement, and socioeconomic status (adjusted relative risk [95% confidence interval]: 1.03 [1.02-1.05] for less than -2 z-score).

Conclusion: In a rural Bangladesh population, smaller than expected CRL for the gestational age was related to subsequent miscarriage.

2.2 Introduction

Spontaneous miscarriage, one of the most common pregnancy complications, is not only associated with morbidity or mortality (1), but also has a significant social and psychological impact on women (2). The incidence of spontaneous miscarriages in pregnancies was reported to be as high as 15%, and at least 80% of those occurred in the first trimester of pregnancy (3).

Chromosomal abnormalities followed by uterine malformations are the most common etiologies of spontaneous miscarriage in early pregnancy (4). Advanced maternal age, smoking, alcohol consumption, drug abuse, vaginal bleeding, and previous history of miscarriages are the commonly reported risk factors of spontaneous miscarriages (4-5). Early growth restriction, fetal heart rate, gestational sac diameter, and yolk sac diameter have been used as early predictors of subsequent miscarriage (9, 10). Conversely, the crown rump length (CRL) measurement is also clinically used for predicting adverse pregnancy outcomes of threatened abortion or predictor of spontaneous miscarriage in early pregnancy (11-13). Most of these studies were hospital-based and conducted in developed countries with a small number of selected populations. Two population-based studies, conducted in the United Kingdom and Sweden, examined the association between maternal risk factors and miscarriages (14, 15). However, these studies did not examine any ultrasound parameters for predicting subsequent miscarriage in early pregnancy.

Hospital-based studies may also be limited in their ability to consider the reproductive outcomes among a general healthy population. It requires a routine data collection system that can cover both the full range of miscarriages and link to individual-based data (14). The data, however, is scarce from developing countries. Moreover, no community-based study was conducted to evaluate the effect of factors related to miscarriage in developing countries where malnutrition is prevalent and socioeconomic status is different. Therefore, in this population-based prospective study, carried out in rural Bangladesh, we aim to examine whether smaller than expected CRL is the predictor for subsequent miscarriages.

2.3. Materials and methods

Study area and population

The present study was conducted as part of a large-scale randomized trial of nutrition interventions in pregnancy: the Maternal and Infant Nutrition Interventions Trial in Matlab, Bangladesh (MINIMat) study (trial registration: isrctn.org identifier: ISRCTN16581394). Matlab is located 53 km south of the capital, Dhaka. The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), has been running a health and demographic surveillance system (HDSS) in Matlab since 1966 that covers a population of about 225,000 in 142 villages. The icddr,b provides health care to women of reproductive age and children less than 5 years of age. This area is divided into 4 administrative blocks, each with a population of about 25,000-27,000.

Each block has a sub-center clinic, where paramedical staff provides maternal and child care, including delivery services 24 hours a day. The clinics are supported by a hospital located at the Matlab center. Community health research workers visit every household on a monthly basis to update information on demographic events, such as marriage, pregnancy, birth, death, and in– and out–migration, as well as to collect information on the morbidity of children below 5 years of age and women of childbearing age. Socioeconomic information, including education and household assets is also recorded by periodic censuses.

Maternal and Infant Nutrition Intervention in Matlab Trial

Recruitment of the women in the MINIMat trial was conducted in the Matlab HDSS area of Bangladesh over 2 year period from November 2001 to October 2003. Food and micronutrient supplementation was continued until birth of their children. In brief, women were recruited early in pregnancy through regular surveillance of the demographic area covered by icddr,b. Consenting women were randomized to two separate nutritional interventions in pregnancy: access to food supplementation or receipt of a micronutrient supplement. For the food intervention, women were randomized to receive encouragement to attend government sponsored local community nutrition centres either early in pregnancy (8-10 weeks gestation) or at a time of their choosing (usually around 20 week gestation). Food supplements that provided 608 kcal/day energy and 18 gram/day of vegetable protein were available to all attending women. Women participating in the MINIMat trial were also randomized to receive one of three micronutrient supplements with identical appearance: 30 mg of iron and 400 µg of folate (Fe30F), 60 mg of iron and 400 µg of folate (Fe60F) or the

combination of 15 micronutrients (MuMs), later is called UNIMAP (UN multiple micronutrient preparation), at or above the recommended daily allowance, which also contained 30 mg of iron and 400 µg of folate (MMS).

Study subject and study procedure

Women in the MINIMat study were recruited from November, 2001 to October, 2003 in the Matlab Health and Demographic Surveillance System area of the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b).

Pregnancy urine test was offered to every woman who reported to the Community Health Research Workers (CHRWs) that her last menstrual period (LMP) was at least 2 weeks overdue, or that she was pregnant. The LMP date was determined by recall during the pregnancy identification interview at routine monthly household visits. A woman with positive pregnancy test was invited to join the study and the date of her LMP was recorded. She was invited to visit a nearby icddr,b clinic for evaluation of viable fetus and measurement of gestational age (GA) by ultrasound examination. To participate in the study, the inclusion criteria for a pregnant woman were: 1) viable fetus, 2) GA less than 14 weeks, and 3) given consent.

A total of 5880 women were identified as eligible for the study. Of these women, 1444 were excluded because of migration out of the study area, refusal to participate, having a fetus whose GA exceeded the limits for the study or no longer having a viable fetus by ultrasound, and other reasons (Fig 3.1). A total of 4436 women were enrolled to follow up with ultrasound examination during clinic visits at 14, 19, and 30 weeks of pregnancy. Of the 4436 women, 532 were excluded from the study for reasons of migration, withdrawal of consent, induced abortion, absence, and other reasons. A

further 291 women had to be excluded due to missing (could not recall) LMP dates (n=47) or erroneous LMP information (n=244). We defined the recalled LMP as erroneous if the first trimester ultrasound-estimated LMP and recalled LMP had a difference more than 21 days. Additionally, 529 women had to be dropped from the analyses because their CRLs were not measured, biparietal diameter (BPD) was measured at 13 gestational weeks (n=409) and early miscarriages before 6 gestational weeks (n=120). Furthermore, 26 twin pregnancies were excluded. Finally, 3058 singleton pregnant women were included in the analysis (Fig 3.1). Of those, 2966 continued with viable pregnancies until delivery and 92 had miscarriages.

Four ultrasound machines (SSA 320A, Justavision-200, Toshiba, Tokyo, Japan) with 3.5 MHz standard convex probes were used for the fetal biometry measurements including CRL. Three measurements were taken and the average value was used for analysis.

Nine sonographers (paramedics) and one supervisor (medical doctor) were trained by a highly qualified local ultrasound consultant. Before beginning field data collection, the team was standardized to assure acceptable intra- and inter-observer variability for all measurements. Ongoing quality control was documented by re-examination of 3% of the total cases by a doctor who did not have access to the previous results and without notification to the study ultrasonographers (16).

Pregnant women were interviewed monthly to identify pregnancy outcomes such as spontaneous abortion, induced abortion, stillbirth, live birth, and survival in infancy. Trained female field workers prospectively collected the outcome information. A study physician reviewed each outcome, and miscarriages were confirmed at the icddr,b

clinics. Miscarriage was defined as an unintended loss of fetus before the first 20 weeks of gestation as determined by the reported LMP. Stillbirth was defined as birth of a dead fetus after 20 weeks of gestation (17). Live birth was defined as birth of a fetus with any sign of viability. Parity is the number of live and/or deceased children before the current pregnancy. Socioeconomic status was assessed by generating scores through principal-components analysis based on household assets, housing structure, land occupation, and income. These scores were then indexed into quintiles, where 1 represents the poorest and 5 the richest (18). Height and weight measurements of the pregnant women were taken at the time of enrollment, between 6 and 13 week of gestation. The Body Mass Index (BMI) (kg/m^2) was categorized as either under nutrition (<18.5), normal (18.5-24.9), or overweight (≥25).

The observed CRL was measured by ultrasound examination at enrollment, between 6 and 13 weeks of gestation. The expected CRL was determined based on an international reference of GA and CRL (19). The difference between the observed and expected CRL was calculated based on the formula: observed value minus expected value divided by standard deviation (SD), and expressed as a z-score. The CRL z-score was compared between the miscarriage and viable pregnancy groups. A grade of 0 on the CRL z-score means that the CRL is the same as expected for the corresponding GA. A positive or negative CRL z-score indicates that CRL is above or below expected for the corresponding GA. The CRL z-score was categorized in different recommended values as follows: 1^{st} (-1 or more), 2^{nd} (-2 to less than -1), 3^{rd} (-3 to less than -2), and 4^{th} (less than -3).

Statistical analysis

Maternal factors and CRL z-score were compared between miscarriage and viable pregnancy groups. For continuous variables, independent t-test was performed to compare the mean between the 2 groups. Chi-square test was used for comparing proportions between categorical variables. Multiple Poisson regression model was used to determine the independent contribution from the CRL to miscarriage by controlling for socioeconomic status, maternal age, parity, gestational age at CRL measurement, and BMI. The strength of association was expressed by adjusted relative risk (aRR) and 95% confidence interval (CI). The significance of an association was considered at *P* value < 0.05 in 2 sides. Statistical analysis was performed using IBM SPSS (version 22.0; New York, USA).

Ethical consideration

The study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the ethical review committees of icddr,b and Uppsala University. Written informed consent was obtained from each participant.

2.4. Results

General characteristics of participants

Table 3.1 presents the general characteristic of the study participants. The mean maternal age at the enrollment was 25.8 ± 5.8 years (range 14-50 years). One third of women (33.7%) were nulliparous. Only 67.9% of women had attended school. The

mean of early pregnancy BMI was 20.2 ± 2.6 kg/m^2 and 27.4% of women were underweight.

Comparison between miscarriage and continued pregnancy

Table 3.2 represents the comparison between two groups of women. The mean z-score of CRL was significantly lower in the miscarriage group of women compared with continued pregnancy (-1.43 vs -0.80, *P* = 0.030). The rate of miscarriages was almost double among the women who had smaller than expected CRL for GA (negative z-scores) as it among the women with larger CRL (3.6% [72/1983] vs. 2.1% [20/1075]; *P* = 0.003). The occurrence of miscarriages was significantly more in the smaller categories of CRL z-score (2^{nd}, 3^{rd}, and 4^{th}) compared with the 1^{st} category (z-score -1 or more; *P* = 0.004). The sensitivity and specificity of CRLs below -2 SDs was 45.0% and 68.0%, respectively. The mean maternal age was significantly older in the group of miscarriage than in the group with continued viable pregnancy (27.4 vs 25.7, *P* = 0.008). The occurrence of miscarriages was more likely to be higher among older women aged ≥ 35 years (*P* = 0.032). The number of overweight women was higher in the miscarriage group than in the continued viable pregnancy group (*P* = 0.021). Socioeconomic status, parity and food supplementation were not significantly different between the two groups. The mean gestational age at enrollment and the mean observed CRL both were significantly lower in the miscarriage group of women compared with the continued pregnancy group of women (P <0.001).

Association with the risk factors

After adjusting for maternal age, parity, early pregnancy BMI, GA at CRL measurement and socioeconomic quintile, we found a similar result, with the smaller

CRL categories having more miscarriages. The adjusted relative risks (aRR) of miscarriage (95% confidence interval: 95% CI) were 1.02 (1.00-1.04), 1.04 (1.01-1.06), and 1.03 (1.01-1.05) in the 2nd, 3rd, and 4th categories of CRL z-score respectively by comparing with the 1st category of CRL z-score (Table 3.3). When the cut-off of -2 z-score was used, aRR (95%CI) was 1.03 (1.02-1.05). The risk of miscarriages was significantly higher in women aged ≥ 35 years than those aged 25-29 years (*P* = 0.021). The occurrence of miscarriages was higher in the poorest quintile of women than the richest (*P* = 0.030).

2.5. Discussion

The present population-based study shows that smaller CRL is related to miscarriages in early pregnancy before 20 weeks. In the present study, nearly half of the pregnancies that subsequently miscarried showed a CRL measurement significantly lower ≤ -2 z-score than expected for the GA. The present study shows that the risk of miscarriage increases in small CRL categories. Women with a fetus of smaller than expected CRL had nearly twice the risk of miscarriage. Despite a lower CRL measurement ≤ -2 z-score from that expected for the GA, one third of the pregnancies ended as viable. These 2 groups of women, however, had significant differences between socio-economic strata, age, and nutritional status.

Previous hospital-based studies have shown an association between a smaller than expected CRL and the increased probability of subsequent miscarriage (9, 10, 13). A hospital-based study in London showed that the pregnancies with CRL smaller than

expected were more likely to be at risk for miscarriages (11). Another hospital-based study in Egypt reported that approximately 60% of pregnancies that ended in subsequent miscarriage had smaller than expected CRL (12). The present study shows that the risk of miscarriage increases with advancing the negative CRL z-score categories.

In women who have conceived naturally, it is assumed that ovulation occurs 14 days after their LMP. The difference between observed and expected fetal size may be due to the timing of ovulation and smaller than expected CRL, and therefore reflects a delayed conception in relation to the LMP rather than a true fetal growth delay (20). By considering these issues, the present study aimed to identify accurate LMP dates through a strong surveillance system and confirmation by study physicians at clinics.
In the present study, advanced maternal age and poor socioeconomic status were also identified as potential risk factors for miscarriage.

Maternal age was an important variable in the prediction of miscarriage. The risk of miscarriage was increased with increasing maternal age. It is well documented that the majority of early fetal deaths are due to chromosomal abnormalities and that there is an exponential increase of the risk for fetal trisomy with increasing maternal age (6). However, in the present study we did not have appropriate data to prove a correlation between chromosomal abnormalities and spontaneous miscarriage. Further community-based studies are required in order to understand clinical and biological phenomenon of spontaneous miscarriages. A study done in the United Kingdom showed that, in comparison to women aged 25-29 years, the occurrence of miscarriage sharply increased among women aged 35 years or more (14). The results of the present study

support these findings, suggesting that, for women in rural Bangladesh, increased maternal age is a significant risk factor for miscarriage. Miscarriages were significantly higher in the poor socioeconomic group of women. A similar finding was also observed in the United States (7).

A previous study conducted in the United Kingdom showed that low BMI was a risk factor for miscarriage (14), however a Finish study showed that both low and high BMI were risk factors for miscarriage in early pregnancy (21). In the present study, univariate analysis shows that BMI is related to miscarriage. After adjusting for maternal age and parity, however, BMI was not associated with miscarriage. This might be due to the small percentage of women in the overweight group of our study, and thus the limited statistical power.

The present study has several strengths. Firstly, it showed the incidence of natural phenomenon of miscarriage in a healthy population in which trained research members, using a strong surveillance system, followed up on the outcome of each pregnancy. Secondly, for quality assurance, an independent team of data collectors randomly selected 5% of the sample and repeated the interviews and measurements and compared the data with 2 interviews.

There are some limitations to this study. This study does not report some of the important factors that may influence early miscarriage such as previous history of miscarriage, vaginal bleeding, abdominal pain, non-steroidal anti-inflammatory drugs use, maternal diabetes, and hypertension. Another limitation was that this study could not measure the CRLs of all the participant's fetuses. This study focused on

miscarriages with measured CRLs and did not cover all miscarriages, for example, early miscarriages (occurring before 6 weeks) cannot be discussed in the present study.

Conclusions

This study suggests that, in rural Bangladesh, smaller CRL is associated with the occurrence of subsequent miscarriage. The CRL is usually measured at the first consultation in health facilities, especially in developing countries. Ultrasound biometry information together with careful clinical assessment should provide much needed attention and care for pregnant women. Further studies are required to identify related risks of first-trimester growth restriction and miscarriage in developing countries.

