**Chapter-1: Introduction**

Nutrition is one of the most important factors influencing the quality of life. In human being, it is very much critical to maintain at standard level especially in pregnant women. Expected mother needs balanced nutrition for their better health and pregnancy outcome, because maternal nutritional status directly affects the fetal growth and development. For example, anaemia and vitamin-A deficiency are highly prevalent nutrient deficiency encountered by pregnant women, affecting 55.8% and 6.8%on global scale, respectively. (Christoph et al., 2006).

Pregnancy is a period of rapid growth and cell differentiation, both for the mother and the fetus she carries. Consequently, both are very susceptible to alterations in dietary supply, particularly nutrients which are marginal under normal circumstances during this period. Imbalanced nutrition is not only caused an increased risk of death in utero, as well as responsible for changes in birth weight and functions in the neonatal organs. For instance, the baby who is small at birth is at an increased risk of cardiovascular disease and diabetes as adults (Barker, 1992), who are born to zinc deficient mothers have a compromised immune system (Keen et al.,1989) due to changes in gene expression in the fetus. Various factors are associated with the healthy mother and fetus. For example, life style behavior, socioeconomic status, education, parity, age, anthropometric parameters. Among these nutrients are the most vital for maintenance of good health of pregnant women. Previous study shows that serious health consequences increased mortality and morbidity of both mother and child (Kennedy et. al., 2004). Globally nearly 15 million women are physically immature and still in a state of growth, face even more difficulties in meeting their nutritional requirements. Earlier study depicted that protein energy malnutrition (PEM), iron deficiency anaemia (IDA), vitamin A deficiency (VAD) were 15.1%, 6.3% and 3.3% , respectively were found in pregnant women(Christoph et. al.2006).

Nutrients are chemical compounds in food that are used by the body to function properly and maintain health. Examples include proteins, fats, carbohydrates, vitamins, and minerals. Now-a-days in Bangladesh people are aware about the maternal health status due to many activities have conducting by government and non-government organizations throughout the country. These activities include counseling for balance diet and immunization of women before and after conception of baby. During this period micro-nutrients, in serum, are play important roles for maintenance of maternal health (Reddyys et al., 2014). And micro-nutrients include copper, zinc, calcium, magnesium, iron and iodine etc. (Frias-Espericutamg, et al., 2014), which does specific function. For example, copper protects the cells from the toxic superoxide anion, ensure normal fetal growth and immune function, and copper deficient pregnant mother are prone to preterm delivery, premature rupture of membranes and damage of fetal nervous system (Ergazz et al., 2012). Zinc is a cofactor of a variety of enzymes and nucleic acid and involved in the human immune system and the development of the fetal nervous system (Dickinson, et al., 2014). Furthermore, lack of zinc in-take in late pregnancy can cause fetal growth retardation, congenital malformations, and even fetal death (Dickinsonn et al., 2009). Calcium is the pivotal component of the teeth and skeleton and it plays an important role in the activation of muscle-keeping, nervous excitement and enzyme activation. Calcium deficiency in pregnant women can cause gestational hypertension (Esteban et al., 2012) and aggravate postpartum hemorrhage, and also causeossification disorder, when maternal calcium mobilization compensate for the lack of fetal calcium, which may lead to an in-creased maternal deficiency (Taylor et al., 2014). Iron is the main component of hemoglobin and myoglobin; and performs many functions including oxygen transport, storage and use and synthesis of cytochrome enzymes, peroxidase enzymes and hormones. Previous studies have shown that anemia occurs in pregnant women who have a lack of iron, leading to fetal chronic hypoxia, premature birth and perinatal fetus mortality (Kapilu et al., 2013). The imbalance of these micro-nutrients caused a number of complications during pregnancy such as miscarriage, preterm delivery, stillbirth, intrauterine growth restriction, fetal malformations, premature rupture of membranes and other adverse pregnancy outcomes. Furthermore, magnesium (Mg) also is closely associated with hundreds of body physiologies involving over 350 enzymes. Dietary Mg intake has been related to several health outcomes including those related to metabolic and inflammatory processes such as hypertension, metabolic syndrome, type 2 diabetes, cardiovascular diseases, osteoporosis and some cancers (for example, colon, breast). For the above disorders, one suggested mechanism for the beneficial effect of Mg intake is that Mg may reduce the levels of C-reactive protein (CRP)-a well-documented indicator of a low-grade or chronic inflammation.  Mg deficiency to acute inflammatory response mediated by calcium*, N*-methyl-D-aspartic acid or *N*-methyl-D-aspartate, interleukin-6 (IL-6) and tumor necrosis factor-alpha. CRP is an acute acute phase reactant is synthesized in the liver. Serum CRP levels increase following a variety of pro-inflammatory events such as infection, tissue necrosis, trauma, surgery and malignancy. CRP levels can increase quickly and dramatically (often 100-fold) during inflammation.