2.6 References

1.Trinder J, Brocklehurst P, Porter R, Read M, Vyas S, Smith L. Management of miscarriage: expectant, medical, or surgical? Results of randomised controlled trial (miscarriage treatment (MIST) trial). BMJ 2006;332(7552):1235-40.

2. Lok IH, Neugebauer R. Psychological morbidity following miscarriage. Best Pract Res Clin Obstet Gynaecol 2007;21(2):229-47.

3. Groden J, Gocha AS, Croce CM. Human Basic Genetics and Patterns of Inheritance. In: Creasy RK, Resnik R, Iams JD, Lockwood CJ, Moore TR, Greene MF, eds. Creasy and Resnik's Maternal-Fetal Medicine. China: Elsevier Saunders; 2014:3-36.

4. Garcia-Enguidanos A, Calle ME, Valero J, Luna S, Dominguez-Rojas V. Risk factors in miscarriage: a review. Eur J Obstet Gynecol Reprod Biol 2002;102(2):111-9.

5. Bottomley C, Bourne T. Diagnosing miscarriage. Best Pract Res Clin Obstet Gynaecol 2009;23(4):463-77.

6. Makrydimas G, Sebire NJ, Lolis D, Vlassis N, Nicolaides KH. Fetal loss following ultrasound diagnosis of a live fetus at 6-10 weeks of gestation. Ultrasound Obstet Gynecol 2003;22(4):368-72.

7. Siddiqi TA, Caligaris JT, Miodovnik M, Holroyde JC, Mimouni F. Rate of spontaneous abortion after first trimester sonographic demonstration of fetal cardiac activity. Am J Perinatol 1988;5(1):1-4.

8. Hill LM, Guzick D, Fries J, Hixson J. Fetal loss rate after ultrasonically documented cardiac activity between 6 and 14 weeks, menstrual age. J Clin Ultrasound 1991;19(4):221-3.

9. Papaioannou GI, Syngelaki A, Maiz N, Ross JA, Nicolaides KH. Ultrasonographic prediction of early miscarriage. Hum Reprod 2011;26(7):1685-92.

10. Choong S, Rombauts L, Ugoni A, Meagher S. Ultrasound prediction of risk of spontaneous miscarriage in live embryos from assisted conceptions. Ultrasound Obstet Gynecol 2003;22(6):571-7.

11. Mukri F, Bourne T, Bottomley C, Schoeb C, Kirk E, Papageorghiou AT. Evidence of early first-trimester growth restriction in pregnancies that subsequently end in miscarriage. BJOG 2008;115(10):1273-8.

12. Abuelghar WM, Fathi HM, Ellaithy MI, Anwar MA. Can a smaller than expected crown-rump length reliably predict the occurrence of subsequent miscarriage in a viable first trimester pregnancy? J Obstet Gynaecol Res 2013;39(10):1449-55.

13. Reljic M. The significance of crown-rump length measurement for predicting adverse pregnancy outcome of threatened abortion. Ultrasound Obstet Gynecol 2001;17(6):510-2.

14. Maconochie N, Doyle P, Prior S, Simmons R. Risk factors for first trimester miscarriage--results from a UK-population-based case-control study. BJOG 2007;114(2):170-86.

15. Blohm F, Friden B, Milsom I. A prospective longitudinal population-based study of clinical miscarriage in an urban Swedish population. BJOG 2008;115(2):176-82.

16. Neufeld LM, Wagatsuma Y, Hussain R, Begum M, Frongillo EA. Measurement error for ultrasound fetal biometry performed by paramedics in rural Bangladesh. Ultrasound Obstet Gynecol 2009;34(4):387-94.

17. Cunningh FG, Lenevo KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL et al. Williams Obstetrics. Stamford, Connecticut, USA: The McGraw-Hill Companies; 2014.

18. Rahman A, Persson LA, Nermell B, El Arifeen S, Ekstrom EC, Smith AH et al. Arsenic exposure and risk of spontaneous abortion, stillbirth, and infant mortality. Epidemiology 2010;21(6):797-804.

19. Robinson HP, Fleming JE. A critical evaluation of sonar crown rump length measurement. Br J Obstet Gynaecol 1975;82(9):702-10.

20. Smith GC. First trimester origins of fetal growth impairment. Semin Perinatol 2004;28(1):41-50.

21. Veleva Z, Tiitinen A, Vilska S, Hyden-Granskog C, Tomas C, Martikainen H et al. High and low BMI increase the risk of miscarriage after IVF/ICSI and FET. Hum Reprod 2008;23(4):878-84.

Table 2.1: General characteristics of study subjects (n=3058)

Variables	n (%)
Maternal age [Mean ± SD]	[25.8 ± 5.8][a]
Age group, y	
14-19	470 (15.4)
20-24	895 (29.3)
25-29	872 (28.5)
30-34	560 (18.3)
≥35	261 (8.5)
Parity	
0	1030 (33.7)
≥1	2028 (66.3)
Educational status	
Illiterate	983 (32.1)
Literate	2075 (67.9)
BMI, kg/m^2 [mean ± SD]	[20.2 ± 2.6][a]
BMI, kg/m^2	
<18.5	838 (27.4)
18.5-24.9	2036 (66.6)
≥25.0	184 (6.0)
Socioeconomic quintile	
1st (poorest)	593 (19.4)
2nd	608 (19.9)
3rd	605 (19.8)
4th	618 (20.2)
5th	634 (20.7)

Abbreviation: BMI; body mass index
[a] Values are given as [mean ± SD]

Table 2.2: Comparisons between miscarriage and continued pregnancy groups (n=3058)

Variables	Pregnancies with miscarriage n=92 (%)	Continued Pregnancies n=2966 (%)	*P* value
CRL z score [mean ± SD]	[-1.43 ± 2.7][a]	[-0.80 ±2.7][a]	0.030[b]
CRL z-score categories			
1st (-1 or more)	31 (33.7)	1534 (51.7)	0.004[c]
2nd (-2 to less than -1)	19 (20.7)	484 (16.3)	
3rd (-3 to less than -2)	20 (21.7)	375 (12.6)	
4th (less than -3)	22 (23.9)	573 (19.3)	
Maternal age [mean ± SD]	[27.4 ± 6.6][a]	[25.7±5.8][a]	0.008[b]
Maternal age, y			
14-19	12 (13.0)	458 (15.4)	0.032[c]
20-24	21 (22.8)	874 (29.4)	
25-29	25 (27.2)	847 (28.7)	
30-34	18 (19.6)	542 (18.3)	
≥35	16 (17.4)	245 (8.2)	
Parity			
0	31 (33.7)	999 (33.7)	0.539[c]
≥1	61 (66.3)	1967(66.3)	
BMI, kg/m^2			
<18.5	18 (19.5)	820 (27.7)	0.021[c]
18.5-24.9	63 (68.5)	1973 (66.6)	
≥25.0	11 (12.0)	173 (5.7)	
Socioeconomic quintile			
1st (poorest)	26 (28.2)	567 (19.1)	0.263[c]
2nd	16 (17.4)	592 (20.1)	
3rd	15 (16.3)	590 (19.8)	
4th	19 (20.7)	599 (20.2)	
5th	16 (17.4)	618 (20.8)	

Abbreviation: BMI; body mass index, CRL; crown rump length
[a] Value are given as [Mean ± SD]
[b] *P* value by t-test
[c] *P* value by chi-square test

Table 2.3 Multivariate analysis in prediction of miscarriages (n=3058)

Variables	aRR [a] (95% CI)	*P* value
CRL z-score category		
1st (-1 or more)	ref	
2nd (-2 to less than -1)	1.02 (1.00 – 1.04)	0.009
3rd (-3 to less than -2)	1.04 (1.01 – 1.06)	<0.001
4th (less than -3)	1.03 (1.01 – 1.05)	<0.001
Maternal age, y		
14-19	0.98 (0.96 – 1.01)	0.316
20-24	0.99 (0.97 – 1.00)	0.385
25-29	ref	
30-34	1.00 (0.98 -- 1.02)	0.501
≥ 35	1.03 (1.00 -1.06)	0.021
Parity		
0	1.01 (0.99 – 1.03)	0.074
≥1	ref	
BMI (kg/m^2)		
<18.5	0.99 (0.97 – 1.00)	0.161
18.5-24.9	ref	
≥25.0	1.02 (0.99 – 1.06)	0.130
Socioeconomic quintile		
1st (poorest)	1.02 (1.00 – 1.04)	0.030
2nd	1.00 (0.98 – 1.02)	0.573
3rd	1.00 (0.98 – 1.01)	0.856
4th	1.00 (0.98 – 1.02)	0.429
5th	ref	

Abbreviation: aRR; adjusted relative risk; CI; confidence interval; ref; reference category

[a] Adjusted by maternal age, parity, gestational age at CRL measurement, BMI and socioeconomic quintile

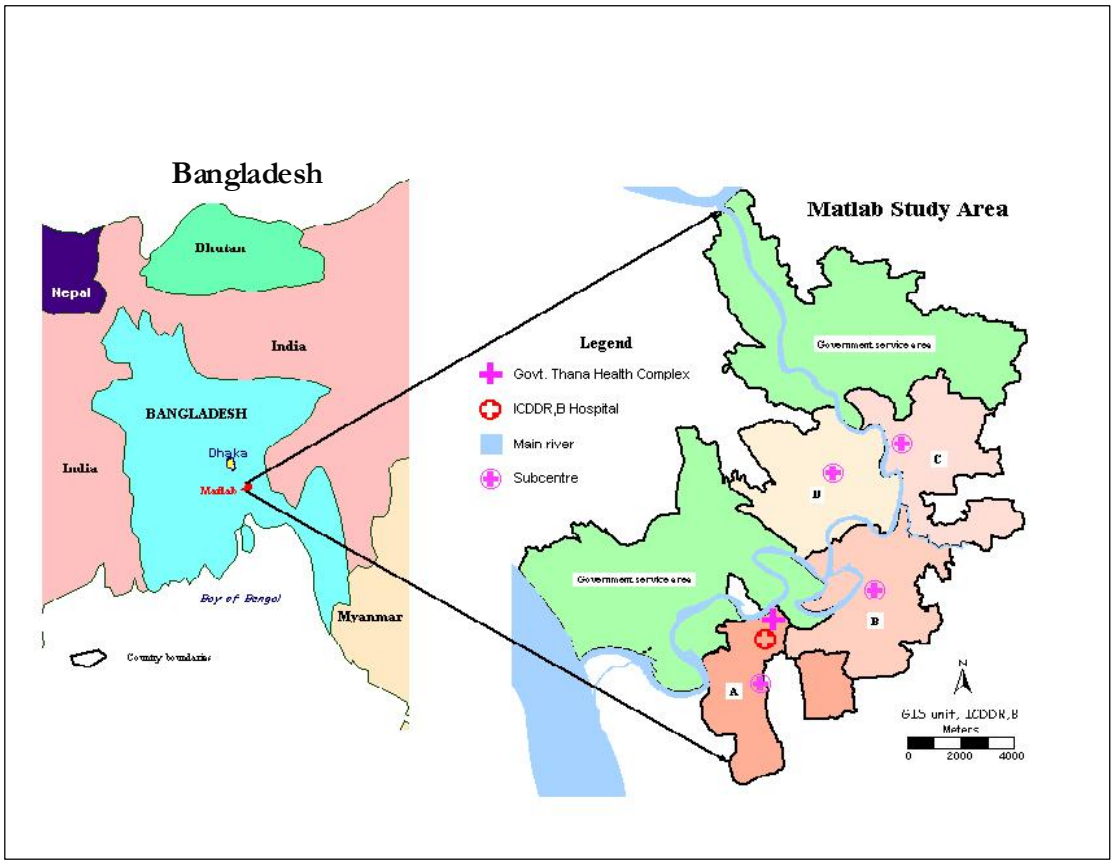


Figure 2.1 Study area

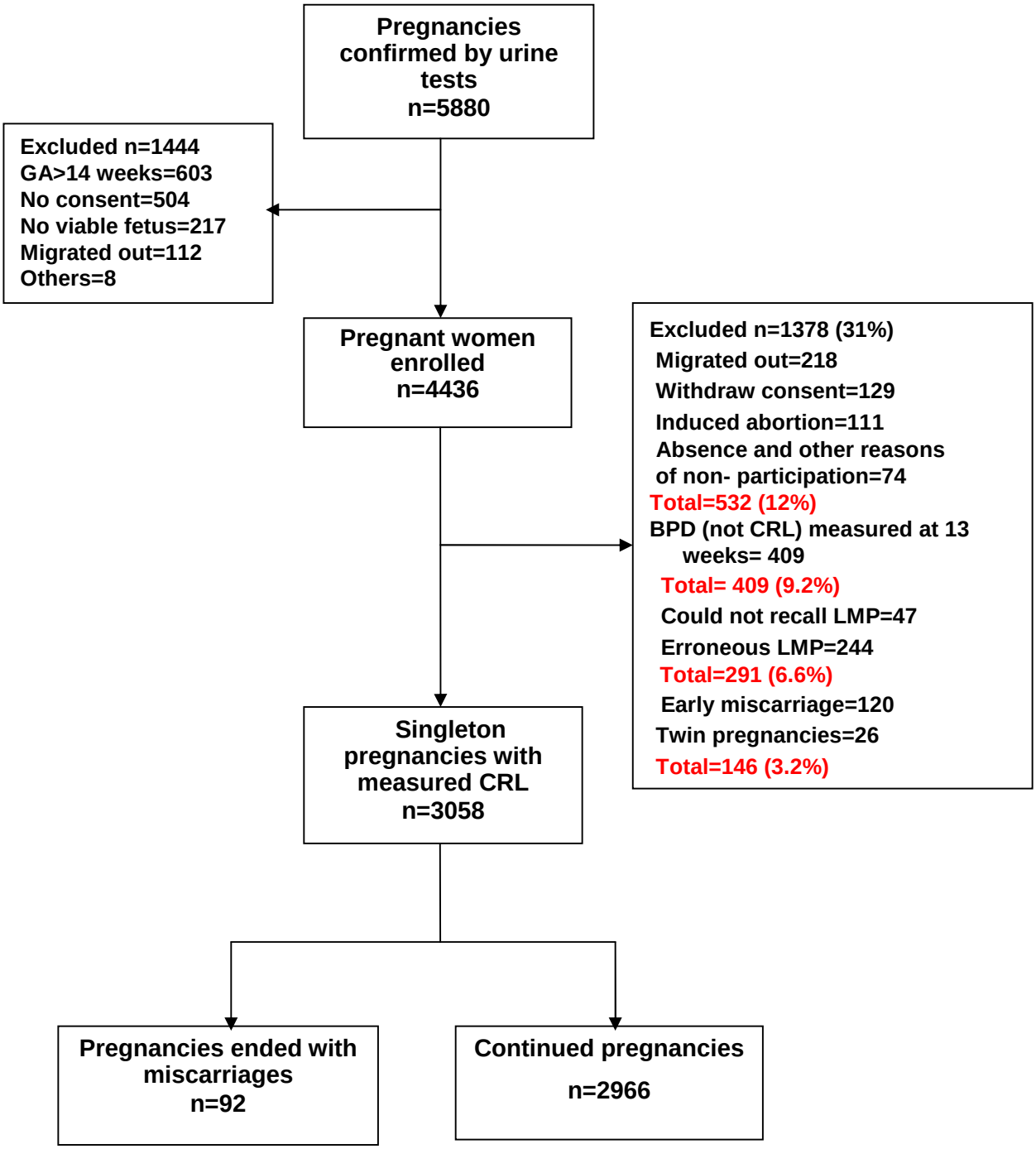


Figure 2.2: Flow charts of study participants

Chapter 3

Fetal growth charts and fetal growth restriction in rural Bangladesh.