Anthropometric parameters are also important for maintenance of good health. Pre-pregnancy body mass index (BMI) is important parameter which reflects the physiology. BMI has been associated with increased risks for adverse pregnancy outcomes such as gestational hypertension, gestational diabetes mellitus (GDM), pre-term birth, and small-and large-for-gestational-age infants (Shin and Song, 2015). Elevated inflammation during pregnancy has also been found as a risk factor for pregnancy complications such as GDM (Wolf et al., 2003; Qiu et al., 2004), pre-term delivery (Bullen et al., 2013) and pre-eclampsia. A study showed that pregnant women with elevated CRP during the first trimester had an increased risk of developing GDM (Wolf et al., 2003) and pre-pregnancy BMI and the risk of preeclampsia was partially mediated by inﬂammation during pregnancy (Bodnar, et al., 2005). Interestingly, pre-pregnancy BMI and inﬂammation are both associated with dietary factors. Women with obese pre-pregnancy BMIs had signiﬁcantly associated with having lower quality diets during pregnancy compared with women with normal pre-pregnancy BMIs (Shin et al., 2016; Laraia et al., 2007; Tsigga et al., 2011). And the diet is a major modiﬁable determinant for inﬂammation. Fruit and vegetable intake (Esmaillzadeh, et al., 2006) and dietary ﬁber (Ma et al., 2006) were negatively associated with plasma CRP concentrations, which may be due to antioxidants present in fruit and vegetables (Liu et al., 2013) and decreased lipid oxidation levels.

More to the point, low-grade inflammation is associated with [endothelial dysfunction](https://www.sciencedirect.com/topics/medicine-and-dentistry/endothelial-dysfunction), leading to vascular dysfunction and suboptimal [placental development](https://www.sciencedirect.com/topics/medicine-and-dentistry/placenta-development). Maternal systemic inflammation might also be a response to [ischemia](https://www.sciencedirect.com/topics/medicine-and-dentistry/ischemia) of the placenta, due to suboptimal placentation (Lam, 2005; Redman, 2004). Subsequently, suboptimal placental development might predispose mothers to increased risks for various pregnancy complications (Redman, 2009; Roberts, 2010).

However, owing to differences between races, difference in diet and living environments, changes of the trace elements during pregnancy in Bangladeshi women is not well under-stood. Lack of trace elements in pregnant women and the way they affect pregnancy outcome is also not clear. Our research will prospectively observe changes of maternal serum trace elements in pregnant Bangladeshi women and analyze their relationship with inflammatory biomarker CRP.

Micronutrients support vital aspects of maternal and fetal health during pregnancy. Under nutrition and poor health from preventable causes disproportionately affect well-being of millions of people in the developing world. Factors at individual, household and community level or a combination of these factors may contribute to poor nutrition and health status. Of particular, women are more likely to suffer from nutritional deficiency than men for several reasons, including their reproductive biology, low social status, poverty and lack of education. Adequate nutritional status of women is important for good health and increased work capacity of women themselves as well as for the health of their off springs. Severely malnourished mothers have reduced lactation performance contributing to the increased risk of child mortality. To achieve the sustainable development goals, maintenance of mother and child health is very much crucial. Although Bangladesh has improved the indicators of mother and child health status set by the United Nation as compared to other developing countries, there is scarcity of information on nutritional status specially micronutrients of pregnant women in the context of Bangladesh.

Considering the backgrounds, the present study was undertaken with the aim of following objectives:

1. To study the socioeconomic status of pregnant women, who visit the doctor at hospitals
2. To study the life style behavior and anthropometric parameters
3. To know the physiological parameters including hematological and biochemical indices specially trace elements and inflammatory biomarkers
4. To find out the association between trace element (Mg), life style behavior (resting time) and inflammatory biomarker (CRP).

**Chapter-2: Review of Literature**

The world population is around 7.8 billion. Bangladesh belongs to 2.11% of the world population (UN data 2020), which is equivalent to 165,918,701. In Bangladesh the male versus female is 50.60%:49.4%. Pregnant women, nursing mothers and children are particularly vulnerable to the incidence of malnutrition. The adverse effects of malnutrition are maternal depletion, low birth weight, anaemia, toxiaemia of pregnancy, postpartum hemorrhage which result inmortality and morbidity(Park,2011). Maternal diets during pregnancy need to provide energy and nutrients for the mother as well as fetal growth(King,2000;Ziegler,1996). Poor maternal nutrition during third trimester is a major cause of low birth weight in developing country (Yip, 2001;Ladipo,2000). Inadequate specific nutrients intake during pregnancy leads to a variety of poor maternal and infant outcomes(Lumey,1992).

**2.1 Measurement of variables**

**2.1.1Anthropometric parameters**

* Age- 15-49 years (Reproductive Age)
* Weight: Pregnancy is a change within the body. It is normal to gain some weight during pregnancy due to the growth of the baby, placenta and amniotic fluid around the baby.
* Height: Maternal height at the time of pregnancy is a major determinant of gestational duration and hence short stature would be associated with increased risk of preterm labour.
* BMI (Body Mass Index)

**Table-1:** The World Health Organization's (WHO) recommended body weight based on BMI values for adults. It is used for both men and women, age 18 or older.

|  |  |
| --- | --- |
| **Category** | **BMI range - kg/m2** |
| Severe Thinness | < 16 |
| Moderate Thinness | 16 – 17 |
| Mild Thinness | 17 - 18.5 |
| Normal | 18.5 – 25 |
| Overweight | 25 – 30 |
| Obese Class I | 30 – 35 |
| Obese Class II | 35 – 40 |
| Obese Class III | > 40 |

Source: World Health Organization's (WHO) in 1992

**Table-2:** The Institute of Medicine (IOM) guidelines for total weight gain during a full-term pregnancy recommended that range for weight gain during pregnancy (carrying a single fetus), by BMI at the beginning of the pregnancy.

|  |  |  |
| --- | --- | --- |
| BMI category at the beginning of the pregnancy | BMI status | Weight gain during the entire pregnancy |
| BMI < 18.5 | Underweight | 12.5 – 18 kg |
| 18.5 ≤ BMI < 24.9 | Adequate weight | 11.5 - 16 kg |
| 25 ≤ BMI < 29.9 | Over weight | 7 - 11.5 kg |
| 30 ≤ BMI | Obese | 5 - 9 kg |

For the initial three months a 0.5-2.0kg gain of is taken into account.

Source: Institute of Medicine (IOM),National Research Council(NRC)

**MUAC:** Mid Upper Arms Circumferenceis a good indicator of the protein reserves of a body and a thinner arm reflects wasted teanmass. i.e. malnutrition. MUAC is the circumference of the left upper arm and is measured at the midpoint between the tip of the shoulder and elbow. To measured: Bend the left arm, find and mark with a pen the olecranon process and acromion. Mark the mid-point between these two marks. (**Anamaría, 1998)**

**Waist Circumference:** We can estimate WC at maximum a woman's waist will likely grow by 1 centimeter (0.39 inches) per week, on average. This gives us an average maximum waist growth of 20 centimeters or around 8 inches (rounded) from week 20 to week 40. This is a very rough estimation. (Allison, 2017)

**2.1.2 Lifestyle Behavior**

Healthy weight and healthy lifestyle behavior are considered as essential prerequisites for a successful pregnancy. The importance of maternal lifestyle including nutrition and physical activity in relation to the short- and long-term birth outcomes is increasingly featured in the literature(Debbie Smith et. al 2017**)**

**2.1.3 Religion**: The major religion in Bangladesh is Muslim (90.4%) but significant percentage of the population adheres to Hinduism (8.5%) other religions groups include Buddhists (6%), Christen (0.3%).