3.1 Abstract

Background: Fetal growth restriction and low birth weight (LBW) are serious public health problem. In developing countries the incidence of LBW is predominantly the results of intrauterine growth restriction (IUGR), and both are associated with neonatal death and later life growth and development. Fetal growth charts are important for assessing the size during pregnancy.

Objective: The aim of this study was to describe the fetal growth pattern of Bangladeshi population, and documented the timing of growth restriction by comparing with international reference values.

Methods: The study was conducted within the Maternal and Infant Nutrition Interventions Trial in Matlab (MINIMat study), Bangladesh. Linear-cubic model was fitted with the data. The values derived from the model were compared with international reference values.

Results: A total of 2678 singleton pregnant women were included in analyses. The growth for all parameters except femur length was significantly smaller throughout the pregnancy compared with the reference values ($P < 0.001$). The growth was deviated for BPD from 13 to 37 weeks, OFD from 17 weeks to onwards, HC from 18 weeks to the end of the pregnancy, AC from 14 weeks to onwards and FL from 34 weeks to end of the pregnancy. The growth of BPD, OFD and AC were significantly lower during the third trimester of pregnancy compared with the 50^{th} percentiles of reference values ($P < 0.05$).

Conclusion: The present population-based study showed that the fetal growth was smaller at the last trimester of pregnancy compared with the reference values. The

growth faltering was started at different gestational points for different biometric parameters. These findings are useful for the assessment of fetal size and growth during pregnancy.

3.2 Introduction:

Low birth weight (LBW) of newborns is a challenging problem in developing countries. LBW is considered when babies born with weight less than 2.5 kg (1) which is a cause of infant mortality and impaired psychological development. Nearly, 20 million infants annually have born worldwide with LBW (15.5% of all births). 95.6% of them are from developing countries. The level of LBW in developing countries (16.5%) is more than double the level in developed regions (7%). Half of low birth weight babies are born in South Asia, where more than a quarter (27%) of infants weighs less than 2,500 g at birth (2). The incidence of LBW in Bangladesh was 21.6% and preterm birth was 14% (3). The incidence of LBW in Bangladesh, predominantly the results of intrauterine growth restriction (IUGR) is among the highest in the world (4). During pregnancy, maternal under-nutrition results in IUGR and newborns with LBW. IUGR is associated with the increased risk of perinatal morbidity and mortality (5).

A study in Bangladesh reported that the risk of neonatal death was several-folds higher in preterm infants than in full-term infants whose growth had been restricted during pregnancy (6). A study in Sweden showed that small fetus was 10 times more risk for fetal death compared with normal fetuses. Another study showed that fetuses with growth restriction were more likely to increase the risk of still birth. Fetuses with

IUGR who survive the compromised intrauterine environment are at increased risk for neonatal morbidity (7). Early detection of fetal growth restriction may help to reduce associated morbidity and mortality. It was reported that 80% risk factors can be prevented by developing the appropriate fetal growth reference charts and timely detection of growth restriction (7).

Ultrasonography is a useful tool to provide good prediction of IUGR and majority of such fetuses can be identified during pregnancy. Measurements of the fetal biparietal diameter (BPD) and head circumference (HC) are used in assessing fetal growth (8) and dating pregnancies (9, 10). Many investigators have constructed charts of fetal head dimensions (11, 12) and their measurements are widely used in obstetric ultrasound examinations. The measurement of the fetal abdominal circumference (AC) was first described in 1975 and is widely used as a single parameter to estimate fetal size and weight (13). Measurement of the femur can be used for the determination of gestational age and fetal size as well as fetal abnormalities (11). The measurements of fetal growth in different gestation age are important for tracking the fetal size during pregnancy.

Numerous studies have been conducted to derive reference charts for fetal size. Most of them have a suboptimal design, using hospital patients or having an inappropriately small sample size (14).

Fetal growth charts are used to identify any deviation from normal by plotting the measurement on charts. Such practices have proven to be effective to prevent adverse outcomes (15). A limited number of studies were conducted in tertiary level hospitals with a small number of subjects in Bangladesh, to develop the fetal growth reference

charts but these studies neither compared with the international reference values nor did not mention the timing of growth restriction.

Although the fetal growth charts are important for assessing the fetal growth but there have not been established in these charts in most of the developing countries. As a result the obstetrician and sonographers followed the fetal growth charts that have been generated by the studies on western population where the socio-economic status and nutritional status are different. It is important to understand the magnitude of various types of IUGR among fetuses in rural Bangladesh where the prevalence of malnutrition and LBW are high. Therefore, the aim of this study was to describe the fetal growth parameters in Bangladeshi population and to examine the deviation from the international growth chart.

3.3 Materials and methods

Study area and population:

The study was carried out in the sub-district of Matlab, Bangladesh, located 53 km south of the capital, Dhaka. The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), has been running a health and demographic surveillance system (HDSS) in the area since 1966 that covers a population of about 225,000 in 142 village of Matlab, where icddr,b provides health care to women of reproductive age and children less than 5 years of age. This area is divided into 4 administrative blocks, each with a population of about 25,000-27,000. Each block has a sub-center clinic, where paramedical staff provides maternal and child care, including

delivery services 24 hours a day. The clinics are supported by a hospital located at the Matlab center. Community health research workers (CHRWs) visit every household on a monthly basis to update information on demographic events, such as marriage, pregnancy, birth, death, and in– and out–migration, as well as to collect information on the morbidity of children below 5 years of age and women of childbearing age. Socioeconomic information, including education and household assets is also recorded by periodic censuses.

Study subjects and procedure

The study was conducted where a maternal food and micronutrient supplementation study (MINIMat study) was on going in Matlab, a rural area of Bangladesh, as well as where the health and demographic surveillance system is maintained. As a part of this study, all women who were identified as pregnant with two urine pregnancy tests from November, 2001 to October, 2003 were examined by ultrasound (trial registration: isrctn.org identifier: ISRCTN16581394).

Pregnancy urine test was offered to every woman who reported to the Community Health Research Workers (CHRWs) that her last menstrual period (LMP) was at least 2 weeks overdue, or that she was pregnant. The LMP date was determined by recall during the pregnancy identification interview at routine monthly household visits. A woman with positive pregnancy test was invited to join the study and the date of her LMP was recorded. She was invited to visit a nearby icddr,b clinic for evaluation of viable fetus and measurement of gestational age (GA) by an ultrasound examination. The inclusion criteria for a pregnant woman were viable fetus, GA less than 14 weeks, and consented to participate in the study.

A total of 5880 women were identified as eligible for the study. Of these women, 1444 were excluded because of migration out of the study area, refusal to participate, having a fetus whose GA exceeded the limits for the study or no longer having a viable fetus by ultrasound, and other reasons (Fig 3.1). A total of 4436 women were enrolled to follow up with ultrasound examination during clinic visits at 14, 19, and 30 weeks of pregnancy. Of the 4436 women, 756 were excluded from the study for reasons of migration, induced abortion, withdrawal of consent, spontaneous miscarriage, absence, and other reasons.

A further 377 women dropped out due to no measurement of birth weight (288) because of migration out of the study area, inability of the study personnel to locate them or weigh their newborn within 30 days of birth, refusal to participate, or fetal death and 89 were still birth. These 3303 women had live newborns. Of which 36 who were members of twin pairs at birth were excluded from the analysis of fetal growth, leaving 3,267 newborns who contributed data.

A further 269 women had to be excluded due to missing (could not recall) LMP dates (n=50) or erroneous LMP information (n=219). We defined the recalled LMP as erroneous if the first trimester ultrasound-estimated LMP and recalled LMP had a difference more than 21 days. Additionally 320 were excluded not to complete three scheduled visits. Therefore the finally valid 2678 singleton women who had valid LMP and successfully completed the three scheduled visits were included in the analysis for fetal growth (Fig.3.1).

Fetal biometry

All enrolled women were examined by ultrasound during the sub-centre visits. The first ultrasound was conducted at enrolment at 8-13 weeks of gestational age to measure the crown-rump length (CRL) or biparietal diameter (BPD) for larger fetuses to provide ultrasound gestational age estimate. All women were invited for the ultrasound examinations around 14, 19 and 30 weeks of gestation. Each woman was taken three measurements and examination took approximately 10 minutes.

Four ultrasound machines (SSA 320A, Justavision-200, Toshiba, Tokyo, Japan) with 3.5 MHz standard convex probes were used for fetal biometry measurements. One ultrasound machine was placed in each sub-centre clinic. The following five parameters were measured at the subsequent examinations around 14, 19 and 30 weeks of gestation age: biparietal diameter (BPD), occipital frontal diameter (OFD), head circumference (HC), abdominal circumference (AC) and femur length (FL), Based on the international reference values on fetal biometry for each parameter, fetuses were compared for the adequacy of growth at their gestational age.

Nine sonographers (paramedics) and one supervisor (medical doctor) were trained by a highly qualified local ultrasound consultant. Before beginning field data collection, the team was standardized to assure acceptable intra- and inter-observer variability for all measurements. On-going quality control was documented by re-examination of 3% of the total cases by a doctor who did not have access to the previous results and without notification to the study ultrasonographers (17).

Women were formally invited to the sub-center one week before they completed the scheduled numbers of week gestation based on the first trimester ultrasound LMP.

The ultrasound-based LMP was used for scheduling of clinic visits since some women could not recall their LMP. The woman was asked whether she is able to attend, and if not, she was asked to attend either the week before or the week after. If women cannot attend during these weeks, they were asked to visit the clinic at least before the next scheduled time. In this manner, while providing maximum opportunity for each woman to assist at her examination, the study provided the repeated longitudinal measurement data with three points per women spreading throughout the gestation weeks.

The information on women's age, parity, education, and household assets were collected from the surveillance system databases and from interviews with the study participants. Parity is the number of live or dead children before the current pregnancy. Women who cannot read or write were defined as no education. Economic status was assessed by generating scores through principal-components analysis based on household assets, housing structure, land occupation, and income. These scores were then indexed into quintiles, where 1 represents the poorest and 5 the richest.

Height and weight of pregnant women were measured at the enrollment of 6-13 week of gestation. Weight was measured by electronic scales (SECA, Hamburg, Germany) with a precision of 100 g, and height was measured with locally made wooden scales with a precision of 0.1 cm. The Body Mass Index (BMI) (kg/m^2) was categorized as under nutrition (<18.5), normal (18.5-<25), and overweight (≥25).

Statistical analysis

The curves were fitted using repeated measurement examinations. The statistical method was used as described in previous studies (19, 20). The mean and standard deviation (SD) of each fetal parameter value was separately fitted with polynomial regression model against its gestational age and generated the regression formula (19, 20). The data points more than 6 SD from the regression line, fitted with raw data were considered to be unrealistic and therefore were removed. The best fitting polynomial curves were chosen by comparing the deviances and visually checking the goodness of fit. The linear-cubic model was fitted with the raw data for mean and linear model fitted for SD. The regression lines were fitted for the dependency of the residual score on gestational age. Subsequently, the standard deviation score (SDs) was fitted against gestational age to assess correctness of the model (19, 20). The normal plot of SD score also used to see the correctness of the curve. The percentile curve has been calculated based on the established formula (20). Raw data fitted with 5^{th} and 95^{th} percentiles against gestation age to observe the fitness of the curve (19, 20). Mixed linear model was preformed to examine the line differences between the fitted line and the reference chart line. The z-score was calculated to observe the deviation of derived value from the expected of reference values at each gestational age. Independent t test was performed to compare between two groups. Statistical analysis was performed using IBM SPSS (version 22.0; New York, USA).

Ethical consideration

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by

the research review committees and ethical review committees of icddr,b and Uppsala University. Written informed consent was obtained from each women or the legal guardian prior to participation in the study.

3.4 Results

General characteristic of study participants

A total 2678 singleton pregnant woman was used in the analyses. The mean of maternal age was 25.7 ± 5.8 (standard deviation SD) years (range 14-47 years). The mean parity was 1.32 ± 1.3(SD). One third of women (32.9%) were nulliparous. Only 68.3% of women had attended schools. The mean maternal height was 149.8 ± 5.8 (SD) cm. The mean of early pregnancy BMI was 20.1 ± 2.6 (SD) kg/m^2 and 27.4% of women were underweight (Table 3.1). The mean and SD of raw data for five fetal biometry parameters were presented in Table 3.2.

Curve fitting procedures

The Liner-cubic polynomial regression models were fitted to the mean and liner model to the SD with the raw data of all biometric parameters (BPD, OFD, AC, HC and FL). The linear-cubic models gave a good fit with the data. The coefficient of multiple correlation (R^2) were 0.96 for BPD, 0.96 for OFD, 0.96 for HC, 0.96 for AC and 0.96 for FL (P <0.001 for all indicated the best correlation between biometric parameters and

gestational age). The fitted standardized residual of SD score with regression line against the gestational age shows more than 90% of observations lie within the fitted line for all parameters. The normal plots of standard deviation score of each parameter appear fairly in linear pattern. The data with fitted percentiles of 5^{th}, 50^{th} and 95^{th} for each parameter appear that more than 90% value lie within the fitted line (Fig 3.7-3.11). The percentiles of each parameter were calculated based on the established equation. The fetal growth charts were developed based on the fetal growth equation derived of the present study. Table 3.3 showed the fetal growth equations of the mean and SD was derived for each parameter for this population.

Growth charts and compared with international reference values

The number of measurements at different weeks of gestation along with their mean, SD and fitted percentile (5^{th}, 50^{th} and 95^{th}) of five parameters were presented in Table 3.4-3.8. Table 3.9 shows the deviation of the growth of fetal parameters throughout the pregnancy. It was observed that all parameters were significantly smaller compared with international reference values ($P < 0.001$) except femur length which was slightly larger compared with the reference values ($P < 0.001$). Table 3.10-3.14 shows the deviation of our derived value from the expected of international reference values in each gestational age. It was found that BPD was smaller than the expected of reference values from 13 weeks to 37 weeks of the pregnancy, AC from 14 weeks to the end of the pregnancy, OFD from 17 weeks to onwards, HC was smaller than the expected of

the reference value from 18 weeks and FL from 35 weeks to onwards respectively. It was found that the deviation of our value was increased with increased gestational age.

Figures 3.2-3.6, showed the comparison of each parameter derived of this study with international reference values. The figure 3.2 showed that BPD was consistently smaller than the reference curve throughout the observed period and significantly smaller than 50^{th} percentiles of reference value at 22-23 and 27-37 weeks of gestation ($P < 0.05$).

Figure 3.3, showed that OFD was close and around the reference curve up to 16 weeks then started faltering throughout the observed period and significantly smaller compared to reference value at 27 to 34 weeks of gestation ($P <0.05$),

Figure 3.4 showed that Head circumference was similar to the reference curve up to 17 weeks then becomes significantly smaller until 37 weeks compared to the 50^{th} percentile of reference value (not statistically significant).

Figure 3.5 showed that AC consistently smaller than the reference curve throughout the pregnancy and significantly smaller than the 50^{th} percentile of reference value at 27-37 weeks of gestation ($P <0.05$).

Femur length (Figure 3.6) was around the reference curve up to 34 weeks then slightly smaller compared to the reference (not statistically significant).

3.5 Discussion

The standards of fetal ultrasound measurements built with high interest in the late 1970s and 1980s. Since then many reference charts and tables have been

published (8, 13, 17,18). In the last 12 years the quality of the ultrasound imaging has improved remarkably regarding its resolutions, velocities and measurement techniques. Improvement of methodologies, statistical methods of analysis, consideration of changing variability of measurement with gestation and presentation of scatter diagrams of the data with fitted percentile contributed in the development of good quality of fetal growth charts.

The present study used the methods of previous studies (19, 20) to overcome the methodological weakness for fitting the curves. Fetus who received three longitudinal measurements of all variables was included for construction of fetal growth charts. All steps of statistical methods used which gave proper attention to the changing variability with increasing gestation and carefully assessed the goodness of fit of the models obtained (19).

Different polynomial regression models were checked to develop the fetal growth equation and compared with the liner-cubic model to get the best model fitted with the data. Finally linear-cubic model was used as the best model in the present study as the plot of standard deviation score (SDS) against gestation age fitted with the regression line, normal plot of SDS fairly appear to the normal distribution and separately check of the raw data of all parameters with fitted percentile showed that more than 90% observation fell within the fitted line (19).

The present study developed the fetal growth charts for BPD, OFD, HC, AC and FL from 13 to 37 weeks of gestation. The present study has chosen the international fetal growth reference values (21-23) to compare with our derived value. This reference was chosen because it provides model-derived fetal biometry values for each gestation

week with standard deviation for all the fetal size parameters, which is usually not the case for other international references published for fetal size.

The fetal growth pattern with fitted percentiles showed that the growth of each biometric parameter increased with increasing the gestational age. It was found that the growth was smaller in the third trimester compared with the second trimester of pregnancy. Average growth of BPD per week of 3-4 mm up to 29 weeks turned to slow down to 2 mm after 30 weeks of gestation.. The similar growth pattern was found for OFD and AC: the growth per week of 10-12 mm up to 28 weeks declined 7-9 mm towards 37 weeks; HC: 10-13 mm up to 27 weeks declined 9-4 mm towards 37 weeks of gestation. The average growth of FL per weeks was 3-4 mm up to 25 weeks, then 1-2 mm up to 37 weeks. Throughout the pregnancy significantly smaller growth was observed except femur length compared with the reference values. After comparing with the international reference values, it was found that the growth faltering was started at different gestational weeks for different fetal parameters. It was also observed that the growth faltering was gradually increased with increasing gestational age.

Several factors may be responsible for the observed difference in this present study from the reference population. The differences are likely to be raised by different population characteristics (24). Maternal malnutrition might be another explanation for this growth faltering of the present study. Maternal stature was markedly different, as Bangladeshi women were lighter and shorter compared to western women. Both these two factors might affect to the fetal growth during pregnancy (25). Placental-fetal development study showed that placenta exchanged gas, nutrient and waste through maternal-fetal circulations. Trans-placental exchange depends on the uterine, placenta

and umbilical blood flows. Maternal nutrition factors associated with the placental homeostasis and influence the fetal growth. Inadequate nutrition during pregnancy hampers this normal process and causes fetal growth restriction (26).

Animal studies also show that both maternal under- and over-nutrition reduce placental-fetal blood flows and cause stunted fetuses. Impaired placental syntheses of nitric oxide and polyamines may provide unified explanation for intrauterine growth restriction in response of two extremes of nutritional problems in the same pregnancy outcome (27).

The present study had several strengths. Firstly, this study included larger observations than most other studies that developed fetal growth charts. Furthermore, this study is a population-based study with longitudinal fetal growth measurement with pregnancies with singleton live births. Fetuses were followed from early fetal life and confirmed by three scheduled visits that spread widely from 13 to 37 weeks of gestation, thus enable us to create fetal growth charts. The present study compared with internationally published recommended reference charts with derived means and SDs.

There are some limitations to this study. In this study, the measurement of fetuses was not equally distributed at all gestational age as the scheduled visits. The study was conducted only one location in Bangladesh.

Conclusions:

. The present study developed the fetal growth charts for Bangladeshi population. The growth was smaller for all parameters at the third trimesters compared to the

international reference values. The growth restriction starts at different gestational age for different parameters. The growth faltering occurs as early as during the first trimester. These findings suggest the importance of improved nutritional status for reproductive age women in developing countries.

3.6 Reference

1. Shirin F, Mehdi T, Alam MM, Nath RK, Hoque MM**.** Effect of gestational homocystein on fetal growth in Bangladeshi women. Ibrahim Med Coll J 2009; (1): 13-16.

2. World Health Organization. Low birth weight, country, regional and global estimate, 2004. *http://apps.who.int/iris/bitstream/10665/43184/1/9280638327.pdf.* Accessed 10th, August 2016

3. World Health Organization. Health at a glance, measuring progress towards universal health coverage. Asia/pacific report, 2014. *http://www.oecd.org/health/health-at-a-glance-asia-pacific-23054964.htm.* Accessed 23rd July, 2016.

4. Arifeen S, Black RE, Caulfield LE, Antelman G, Baqui AH, Nahar Q, Alamgir A, and Mahmud H. Infant growth patterns in the slums of Dhaka in relation to birth weight, intrauterine growth retardation, and prematurity. Am J Clin Nutr 2000; 72:1010-7

5. Belkacemi L, Nelson DM, Desai M, Ross MG. Maternal undernutrition influences placental-fetal development. Biol Reprod 2010; 83(3):325-31.

6. Lawn JE, Cousens S, Zupan J; Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: when? Where? Why? Lancet. 2005; 5-11;365(9462):891-900.

7. Fetal growth restriction, 2015. *http://emedicine.medscape.com/article/261226-overview.* Accessed, July, 29, 2016.

8. Campbell S & Thomas A. Ultrasound measurement of fetal head to abdominal circumference ratio in the assessment of fetal growth retardation. Br J Obstet Gynaecol 1977; 84: 165-74.

9. Campbell S. The prediction of fetal maturity by ultrasonic measurement of biparietal diameter. J Obstet Gynaecol Br Commwlth 1969; 76: 603-9.

10. Hadlock FP,Deter RL, Harrist RB, Park SK. Fetal biparietal diameter: A critical reevaluation to menstrual age by real time ultrasound. J Ultrasound Med 1982a; I: 97104.76.

11. Deter RL, Harrist RB, Birrholz JC, Hadlock FP. Quantitative Obstetrical Ultrasonography. Wiley l986; New York.

12. Kurtz AB & Goldberg BB. Obstetrical Measurements in Ultrasound. A Reference Manual. YearBook, Medical Publishers Inc. Chicago, Illinois 1988; pp. 64-74.

13. Campbell S & Wilkin D. Ultrasonic measurement of fetal abdomen circumference in the estimation of fetal weight Br.J Obstet Gynaecol 1975; 82: 689-97.

14. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol 2008;31(4):388-96.

15. Tinelli A, Bochicchio MA, Vaira L, Malvasi A. Ultrasonographic fetal growth charts: an informatic approach by quantitative analysis of the impact of ethnicity on diagnoses based on a preliminary report on Salentinian population. Biomed Res Int. 2014; 2014:386124.

16. Neufeld LM, Wagatsuma Y, Hussain R, Begum M, Frongillo EA. Measurement error for ultrasound fetal biometry performed by paramedics in rural Bangladesh. Ultrasound Obstet Gynecol 2009;34(4):387-94.

17. Queenan JT, O'Brien GD, Campbell S. Assessment of gestational age in the second trimester by real-time ultrasound measurement of the femur len. gth. Am J Obstet Gynecol 1981; 138: 297-302.

18. Hadlock FP,Deter RL, Harrist RB, Park SK. Fetal head circumference, relation to menstrual age. AJR 1982b; 138: 649-53.

19. Altman DG, Chitty LS**.** Charts of fetal size: 1. Methodology. Br J Obstet Gynaecol 1994; 101(1):29-34.

20. Royston P, Wright EM. How to construct 'normal ranges' for fetal variables. Ultrasound Obstet Gynecol 1998; 11(1):30-8.

21. Chitty LS, Altman DG, Henderson A, Campbell S. Charts of fetal size: 2. Head measurements. Br J Obstet Gynaecol 1994; 101(1):35-43.

22. Chitty LS, ,Altman DG, Henderson A, Campbell S. Charts of fetal size: 3. Abdominal measurements. Br J Obstet Gynaecol 1994;101(2):125-31.

23. Chitty LS, Altman DG, Campbell S. Charts of fetal size: 4. Femur length. Br J Obstet Gynaecol 1994;101(2):132-5.

24. Johnsen SL, Wilsgaard T, Rasmussen S, Sollien R, Kiserud T. Longitudinal reference charts for growth of the fetal head, abdomen and femur. Eur J Obstet Gynecol Reprod Biol 2006; 127(2):172-85.

25. Spencer JA, Chang TC, Robson SC, Gallivan S. Fetal size and growth in Bangladeshi pregnancies. Ultrasound Obstet Gynecol 1995; 5(5):313-7.

26. Belkacemi L, Nelson DM, Desai M, Ross MG. Maternal undernutrition influences placental-fetal development. Biol Reprod 2010; 83(3):325-31.

27. Wu G, Bazer FW, Cudd TA, Meininger CJ, SpencerTE. Maternal nutrition and fetal development J Nutr 2004; 134(9):2169-72.

Table 3.1: Characteristics of study subjects (n=2678)*

Variables	n (%)
Maternal age [Mean ± SD]	[25.9 ± 5.8]**
Age group, y	
20-24	785 (29.3)
25-29	763 (28.5)
30-34	490 (18.3)
≥35	228 (8.5)
Parity	
0	880(32.9)
≥1	1798 (67.1)
Height	149.8 ± 5.3]
Educational status	
Illiterate	848 (31.7)
Literate	1830 (68.3)
BMI, kg/m^2 [mean ± SD]	[20.15 ± 2.66]*
BMI, kg/m^2	
<18.5	734 (27.4)
18.5-24.9	1783 (66.6)
≥25.0	161 (6.0)
Socioeconomic quintile	
1st (poorest)	513 (19.2)
2nd	525 (19.5)
3rd	541 (20.2)
4th	545 (20.4)
5th	554 (20.7)
Infant characteristics	
Birth weight (g) [Mean ± SD]	2697.4±407.6*
Birth length (cm) [Mean ± SD]	47.7±2.2*
Gestational age at birth (weeks)	39.3±1.9*

Abbreviation: BMI; body mass index

*Analysis was performed if the difference between the recall-based LMP and ultrasound-based LMP was within 21 days. **Values are given as [mean ± SD]

Table 3.2: Mean and standard deviation of different fetal parameters (raw data)

Weeks of gestation	No of fetus	BPD		OFD		HC		AC		Fl	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
13	731	26.2	2.2	34.4	3.1	99.8	7.2	81.8	6.0	15.0	1.7
14	879	27.2	2.4	36.2	3.3	105.0	8.4	87.3	7.6	16.0	1.9
15	637	29.4	2.7	38.2	3.7	110.6	9.6	92.1	8.7	17.0	2.2
16	318	30.6	4.1	41.5	6.9	114.8	14.0	95.2	12.3	18.0	3.2
17	208	38.5	6.2	51.8	8.0	145.2	23.2	121.1	20.6	25.3	6.0
18	522	43.6	2.9	57.6	3.8	164.6	10.3	139.8	10.7	31.0	2.7
19	792	44.9	2.4	59.4	3.2	169.9	8.8	144.2	9.4	32.3	2.3
20	644	46.5	2.8	61.5	3.6	176.0	10.0	148.5	9.7	33.4	2.4
21	352	48.3	3.6	63.8	4.5	182.3	12.5	153.8	11.9	34.9	2.9
22	170	50.4	4.0	67.0	4.9	191.0	14.0	162.4	14.8	37.0	3.6
23	66	53.8	4.4	70.9	4.7	202.9	13.9	171.0	14.8	39.8	3.5
24	25	58.1	6.9	75.5	8.1	215.1	22.8	183.0	23.0	42.2	4.6
25	7	57.9	3.7	78.0	4.4	220.5	10.5	192.4	15.8	43.7	2.4
26	4	64.5	8.7	88.6	12.4	240.0	32.1	210.5	26.4	47.3	4.8
27	15	72.2	3.9	98.2	5.0	272.8	20.1	243.1	20.9	56.8	4.8
28	117	74.7	3.0	99.9	3.8	282.9	11.3	250.9	15.8	58.4	2.6
29	542	75.0	3.3	100.0	3.7	283.3	10.7	250.3	13.7	58.5	2.7
30	836	76.1	3.3	100.8	3.9	286.5	10.7	253.3	14.0	59.1	2.6
31	615	76.9	3.6	102.0	3.9	289.9	11.2	257.0	15.3	60.0	2.5
32	311	77.7	4.0	102.7	4.1	291.6	11.8	260.2	17.3	60.6	3.0
33	136	80.0	4.1	105.0	4.3	299.4	12.6	271.8	19.3	62.5	3.4
34	50	81.5	3.9	106.7	4.9	303.7	12.8	279.6	22.4	64.0	3.4
35	34	84.0	4.0	108.5	4.7	310.5	12.7	286.0	19.5	65.5	3.8
36	11	82.3	4.8	110.0	6.0	310.8	18.5	283.0	22.3	65.7	2.7
37	12	84.0	4.4	109.7	5.8	313.7	13.6	292.7	18.6	66.4	3.1
Total	8034	8015		8010		8003		7975		8014	

Table 3.3: Regression formula used to generate ultrasound biometry charts and tables of biparietal diameter (BPD), occipito-frontal diameter (OFD), head circumference (HC), abdominal circumference (AC) and femur lngth (Fl). GA= gestational age

Biparietal diameter (BPD)

Mean= $-25.703+3.743*GA-0.0004973*GA^3$

SD= 2.795+0.047*GA

Occipito-frontal diameter (OFD)

Mean= $-35.395+5.119*GA-0.0007372*GA^3$

SD= 4.469+0.021*GA

Head circumference (HC)

Mean= $-94.926+14.288*GA - 0.002022*GA^3$

SD= 10.803 + 0.117*GA

Abdominal circumference (AC)

Mean= $-79.822+11.775*GA-0.0009931*GA^3$

SD= 3.565 + 0.483*GA

Femur length (FL)

Mean= $-32.789+3.506*GA+ -0.0005463*GA^3$

SD= 2.633+0.021*GA

Table 3.4: Fitted percentiles (5^{th}, 50^{th} and 95^{th}) with SD of biparietal diameter (BPD, mm)

Weeks of gestation	No of fetus	5^{th}	50^{th}	95^{th}	SD
13	725	16.26	21.86	27.47	3.41
14	878	19.65	25.33	31.01	3.45
15	635	23.01	28.76	34.52	3.50
16	318	26.31	32.15	37.98	3.55
17	204	29.57	35.48	41.40	3.59
18	521	32.78	38.77	44.76	3.64
19	791	35.94	42.00	48.07	3.69
20	643	39.03	45.18	51.32	3.74
21	350	42.07	48.29	54.52	3.78
22	170	45.05	51.35	57.65	3.83
23	66	47.96	54.34	60.71	3.88
24	25	50.80	57.25	63.71	3.92
25	7	53.57	60.10	66.63	3.97
26	4	56.27	62.87	69.48	4.02
27	15	58.88	65.57	72.25	4.06
28	117	61.42	68.18	74.95	4.11
29	542	63.88	70.72	77.56	4.16
30	836	66.24	73.16	80.08	4.21
31	615	68.52	75.51	82.51	4.25
32	311	70.71	77.78	84.85	4.30
33	135	72.80	79.94	87.09	4.35
34	50	74.79	82.01	89.24	4.39
35	34	76.68	83.98	91.28	4.44
36	11	78.46	85.84	93.22	4.49
37	12	80.14	87.60	95.06	4.53
Total	8015				