**2.1.4 Education:** Bangladesh literature Rate- Historical data.

|  |  |  |
| --- | --- | --- |
| **Year** | **Literacy Rate (%)** | **Annual Change (%)** |
| 2018 | 73.91 | 1.02 |
| 2017 | 72.89 | 0.13 |
| 2016 | 72.76 | 7.62 |

**2.1.5 Family type:** The Families in Bangladesh may be divided into three categories normally

a) Joint

b) Extended

c) Nuclear

**2.1.6 Residence:** The study population comprises pregnant women permanently residing in the study area. Permanent residency is defined by living in the study area for a minimum of six consecutive months preceding the recruitment.

**2.1.7 No. of Family Member:** Small family consist 4 to 6 people.

**2.1.8 Exposure to sun light**: is increase the brains release of a hormone called serotonin. Serotonin is associated with boosting mood and helping a person feel calm & focused.

Exposure to sun light can also benefit those with:

* Premenstrual dysphoric disorder (PMDD)
* Pregnant people with depression

Our body produces Vitamin D when exposed to sun light about 15 minutes in the sun a day. Vitamin-D helps our body maintain calcium & prevent brittle, thin or misshapen bones, relieves stress and improves sleep.

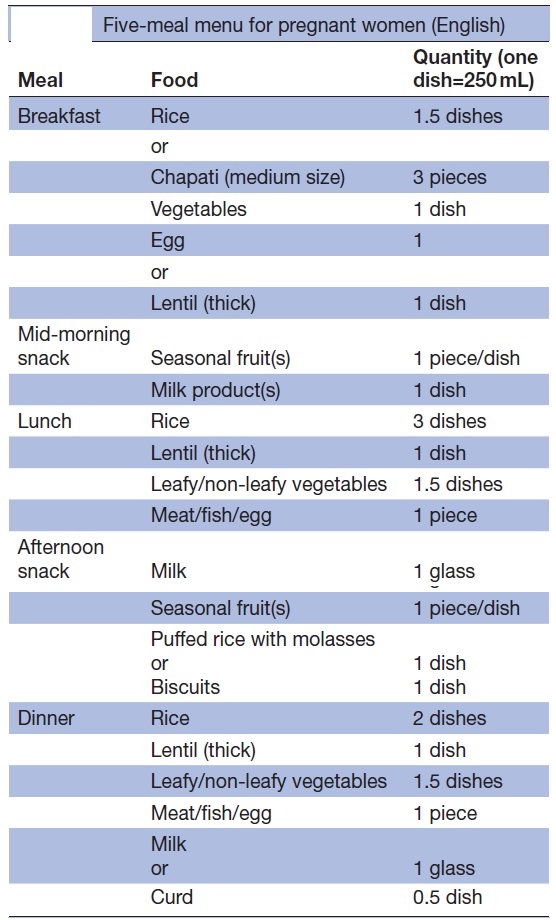
**2.1.9 Exercise**: Exercise is any bodily actively that enhances or maintains physical fitness and overall health and wellness. Exercise is vital for looking after and improving our health. It also relieves stress, improves memory, help our sleep better reduces disease, and strengthens bones and muscle. Exercise also produces an acute increase in various anti-inflammatory mediators, including the cytokine inhibitors, IL-1 receptor antagonist, TNF receptors, IL-10 and IL-8, and macrophage inflammatory proteins 1-alpha and -beta, whereas leukocyte adhesion molecules, such as beta-1 and -2 integrins, decrease. Thus, there is a parallel “protective” anti-inflammatory counter-regulation that also is part of the APR to exercise.

**2.1.10 Eat additional food during pregnancy:** Pregnant women need about 300 extra calories a day. Five food group: Fruits, vegetables, protein, whole grains and dairy products.

**2.1.11Avoid eating of food during pregnancy:** Caffeine, alcohol, fish with levels of mercury, raw meat, raw egg, under cooked food, unwashed product, unpasteurized milk, cheese, fruits juice, junk foods.

**2.1.12 Number of meals:** During pregnancy need to maintain five- meal menu.

**Table-3: Five-meal menu for pregnant women**



Source: Chowdhury M, et al. BMJ Open 2017

**2.1.13 Sleeping time:** Sleep improves our immune system and brain function and its especially important during pregnancy because it regulates growth hormone levels. Women who are already getting 8 hours might need up to 10 while they are pregnant.