Table 3.5: Fitted percentiles (5^{th}, 50^{th} and 95^{th}) with SD of occipito-frontal diameter (OFD, mm)

Weeks of gestation	No of fetus	5^{th}	50^{th}	95^{th}	SD
13	729	21.73	29.53	37.33	4.74
14	879	26.41	34.25	42.08	4.76
15	635	31.03	38.90	46.77	4.78
16	314	35.59	43.49	51.39	4.81
17	203	40.07	48.01	55.94	4.83
18	522	44.47	52.45	60.42	4.85
19	790	48.80	56.81	64.82	4.87
20	643	53.04	61.09	69.13	4.89
21	351	57.20	65.28	73.35	4.91
22	169	61.26	69.37	77.48	4.93
23	65	65.23	73.37	81.52	4.95
24	23	69.09	77.27	85.45	4.97
25	7	72.85	81.06	89.28	4.99
26	4	76.49	84.74	92.99	5.02
27	15	80.02	88.31	96.59	5.04
28	117	83.44	91.75	100.07	5.06
29	540	86.72	95.08	103.43	5.08
30	836	89.88	98.27	106.66	5.10
31	615	92.91	101.33	109.75	5.12
32	311	95.80	104.26	112.71	5.14
33	136	98.55	107.04	115.53	5.16
34	50	101.15	109.68	118.20	5.18
35	34	103.60	112.16	120.72	5.20
36	10	105.90	114.49	123.09	5.23
37	12	108.04	116.67	125.30	5.25
Total	8010				

Table 3.6: Fitted percentiles (5^{th}, 50^{th}, 95^{th}) with SD of head circumference (HC, mm)

Weeks of gestation	No of fetus	5^{th}	50^{th}	95^{th}	SD
13	721	66.10	86.38	106.65	12.32
14	877	79.09	99.56	120.02	12.44
15	635	91.91	112.57	133.23	12.56
16	315	104.55	125.40	146.25	12.68
17	203	116.99	138.04	159.08	12.79
18	521	129.23	150.47	171.70	12.91
19	791	141.25	162.68	184.10	13.03
20	644	153.04	174.66	196.28	13.14
21	351	164.58	186.40	208.21	13.26
22	169	175.87	197.88	219.88	13.38
23	66	186.90	209.10	231.29	13.49
24	24	197.64	220.03	242.42	13.61
25	7	208.10	230.68	253.26	13.73
26	4	218.25	241.02	263.80	13.85
27	15	228.08	251.05	274.02	13.96
28	117	237.59	260.75	283.91	14.08
29	541	246.76	270.11	293.46	14.20
30	836	255.58	279.12	302.66	14.31
31	615	264.03	287.76	311.50	14.43
32	310	272.10	296.03	319.96	14.55
33	136	279.79	303.91	328.04	14.66
34	49	287.08	311.39	335.71	14.78
35	33	293.95	318.46	342.97	14.90
36	11	300.40	325.10	349.80	15.02
37	12	306.42	331.31	356.20	15.13
Total	8003				

Table 3.7: Fitted percentiles (5^{th}, 50^{th}, and 95^{th}) with SD Of abdominal circumference (AC, mm)

Weeks of gestation	No of fetus	5^{th}	50^{th}	95^{th}	SD
13	705	53.63	71.07	87.78	9.84
14	874	64.41	82.30	99.98	10.33
15	634	75.09	93.45	112.07	10.81
16	315	85.66	104.51	124.06	11.29
17	204	96.12	115.47	135.93	11.78
18	520	106.45	126.34	147.68	12.26
19	791	116.66	137.09	159.30	12.74
20	641	126.73	147.73	170.78	13.23
21	351	136.66	158.26	182.13	13.71
22	170	146.43	168.65	193.32	14.19
23	66	156.05	178.92	204.35	14.67
24	24	165.51	189.05	215.22	15.16
25	7	174.79	199.04	225.92	15.64
26	4	183.89	208.87	236.43	16.12
27	15	192.81	218.56	246.77	16.61
28	117	201.53	228.08	256.90	17.09
29	541	210.06	237.43	266.84	17.57
30	833	218.37	246.61	276.57	18.06
31	612	226.47	255.62	286.09	18.54
32	309	234.35	264.44	295.38	19.02
33	135	242.00	273.06	304.44	19.50
34	50	249.41	281.50	313.27	19.99
35	34	256.58	289.72	321.85	20.47
36	11	263.50	297.74	330.19	20.95
37	12	270.16	305.55	338.26	21.44
Total	7975				

Table 3.8: Fitted percentiles (5^{th}, 50^{th} and 95^{th}) with SD of Femur length (FL, mm)

Weeks of gestation	No of fetus	5^{th}	50^{th}	95^{th}	SD
13	728	6.81	11.59	16.37	2.91
14	879	9.98	14.80	19.61	2.93
15	634	13.11	17.96	22.81	2.95
16	315	16.19	21.07	25.95	2.97
17	203	19.21	24.13	29.05	2.99
18	520	22.18	27.13	32.09	3.01
19	792	25.09	30.08	35.07	3.03
20	642	27.94	32.96	37.98	3.05
21	352	30.72	35.78	40.83	3.07
22	170	33.43	38.53	43.62	3.10
23	66	36.08	41.20	46.33	3.12
24	23	38.64	43.80	48.96	3.14
25	7	41.13	46.33	51.52	3.16
26	4	43.54	48.77	53.99	3.18
27	15	45.86	51.12	56.38	3.20
28	117	48.09	53.39	58.69	3.22
29	542	50.23	55.56	60.89	3.24
30	836	52.27	57.64	63.01	3.26
31	615	54.22	59.62	65.02	3.28
32	311	56.07	61.50	66.94	3.31
33	136	57.81	63.28	68.75	3.33
34	50	59.44	64.94	70.45	3.35
35	34	60.96	66.50	72.04	3.37
36	11	62.36	67.94	73.51	3.39
37	12	63.65	69.26	74.87	3.41
Total	8014				

Table 3.9: The deviation of the growth compared with the international reference values

Variables	β coefficient	95% CI	P value
BPD			
Gestational age	2.92	2.18-3.67	<0.001
Derived value	-5.80	-6.40, -5.19	<0.001
Reference value	1	1	
OFD			
Gestational age	3.57	2.80-4.33	<0.001
Derived value	-1.14	-1.43, -0.84	<0.001
Reference value			
HC			
Gestational age	10.86	8.49-13.22	<0.001
Derived value	-2.71	-3.86, -1.55	<0.001
Reference value	1	1	
AC			
Gestational age	10.54	8.14-12.94	<0.001
Derived value	-8.44	-10.27, -6.62	<0.001
Reference value	1	1	
FL			
Gestational age	2.75	2.26	<0.001
Derived value	0.69	0.51-0.875	<0.001
Reference value	1	1	

Table 3.10: Biparietal diameter (BPD) by gestational age compared with international reference values

Weeks of gestation	Bangladesh			International reference*			z-score	P value**
	No of fetus	Mean	SD	No of fetus	Mean	SD		
13	725	21.86	3.41	17	22.0	2.1	-.07	0.866
14	878	25.33	3.45	17	25.6	2.2	-.12	0.748
15	635	28.76	3.50	18	29.3	2.3	-.23	0.516
16	318	32.15	3.55	17	32.8	2.3	-.28	0.460
17	204	35.48	3.59	17	36.3	2.4	-.34	0.356
18	521	38.77	3.64	22	39.8	2.5	-.41	0.190
19	791	42.00	3.69	20	43.2	2.5	-.48	0.149
20	643	45.18	3.74	23	46.5	2.6	-.51	0.094
21	350	48.29	3.78	22	49.8	2.7	-.56	0.066
22	170	51.35	3.83	21	53.0	2.7	-.61	0.057
23	66	54.34	3.88	23	56.1	2.8	-.63	0.049
24	25	57.25	3.92	22	59.2	2.9	-.67	0.062
25	7	60.10	3.97	26	62.1	2.9	-.69	0.144
26	4	62.87	4.02	13	65.0	3.0	-.71	0.267
27	15	65.57	4.06	21	67.8	3.1	-.72	0.007
28	117	68.18	4.11	30	70.5	3.2	-.72	0.005
29	542	70.72	4.16	15	73.1	3.2	-.74	0.028
30	836	73.16	4.21	24	75.7	3.3	-.77	0.003
31	615	75.51	4.25	19	78.1	3.4	-.76	0.009
32	311	77.78	4.30	28	80.4	3.4	-.77	0.002
33	135	79.94	4.35	22	82.6	3.5	-.76	0.007
34	50	82.01	4.39	21	84.7	3.6	-.75	0.016
35	34	83.98	4.44	18	86.7	3.6	-.76	0.030
36	11	85.84	4.49	22	88.6	3.7	-.75	0.049
37	12	87.60	4.53	17	90.3	3.8	-.71	0.053
Total	8015			594				

*Reference values (3.21)
**p value= Independent t test

Table 3.11: Occipito-frontal diameter (OFD) by gestational age compared with International reference values

Weeks of gestation	Bangladesh			International reference*			z-score	P value
	No of fetus	Mean	SD	No of fetus	Mean	SD		
13	729	29.53	4.74	17	28.6	3.00	.31	0.823
14	879	34.25	4.76	17	33.6	3.00	.22	0.055
15	635	38.90	4.78	18	38.6	3.00	.10	0.732
16	314	43.49	4.81	17	43.5	3.00	.00	0.813
17	203	48.01	4.83	17	48.3	3.00	-.10	0.681
18	522	52.45	4.85	22	53	3.00	-.18	0.594
19	790	56.81	4.87	20	57.6	3.00	-.26	0.075
20	643	61.09	4.89	23	62.1	3.10	-.33	0.907
21	351	65.28	4.91	22	66.5	3.10	-.39	0.179
22	169	69.37	4.93	21	70.8	3.20	-.45	0.607
23	65	73.37	4.95	23	74.9	3.30	-.46	0.879
24	23	77.27	4.97	22	79	3.40	-.51	0.357
25	7	81.06	4.99	26	82.9	3.50	-.53	0.155
26	4	84.74	5.02	13	86.6	3.70	-.50	0.607
27	15	88.31	5.04	21	90.3	3.80	-.52	0.003
28	117	91.75	5.06	30	93.7	4.00	-.49	0.000
29	540	95.08	5.08	15	97.1	4.10	-.49	0.000
30	836	98.27	5.10	24	100.2	4.30	-.45	0.000
31	615	101.33	5.12	19	103.2	4.50	-.42	0.000
32	311	104.26	5.14	28	106.1	4.70	-.39	0.002
33	136	107.04	5.16	22	108.7	5.00	-.33	0.007
34	50	109.68	5.18	21	111.2	5.20	-.29	0.003
35	34	112.16	5.20	18	113.5	5.50	-.24	0.873
36	10	114.49	5.23	22	115.6	5.80	-.19	0.251
37	12	116.67	5.25	17	117.5	6.10	-.14	0.987
Total	8010			515				

*Reference values (3.21)
**p value= Independent t test

Table 3.12: Head circumference (HC) by gestational age compared with International reference values

Weeks of gestation	Bangladesh			International reference*			z-score	P value**
	No of fetus	Mean	SD	No of fetus	Mean	SD		
13	721	86.18	12.32	17	83.70	6.90	.38	0.409
14	877	99.60	12.44	17	97.70	7.20	.28	0.531
15	635	112.83	12.56	18	111.50	7.40	.19	0.665
16	315	125.86	12.68	17	125.10	7.60	.10	0.807
17	203	138.68	12.79	17	138.50	7.90	.02	0.955
18	521	151.28	12.91	22	151.60	8.10	-.04	0.696
19	791	163.63	13.03	20	164.40	8.30	-.10	0.761
20	644	175.73	13.14	23	177.00	8.60	-.15	0.646
21	351	187.57	13.26	22	189.30	8.80	-.20	0.547
22	169	199.13	13.38	21	201.30	9.00	-.25	0.471
23	66	210.39	13.49	23	213.00	9.30	-.29	0.393
24	24	221.35	13.61	22	224.30	9.50	-.32	0.403
25	7	231.99	13.73	26	235.30	9.70	-.35	0.469
26	4	242.30	13.85	13	246.00	10.00	-.38	0.561
27	15	252.26	13.96	21	256.30	10.20	-.41	0.322
28	117	261.87	14.08	30	266.20	10.40	-.43	0.117
29	541	271.10	14.20	15	275.70	10.70	-.45	0.214
30	836	279.95	14.31	24	284.80	10.90	-.46	0.100
31	615	288.40	14.43	19	293.40	11.10	-.46	0.135
32	310	296.43	14.55	28	301.60	11.40	-.47	0.063
33	136	304.04	14.66	22	309.40	11.60	-.47	0.105
34	49	311.22	14.78	21	316.70	11.80	-.48	0.132
35	33	317.94	14.90	18	323.50	12.10	-.48	0.181
36	11	324.19	15.02	22	329.80	12.30	-.47	0.260
37	12	329.97	15.13	17	335.60	12.50	-.46	0.230
Total	8003			515				

*Reference values (3.21)
**p value= Independent t test

Table 3.13: Abdominal circumference (AC) by gestational age compared with International reference values

Weeks of gestation	Bangladesh			International reference*			z-score	P value**
	No of fetus	Mean	SD	No of fetus	Mean	SD		
13	705	71.03	9.84	17	70.90	4.70	.02	0.957
14	874	82.46	10.33	16	82.70	5.30	-.03	0.926
15	634	93.80	10.81	18	94.50	5.90	-.09	0.785
16	315	105.03	11.29	18	106.20	6.50	-.15	0.656
17	204	116.15	11.78	20	117.80	7.20	-.20	0.227
18	520	127.15	12.26	26	129.30	7.80	-.25	0.210
19	791	138.03	12.74	20	140.80	8.40	-.30	0.324
20	641	148.78	13.23	22	152.10	9.00	-.35	0.293
21	351	159.39	13.71	24	163.40	9.60	-.40	0.422
22	170	169.85	14.19	21	174.60	10.20	-.45	0.564
23	66	180.16	14.67	24	185.60	10.80	-.49	0.101
24	24	190.31	15.16	20	196.60	11.40	-.55	0.134
25	7	200.29	15.64	27	207.40	12.00	-.60	0.189
26	4	210.10	16.12	14	218.10	12.60	-.65	0.306
27	15	219.74	16.61	23	228.70	13.20	-.70	0.043
28	117	229.18	17.09	28	239.10	13.80	-.75	0.005
29	541	238.43	17.57	13	249.40	14.40	-.79	0.026
30	833	247.48	18.06	21	259.60	15.00	-.85	0.002
31	612	256.32	18.54	19	269.60	15.60	-.90	0.002
32	309	264.94	19.02	25	279.50	16.20	-.96	0.001
33	135	273.35	19.50	20	289.20	16.80	-1.02	0.001
34	50	281.52	19.99	23	298.80	17.40	-1.07	0.001
35	34	289.46	20.47	17	308.20	18.00	-1.14	0.002
36	11	297.16	20.95	24	317.40	18.60	-1.19	0.007
37	12	304.60	21.44	16	326.40	19.20	-1.25	0.009
Total	7975			516				

* Reference values (3.22)
**p value= Independent t test

Table 3.14: Femur length (FL) by gestational age compared with International reference values

Weeks of gestation	Bangladesh			International reference*			z-score	P value**
	No of fetus	Mean	SD	No of fetus	Mean	SD		
13	728	11.53	2.91	18	10.90	1.80	35	0.375
14	879	14.79	2.93	16	14.10	1.90	.36	0.334
15	634	18.01	2.95	18	17.20	1.90	.43	0.247
16	315	21.17	2.97	17	20.30	2.00	.44	0.234
17	203	24.27	2.99	20	23.30	2.10	.46	0.192
18	520	27.32	3.01	25	26.30	2.10	.49	0.095
19	792	30.30	3.03	20	29.20	2.20	.50	0.107
20	642	33.21	3.05	23	32.10	2.20	.50	0.084
21	352	36.05	3.07	24	34.90	2.30	.50	0.074
22	170	38.82	3.10	21	37.60	2.30	.53	0.083
23	66	41.51	3.12	25	40.30	2.40	.50	0.083
24	23	44.12	3.14	22	42.90	2.50	.49	0.151
25	7	46.64	3.16	27	45.50	2.50	.46	0.316
26	4	49.07	3.18	15	48.00	2.60	.41	0.493
27	15	51.41	3.20	23	50.40	2.60	.39	0.292
28	117	53.65	3.22	30	52.70	2.70	.35	0.139
29	542	55.80	3.24	15	55.00	2.80	.29	0.344
30	836	57.84	3.26	24	57.10	2.80	.26	0.272
31	615	59.77	3.28	21	59.20	2.90	.20	0.432
32	311	61.59	3.31	30	61.20	2.90	.13	0.534
33	136	63.30	3.33	23	63.10	3.00	.07	0.788
34	50	64.90	3.35	23	64.90	3.00	.00	1.00
35	34	66.37	3.37	19	66.60	3.10	-.07	0.807
36	11	67.71	3.39	27	68.20	3.20	-.15	0.676
37	12	68.93	3.41	18	69.70	3.20	-.24	0.534
Total	8014			544				

* Reference values (3.23)
**p value= Independent t test

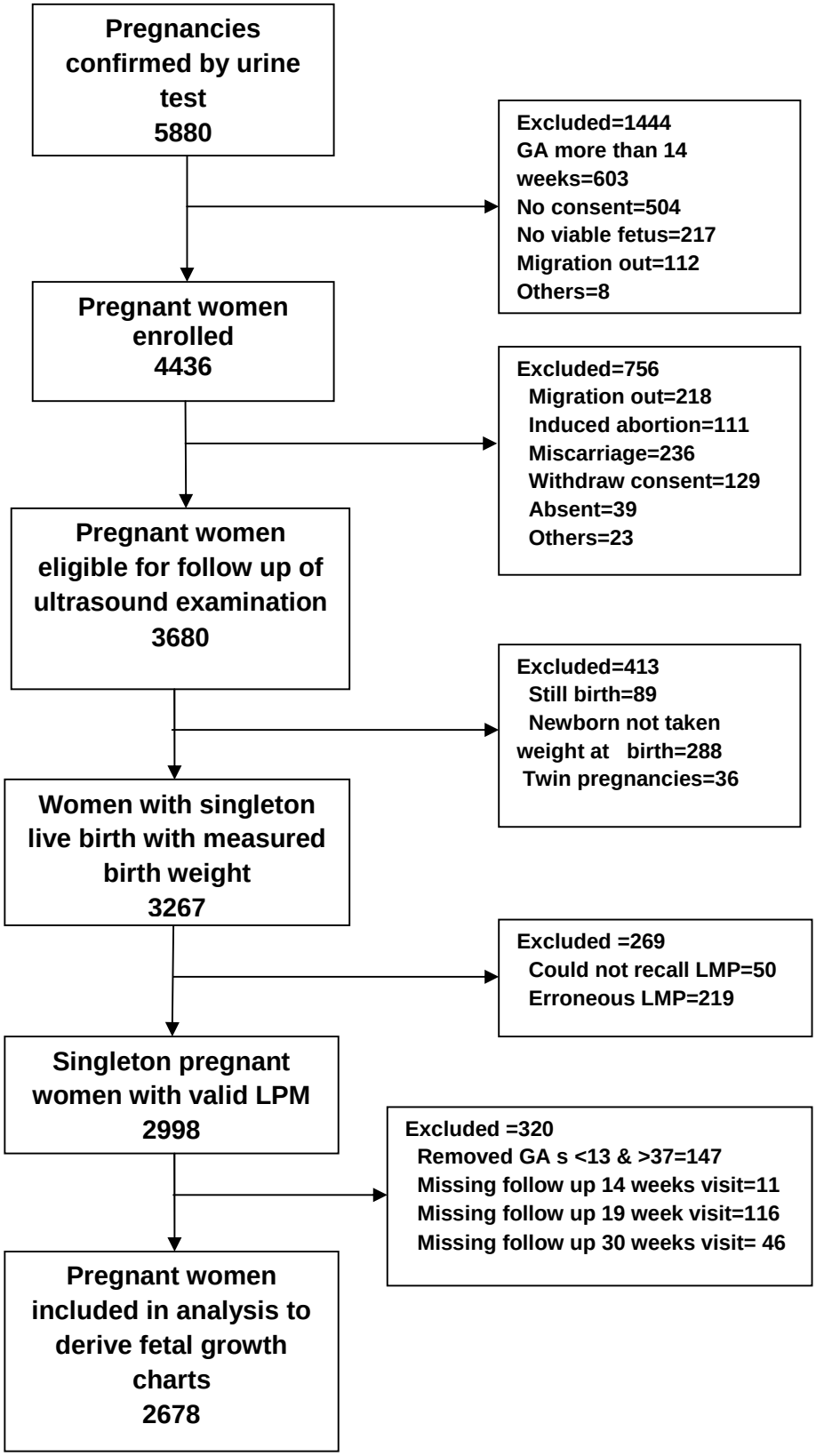


Figure 3.1: Flow chart of study participants

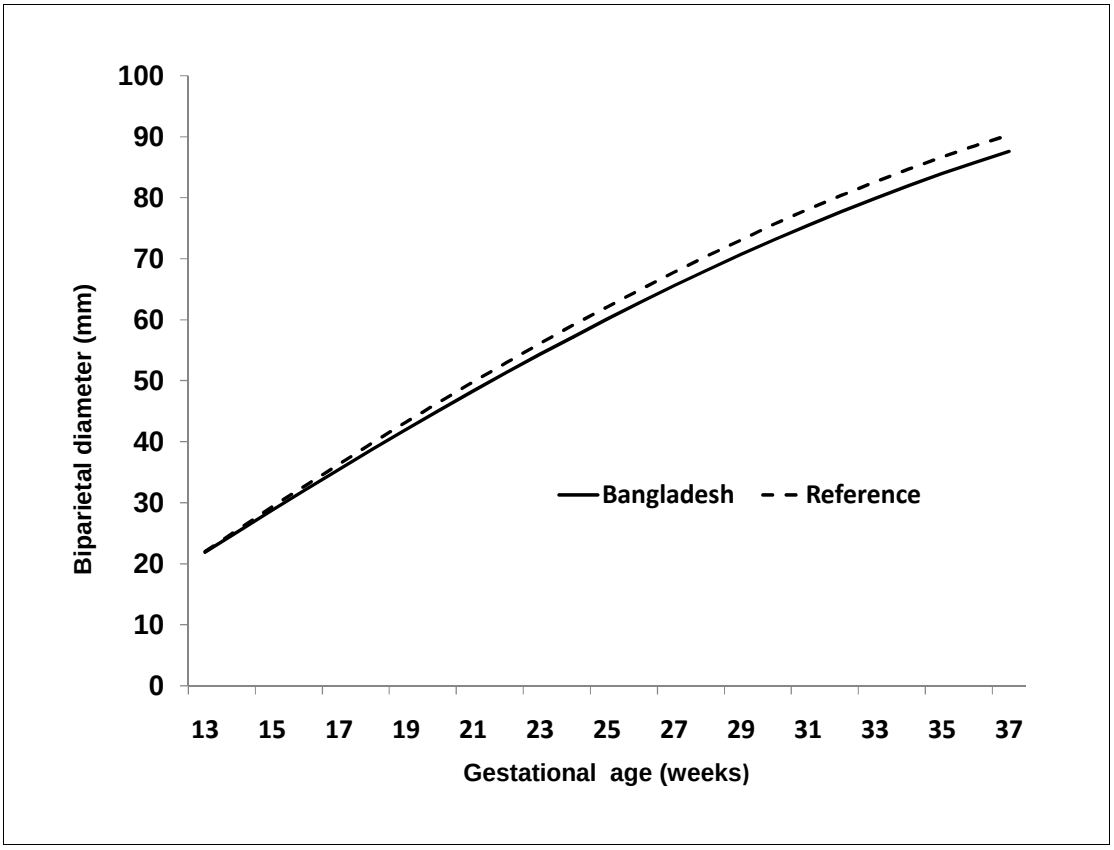


Figure 3.2: Comparison of biparietal diameter (BPD) with international values (dash line) [4.21]

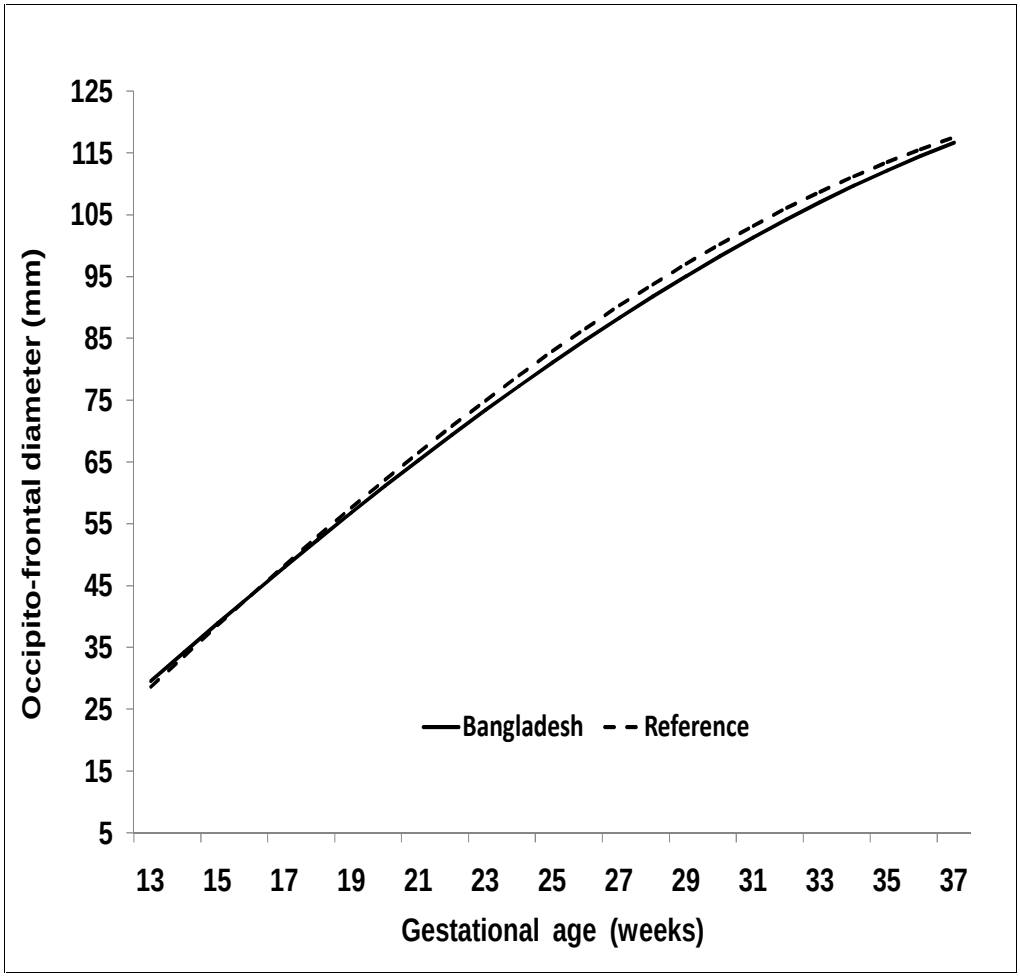


Figure 3.3: Comparison of occipito-frontal diameter (OFD) with International values (dash line) [3.21].

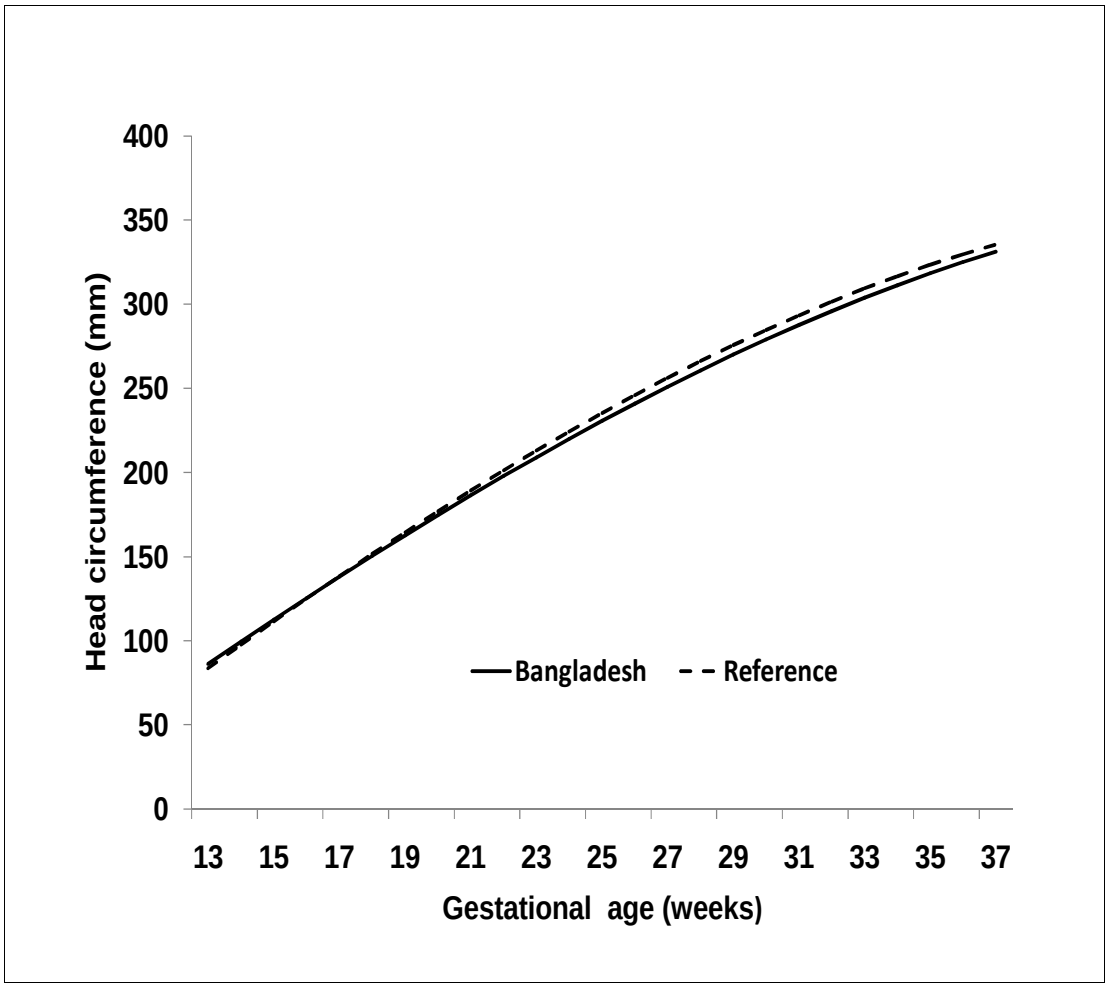


Figure 3.4: Comparison of head circumference (HC) with international value (dash line) [3.21].