**2.1.14 Resting time: Bed rest** is commonly prescribed during pregnancy to alleviate certain [pregnancy complications](https://americanpregnancy.org/pregnancy-complications/most-common/). Some women know it is coming because of their medical history, whereas others are surprised during a routine checkup. Bed rest is common, so don’t be alarmed. Bed rest will differ from woman to woman and may range from simple periodic resting at home to full bed rest with monitoring in a hospital.  Some women will discover that their health care provider places them on bed rest for a brief period to help a complication stabilize, while other women may be placed on bed rest throughout most of their pregnancy if they have what is called, a high-risk pregnancy.

**2.1.15 Practice exclusive breast feeding:** Exclusive breast feeding means that the infant receives only breast milk no other liquids or solids are given not even water. The American Academy of pediatrics recommends that infants be exclusively breastfed for about the first 6 months with continued complimentary food for 2year or longer. Breast feeding is good for both infant and mothers. Breast milk is the source of nutrition for most infant.

**2.1.16 No of antenatal care visit:** The WHO recommends that pregnant women should be receive at least eight antenatal visits to stop and treat problems and give immunizations. Although antenatal care is improve the health of both mother and many women do not receive eight visits. For women whose pregnancies are progressing normally WHO recommends a minimum of visits, ideally at 16 weeks, 24-28weeks, 36 weeks (USAID-2006).

**2.1.17 Parity:** Defined as the number of times that she has given birth to a fetus with a gestational age of 24 weeks or more, regardless of whether the child was born alive or was stillborn.

**2.1.18 History of miscarriage:** Miscarriage is when an embryo or fetus dies before the 20th week of pregnancy.

**2.1.19 Trimester:** In obstetrics, one of the three divisions of three months each during Pregnancy in which different phases of fetal development take place. The first trimester is a time of basic cell differentiation. The second trimester is a period of rapid growth and maturation of body systems. The third trimester marks the final stage of fetal growth.

**2.1.20 Blood Pressure**: Blood pressure is measured in millimeters of mercury (mm Hg) and recorded with the systolic number first, followed by the diastolic number. For example, a normal blood pressure would be recorded as something under 120/80 mm Hg.

**2.1.21 Pulse Rate (PR)**:70 to 100b/min.

**2.1.22 Blood group**: There are 4 main blood groups (types of blood) – A, B, AB and O. Our blood group is determined by the genes inherit from our parents. Each group can be either Rh positive or Rh negative, which means in total there are 8 blood groups.

**2.1.23 History of other disease:** Comorbid describes two or more disorders or illnesses occurring in the same person. Examples: diabetes, heart disease, high blood pressure (hypertension), psychiatric disorders.

**Definition of different types of diseases**

* **Hyperthyroidism:** Hyperthyroidism refers to the over activity of thyroid glands to produce too much thyroxin hormone.
* **Urinary tract infection (UTI):** Urinary tract infection (UTI) refers to the infection of urinary system including kidney, ureter, bladder and urethra. Most of the infections involve the lower urinary tract-bladder and the urethra. And women are at greater risk of developing a UTI than men.
* **Hypertension:** Hypertension refers to the high or raised blood pressure in which the blood vessels have persistently raised pressure. The normal range of systolic and diastolic blood pressure is (80-120 mm Hg)(60-80mmHg)
* **Anaemia:** Anaemia is a disease in which the total amount of red blood cell or haemoglobin in the blood or a lowered ability of the blood to carry oxygen.
* **Oedema:**Oedema is condition of swelling caused by excess fluid trapped our body tissues.
* **Tuberculosis (TB):** Tuberculosis (TB) is caused by infection with *Mycobacterium tuberculosis* (MTB), which is part of a complex of organisms including *M. bouis* (reservoir cattle) and *M. africanum* (reservoir human).
* **Asthma:** Asthma is a chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night and in the early morning.

**2.1.24 Hematological parameters**: Hematological parameters, including red and white blood cell counts and hemoglobin concentration, are widely used clinical indicators of health and disease. These traits are tightly regulated in healthy individuals and are under genetic control.

**2.1.25 Micronutrient deficiencies in pregnancy**

**Calcium:** Calcium supplementation in pregnancy has the potential to reduce adverse gestational outcomes, in particular by hypertensive disorders during pregnancy, which are associated with a significant number of maternal deaths and considerable risk of preterm birth, the leading cause of early neonatal and infant mortality.