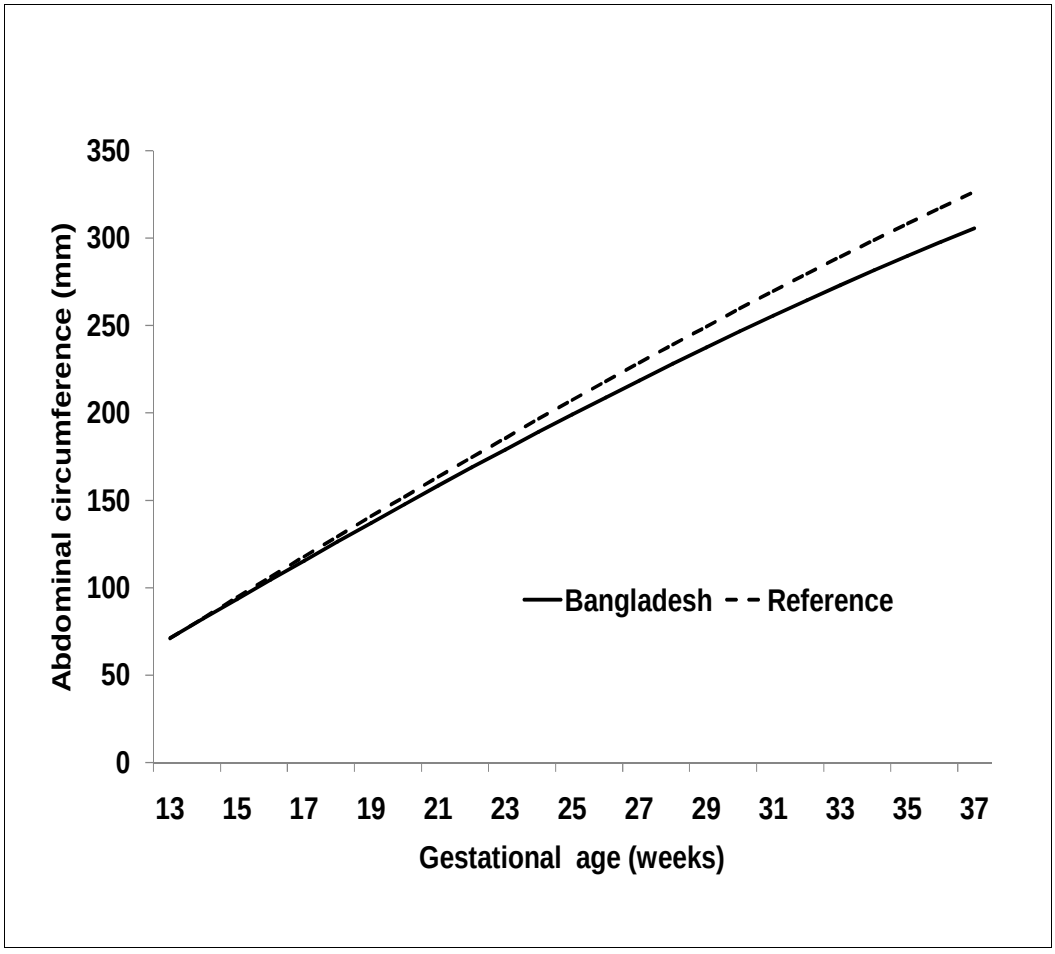


Figure 3.5: Comparison of abdominal circumference (AC) with International value (dash line) [3.22].

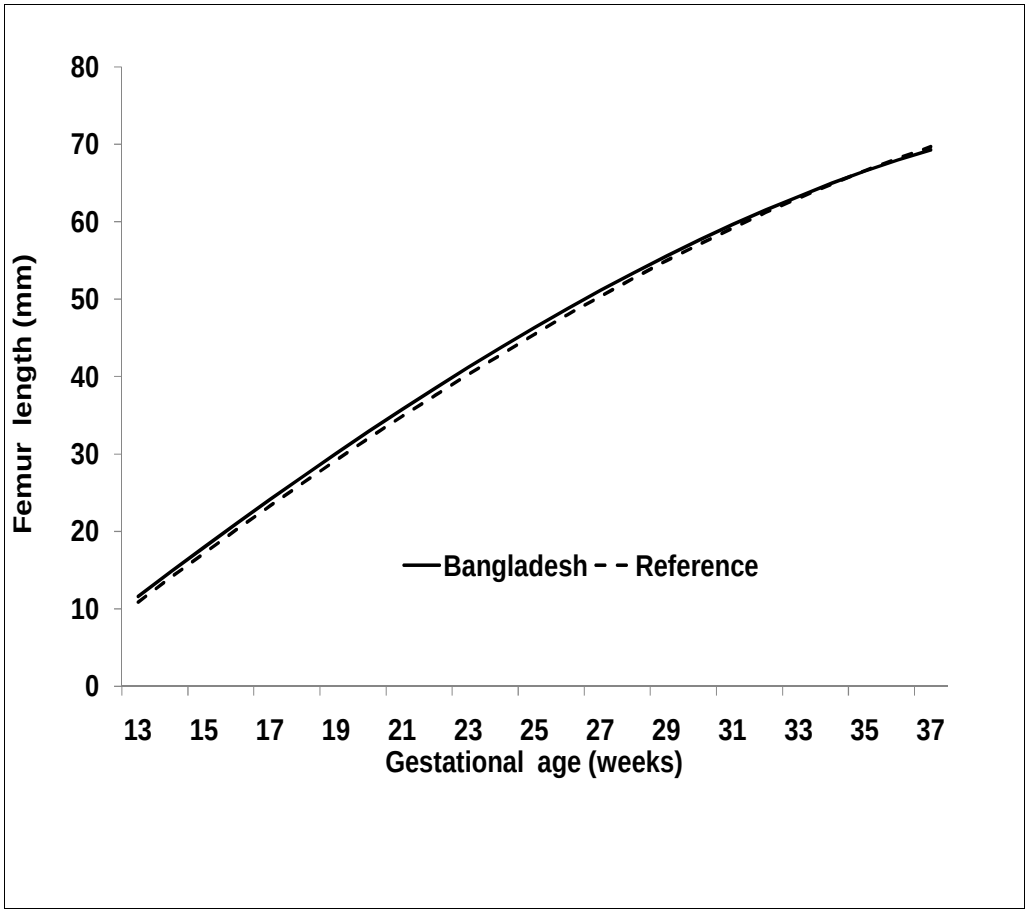
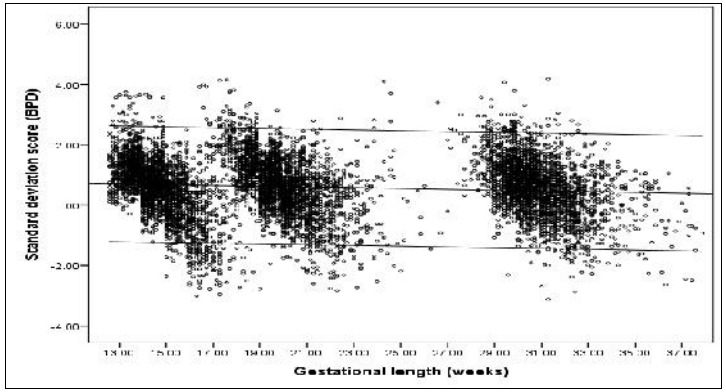
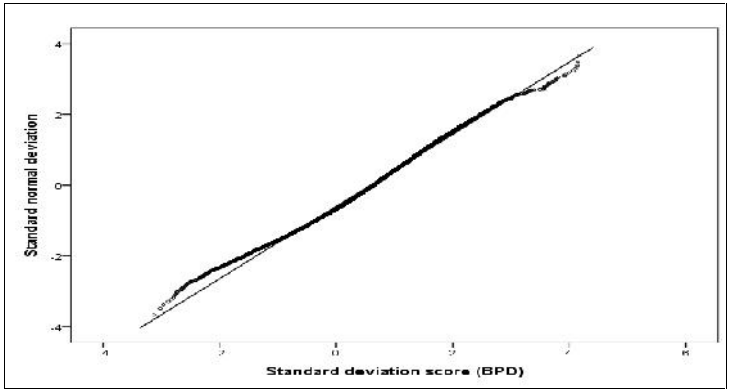


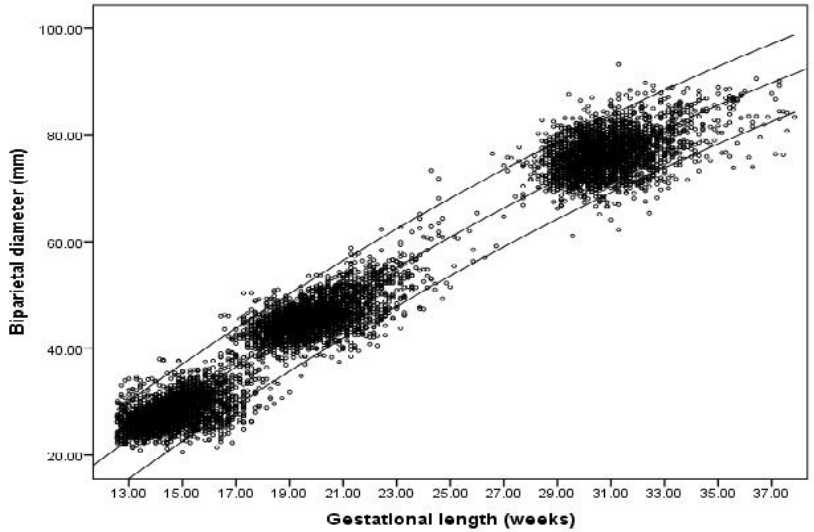
Figure 3.6: Comparison of femur length (FL) with international values (dash line) [3.23].



(a)

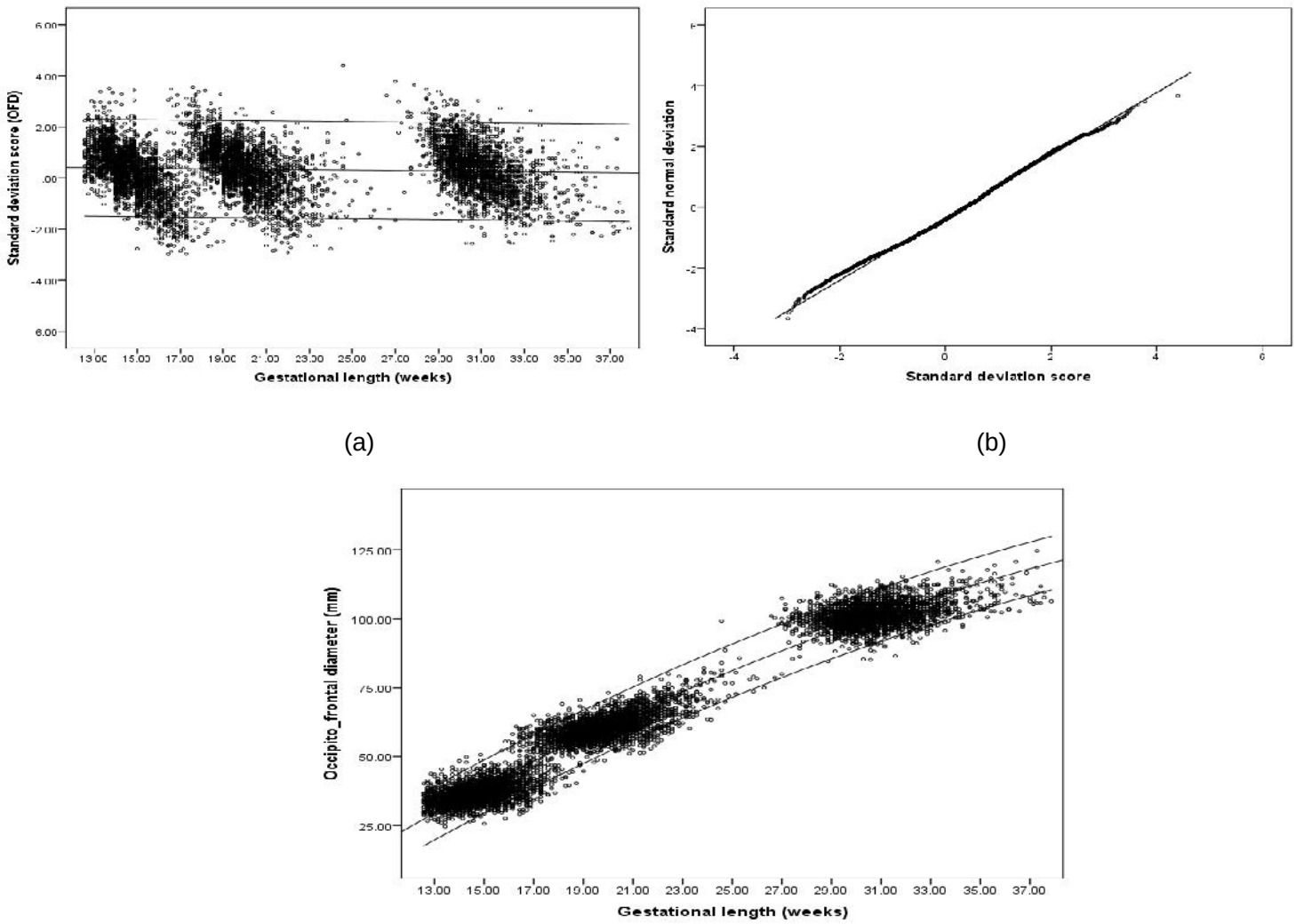


(b)



(c)

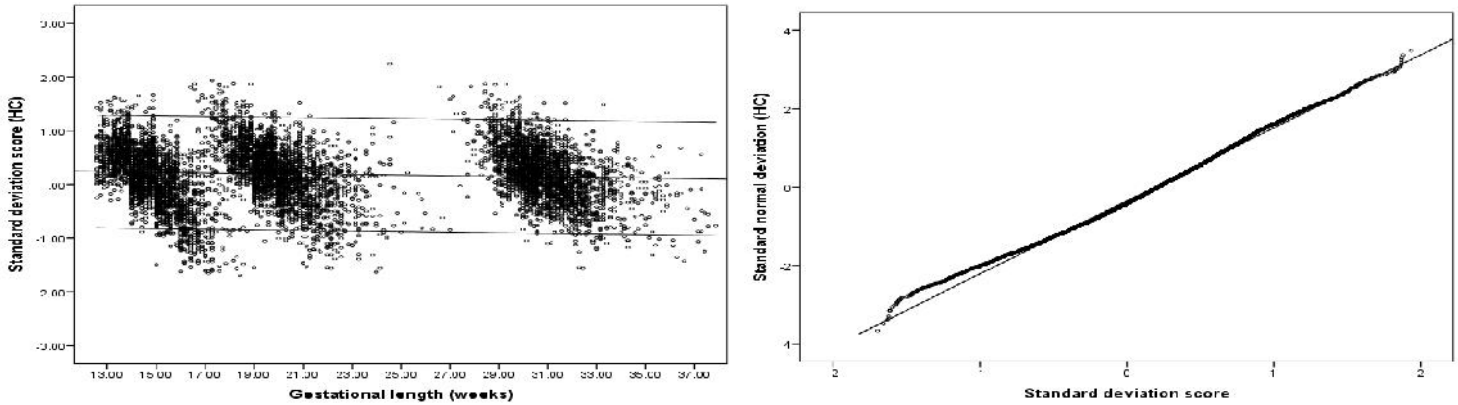
Figure: 3. 7: (a) Plotted of standard deviation score (standardized residual) of BPD fitted with 5th and 95th percentiles against gestational age. (b) Normal plot of SDS. (c) Raw data fitted with 5th and 95th percentile.