Most effective dose for calcium supplementation during pregnancy is 2g/day. Low calcium intakes during pregnancy may stimulate PTH secretion, increasing intracellular calcium and smooth muscle contractibility and release renin from the kidney, leading of sodium and fluid. These physiological changes can lead to development of preeclampsia(Robert,2001).

WHO recommends an intake of 1.5-2g elemental calcium/day with the total daily dosage divided into three doses from 20weeks gestation until the end of pregnancy. Target group includes all pregnant women; particularly those at higher risk of gestational HTN and in are with low calcium intake. Calcium deficiency has been associated with preeclampsia, preterm birth, post-partum hemorrhage, short-term bone changes, and osteoporosis.

**Magnesium:** Mg and calcium work in combination. Mg relaxes muscle while ca+ stimulate muscles to contract. Research suggests that getting adequate Mg during pregnancy can help prevent the uterus from contracting prematurely. Mg also helps strong teeth and bones in our baby. Pregnant women ages 19-30:350mg/day.31and older-360/day. Mg is an essential mineral required to regulate body temperature, nucleic acid and protein synthesis with an important role in maintaining nerve and muscle cell electrical potentials. It may reduce fetal growth restriction and preeclampsia as well as increase birth weight. Total and ionized Mg is inversely associated with gestational age in pregnancy. Mg deficiency has been associated with a higher risk of chronic HTN, preeclampsia, placental dysfunction, gestational diabetes, IUGR, leg cramps.

**Phosphorus:** Phosphorus is a mineral that helps build strong bones in our and our developing baby. About 85% of all the phosphorus in our body is found in our bones. This mineral is also important for muscle contractions, blood clotting, kidney function, nerve conduction, tissue and cell repair and normal heart rhythm. Women age19 and older: 700mg/day. Women age 18 and younger: 1250mg/day.

**Iron:** Iron is essential for the production of haemoglobin, which functions in the delivery of oxygen from lungs to the tissues of the body and to the synthesis of iron enzymes which are required to utilize oxygen for the production of cellular enzyme (Bothwell et al, 1979).It appears that severe anemia in the Bangladeshi population is less frequent, possibly present among only 2-3% of the population. The data on the etiology of anemia reveal that iron deficiency may be a substantial cause of anemia in the Bangladeshi population. At least 27mg of iron is requiring in pregnancy.

**2.1.26 Macronutrients in Pregnancy**

**Energy:** Energy intake is the main determinant of gestational weight gain. During pregnancy, the maternal diet must provide an adequate supply of energy to support the mother’s usual requirements as well as those of the growing fetus. Extra energy is required for the synthesis of new tissue (fetus, placenta and amniotic fluid) and the growth of existing tissue (uterus, breast and maternal adipose tissue). Energy requirements during the first trimester generally do not differ from those of non-pregnant women but increase between 10 and 30 weeks of gestation when the growth of maternal and foetal tissue is greatest. However, energy needs of individual women vary widely during pregnancy, depending on their physical activity levels, pre-pregnancy body mass index (BMI) and metabolic rate, hence, recommendations for energy intake should be tailored accordingly.

Global estimates suggest that energy intake during pregnancy ranges from 7710 to 9260 kJ/day, with higher intakes reported in the Americas and the Eastern Mediterranean compared with Africa, Southeast Asia and the Western Pacific. Appropriate maternal energy intake is important to prevent poor pregnancy outcomes associated with both insufficient and excessive gestational weight gain, although there is very limited evidence from intervention trials examining the effects of energy restriction during pregnancy. A meta-analysis of three trials (n = 384) reported that in women who were overweight or who exhibited excessive gestational weight gain, energy restriction during pregnancy reduced maternal weight gain but had no effect on pregnancy-induced hypertension or preeclampsia (gestational hypertension with proteinuria). However, two of the three trials also reported decreases in neonatal birth weight, suggesting that energy restriction may have adverse effects on birth weight. While preventing maternal obesity is important for reducing the risk of macrosomic infants, obstetric complications and birth trauma, the potential weight loss/retention benefits of energy restriction must be weighed against possible harms including foetal growth restriction. Given the absence of sufficient evidence, energy restriction is currently not advised during pregnancy and any recommendations for energy intake should be individualized based on pre-pregnancy BMI and gestational weight gain targets. (Aya .Mousa et al,2019)

**Table-4: Daily requirements of nutrients for pregnant women**

|  |  |
| --- | --- |
| **Nutrient** | **Daily requirements for pregnant women** |
| Calcium | 1200 milligrams (mg) |
| Folate | 600–800 micrograms (mcg) |
| Iron | 27 mg |
| Protein | 70–100 grams (g) per day, increasing each trimester |

Source: Nutritional Needs During Pregnancy, Catherine Clark - 2020

**Protein:** Protein is involved in both structural (keratin, collagen) and functional (enzymes, protein transport, hormones) biological roles. Globally, the primary sources of protein are plant-based foods such as legumes, grains and nuts (57% of daily intake) followed by animal-based foods such as meat (18%) and dairy (10%), although small amounts may also be derived from alternative sources such as algae, bacteria and fungi (mycoproteins). Protein quality is determined by its digestibility and capacity to meet the nitrogen and indispensable amino acid requirements necessary for growth, repair and maintenance. Animal protein sources are considered “complete proteins” because they provide all nine indispensable amino acids, while plant sources are “incomplete proteins” since they can be deficient in one or more indispensable proteins such as lysine or threonine. Pregnant women in developed countries report consuming 14.7% to 16.1% of total energy from protein, which is adequate based on current recommendations. Adjustments in protein metabolism occur within several weeks of conception in order to maintain maternal homeostasis while accommodating increased foetal demands and preparing for lactation. Whole-body protein turnover studies suggest that protein turnover in early pregnancy is similar in pregnant and non-pregnant women but a 15% and 25% absolute increase in protein synthesis occurs during the second and third trimesters, respectively. Concomitant decreases in maternal amino acid concentrations, urea synthesis and urinary urea excretion occur early in gestation and remain low throughout pregnancy. In well-nourished individuals, these physiological changes conserve protein and nitrogen and promote protein accretion to ensure adequate nutrient supply to the foetus. Consequently, it is a period when both are very susceptible to alterations in dietary supply, especially of nutrients which are marginal under normal circumstances. Inappropriate nutrition leads not only to an increased risk of death in utero, but also to alterations in birth weight and functional changes in the neonatal organs. These changes can have far-reaching consequences. For example, babies who are small at birth are at an increased risk of cardiovascular disease and diabetes as adults, while animals who are born to zinc deficient mothers have a compromised immune system. The underlying mechanisms relate to nutrition effects on gene expression in the fetus. (Aya. Mousa, et al2019)

**2.1.27 Inflammatory Biomarker:**

**CRP:** C-reactive protein is a marker of systemic inflammation. Serum concentrations of CRP in pregnancy are elevated above non-pregnant values with the difference being detected as early as 4 weeks gestation. Higher concentration of CRP in 1st trimester has been associated with preterm delivery, presence of intrauterine infection, preeclampsia, IUGR, intrauterine growth retardation.([Bayar,2014](https://www.ncbi.nlm.nih.gov/pubmed/?term=Najat%20Nakishbandy%20BM%5BAuthor%5D&cauthor=true&cauthor_uid=25332756)).

**Chapter-3: Materials and Methods**

**3.1 Study area and design**

Chittagong is the second largest City and commercial capital of Bangladesh. It is situated on the bank of the Karnaphuli. The population of this City is almost 6 million (Fig 1). A cross-sectional study was conducted on assessment of nutritional status of pregnant women between November 2019 and December 2020. For this purpose, The Father Boudreaus Medical Center, Pathorghata, Kotoali, and Chattagram Maa-O-Shishu Hospital and Medical College (CMOSH), Agrabad, Chattagogram were selected as study sites. The author is a medical officer in Father Boudreaus Medical Center. And a written permission was taken from the CMOSH to conduct the study at the outdoor at CMOSH. Data were collected using a pre-structured questionnaire consisting information on socioeconomic status, demographic information, life style behavior, physical and biological parameters and laboratory investigations. The patient registered herself in the registration desk and visits the referred doctor, where the author was on duty. A total of 140 patients were selected during the visit of pregnant women, who were identified by a history of a missed menstrual cycle. Upon confirmation of urine pregnancy test and ultra-sonography (USG) women were invited to visit the mother and child health institute, a tertiary hospital for ante natal care. The woman who has a history of Type 2 diabetes or history of gestational diabetes mellitus or pregnancy induced hypertension was excluded from this study.