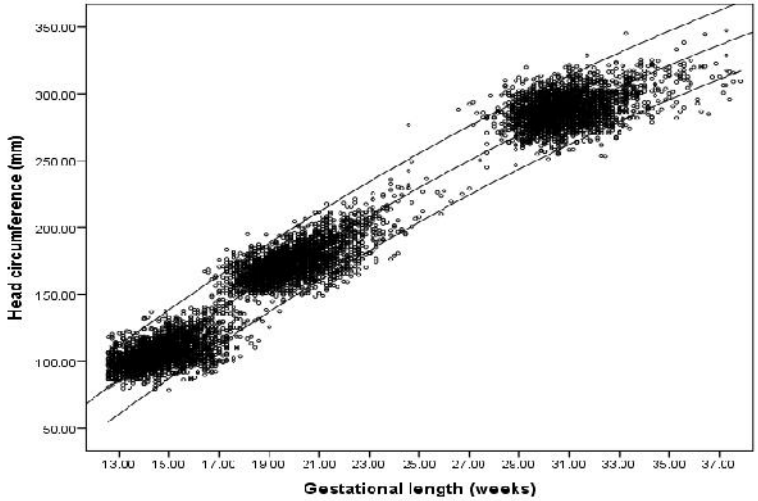
(a) (b)

(c)

Figure 3. 8: (a) Plotted of standard deviation score (standardized residual) of OFD fitted with 5th and 95th percentiles against gestational age. (b) Normal plot of SDS. (c) Raw data fitted with 5th and 95th percentile.

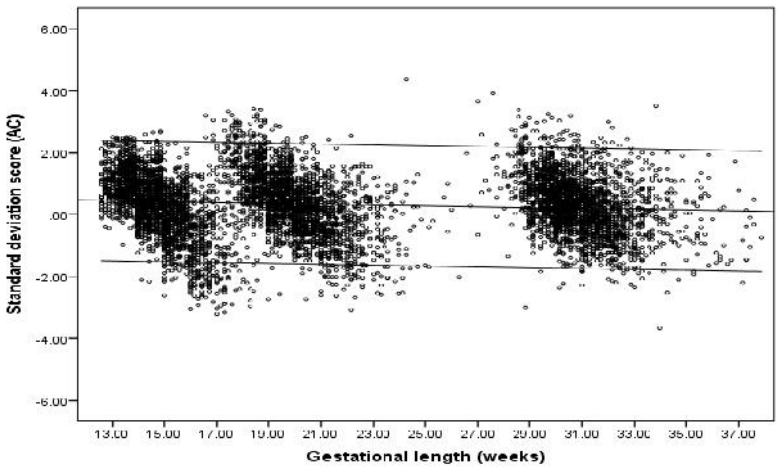


(a) (b)

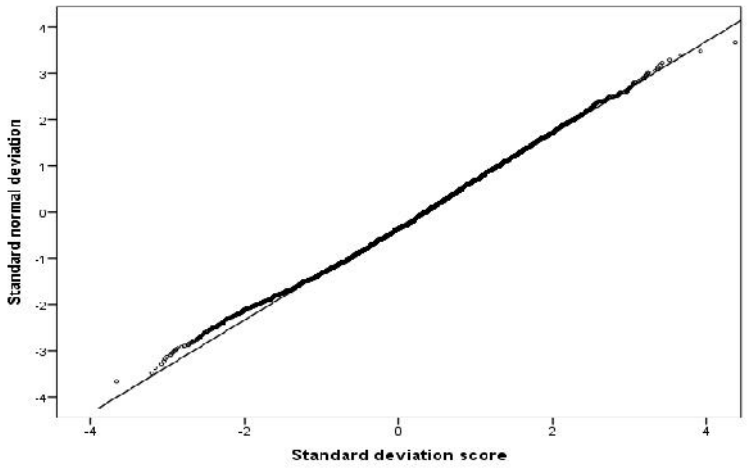


(c)

Figure 3. 9: (a) Plotted of standard deviation score (standardized residual) of HC fitted with 5th and 95th percentiles against gestational age. (b) Normal plot of SDS. (c) Raw data fitted with 5th and 95th percentile.



(a)



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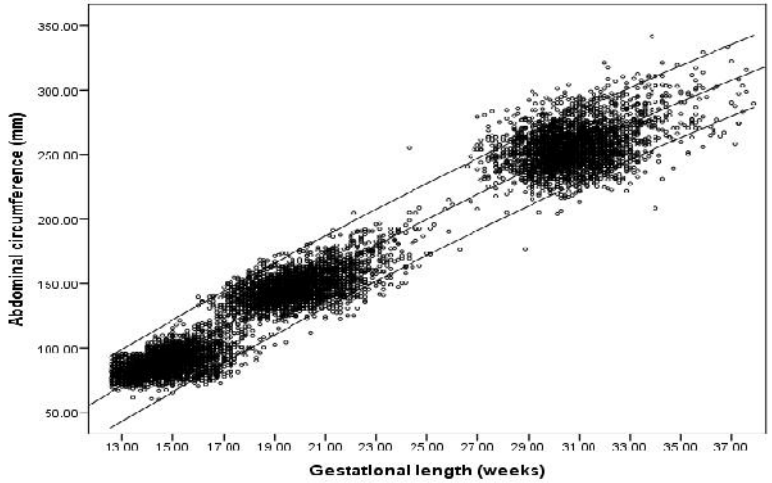
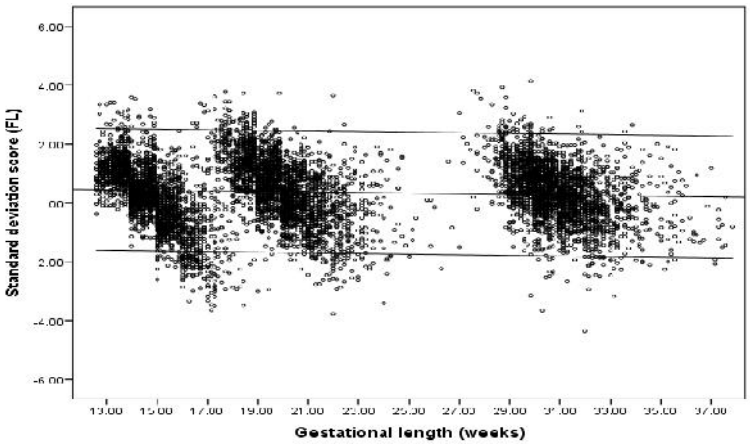
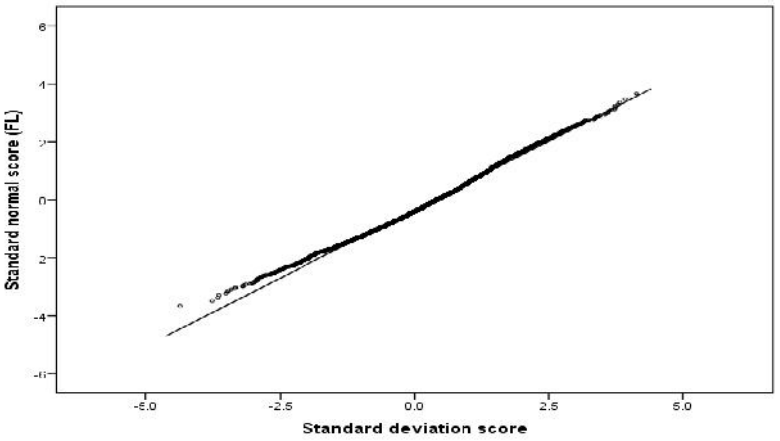


(c)

Figure 3. 10: (a) Plotted of standard deviation score (standardized residual) of AC fitted with 5th and 95th percentiles against gestational age. (b) Normal plot of SDS. (c) Raw data fitted with 5th and 95th percentile.



(a)



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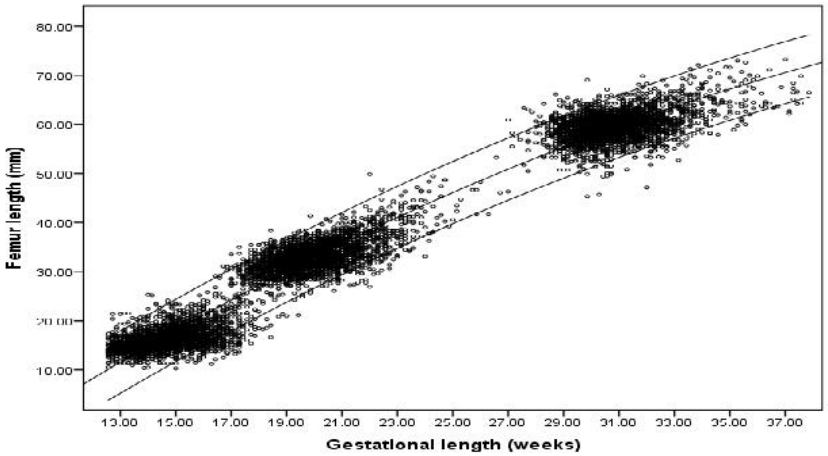
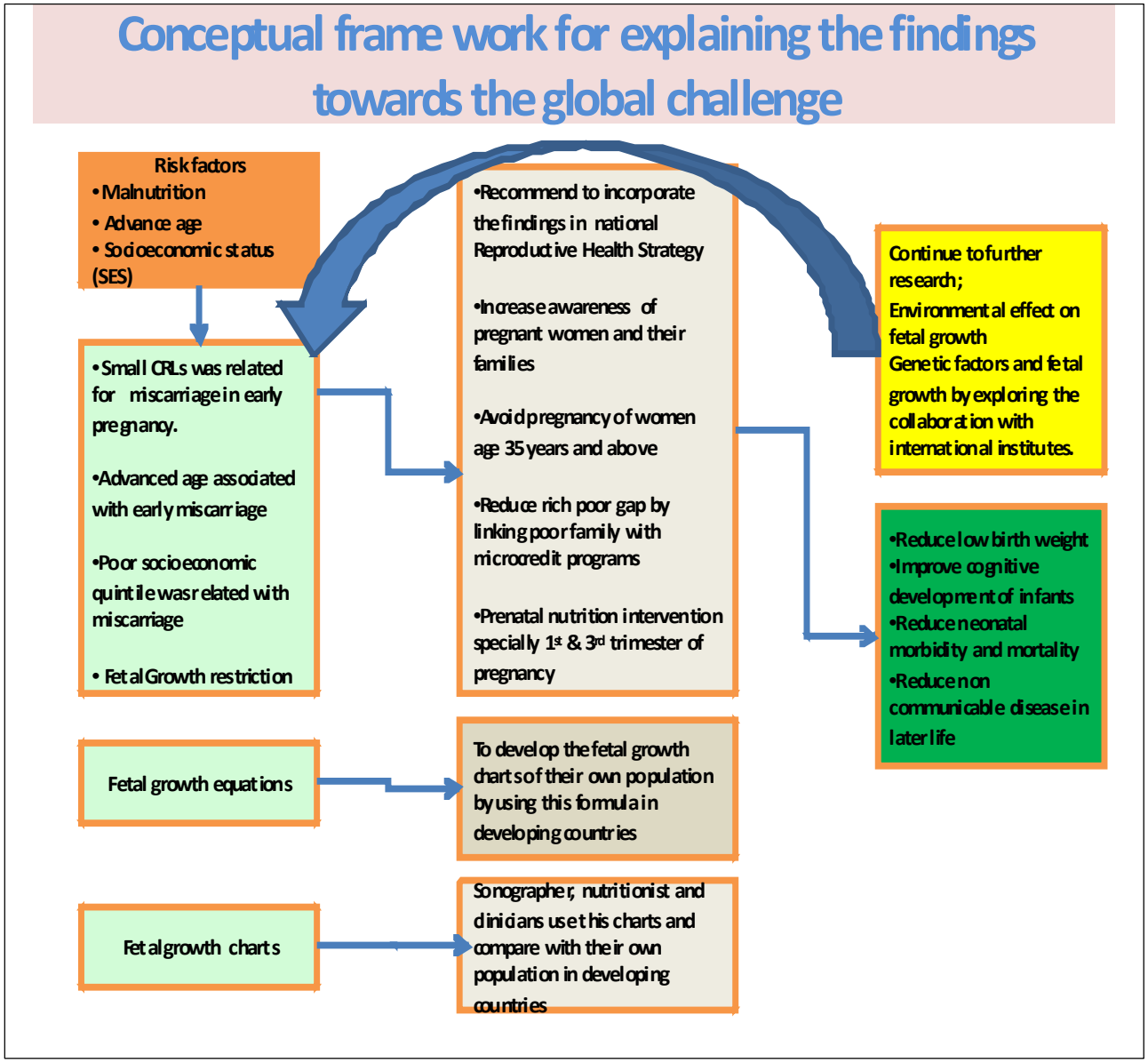


(c)

Figure 3. 11: (a) Plotted of standard deviation score (standardized residual) of FL fitted with 5th and 95th percentiles against gestational age. (b) Normal plot of SDS. (c) Raw data fitted with 5th and 95th percentile.


Conceptual frame work for explaining the findings towards the global challenge
Risk factors
• Malnutrition
• Advance age
• Socioeconomic status (SES)
• Small CRLs was related for miscarriage in early pregnancy.
•Advanced age associated with early miscarriage
•Poor socioeconomic quintile was related with miscarriage
• Fetal Growth restriction
•Recommend to incorporate the findings in national Reproductive Health Strategy
•Increase awareness of pregnant women and their families
•Avoid pregnancy of women age 35 years and above
•Reduce rich poor gap by linking poor family with microcredit programs
•Prenatal nutrition intervention specially 1st & 3rd trimester of pregnancy
Continue to further research;
Environmental effect on fetal growth
Genetic factors and fetal growth by exploring the collaboration with international institutes.
•Reduce low birth weight
•Improve cognitive development of infants
•Reduce neonatal morbidity and mortality
•Reduce non communicable disease in later life
Fetal growth equations
To develop the fetal growth charts of their own population by using this formula in developing countries
Fetal growth charts
Sonographer, nutritionist and diniciansuse this charts and compare with their own population in developing countries

Chapter 4

Conclusions

Intrauterine growth restriction (UGR) has always been a global challenge for epidemiologists, nutritionist and clinicians. Many risk factors have been involved directly and indirectly for the fetal growth during pregnancy. Most important factors leading to high prevalence of IUGR in developing countries are malnutrition and low birth weight. LBW is predominantly results of IUGR. The fetal growth charts and timing of growth faltering is important for assessing the fetal growth and size during pregnancy as well as to identify the role of early growth restriction and miscarriage.

In the present study, I have analyzed two large scale studies, firstly on first trimester growth restriction and occurrence of miscarriage, secondly, to develop the fetal growth charts and growth restriction in rural Bangladesh. Both of studies have clinical as well as public health important.

The study on miscarriage shows that shorter CRL was associated with the miscarriage in early pregnancy among the Bangladesh population. Advanced maternal age and socioeconomic quintile also associated with the miscarriage in early pregnancy.

The study on the fetal growth shows that the growth was smaller at the mid of second trimester to last trimesters compared with reference values. The growth faltering was started at different gestational weeks in different fetal parameters. This is the first time; a large population based longitudinal study and compared with the internationally recommended reference values to observe the deviation of the derived value of the present study. The findings of this study will be useful for reliable assessment of fetal growth in Bangladesh.

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REFERENCE PAPER