**3.2 Socio demographic and life style behavior**

The author recorded demo-graphic information, including age, sex, and region. In addition, data on life style behavior, including smoking, alcohol consumption, and exercise, was recorded on a pre-structured questionnaire. Clinical Measurements of height and weight were per-formed with the participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Waist circumference (WC) was measured at the mid-point between the lower margin of the last palpable rib and the top of the iliac crest at the end of a normal expiration with the arms relaxed at the sides using a measuring tape. Blood samples were collected from each participant and were processed, immediately refrigerated, and transported in cold storage to the Post Graduate Research Laboratory, Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram. Analysis of all blood samples was performed within 24 h after collection in The Father Boudreaus Medical Center, Pathorghata, Kotoali, Chattogram. The author recorded the information of patient on socio-demographic, including age, religion, and education, profession, and lifestyle behavior and family type. Then anthropometric measurement was performed following standard protocol.

**3.3. Maternal data collection:**

Data were collected a pre-structured questionnaire (Annex) followed by physical examination and laboratory investigations. The questionnaire consist of following information on age, place of residence, education, type of occupation, history of chronic diseases including T2DM, HTN, history of participants’ past illness or any chronic disease, history of addiction, physical activity level, nutritional status, and pregnancy-related history from the mother at the baseline visit. Maternal anthropometry including height, weight, waist and mid upper arm circumference (MUAC) were measured (using standardized measurement protocols) at the time of registration. BMI was calculated as weight divided by height squared (kg/m2). Waist circumference was measured by placing a plastic tape at the midpoint between the lower rib margin and the iliac crest. Similarly, hip was measured by taking the extreme end posteriorly and the symphysis pubis anteriorly. In addition, blood pressure was measured in right hand in sitting position. Prior to the measurement, 10-min rest was assured and using standard cuffs fitted with sphygmomanometer minimized variation in measurement.

MUAC is a measure to assess nutritional status. It is measured on a straight left arm, mid-way between the tip of the shoulder and the tip of the elbow. It identifies acute malnutrition and is commonly used in children 6-59 months of age as well as pregnant women. MUAC less than 115 mm indicates severe wasting or severe acute malnutrition (SAM). MUAC greater than or equal to 115 mm and less than 125 mm indicates moderate wasting or moderate acute malnutrition (MAM) (Global Health Learning Center, USUAID).

According to SPHERE Guidelines mid upper arm circumference (MUAC) may be used as a screening tool for pregnancy, e.g. a criterion for entry into a feeding program. The guidelines state that cut-off points for risk vary by country and range from 21-23cm.

**3.4 Maternal blood sample collection and storage:**

Blood sample were collected from median cubital vein with the help of Butterfly needle or direct puncture the vein a sterile syringe put in the tube having anticoagulant (Na2-EDTA) in one tube and without anticoagulant in another tube and collected the serum after centrifugation at 3000 rpm for 10 minutes; stored in ice box and brought to post graduate Research laboratory of Department of Physiology, Biochemistry, Pharmacology, Chittagong Veterinary and Animal Science University within 2-3 hours of collection. The complete blood count was assessed in The Father Boudreaus Medical Center, Pathorghata, Kotoali, using automated blood counting machine. The complete blood counts include erythrocyte sedimentation rate, paced cell volume, haemoglobin, total red blood cell counts, total white blood cell counts, differentiate leukocyte counts (Neutrophil, eosinophil, lymphocyte, monocyte and basophil) and MCV, MCH, MCHC.

**3.5 Analysis of serum samples**

The concentrations of minerals (calcium, magnesium, phosphorus, iron and C-reactive protein) were analyzed by an automatic biochemical analyzer (Humalyzer 3000®, USA) using commercially available kits. Randox kits (Randox Laboratories Limited, London, UK) were used for determination of Ca; Chroma test kits (Bio Analyt, Rheinstraße 17, Teltow, Germany) for P, and Mg; Biorex kits (Linear Chemicals, S.L. Mongat, Barcelona, Spain).

**3.6 Statistical analysis**

Collected data were recorded into MS Excell-2010, sorted out and entered graph into the Statistical Package for Social Science vertion-20 (SPSSinc. Chicago, IL). Result are presented as percentage, mean± SD, unless started otherwise. Students t-test, linear correlation and multiple regressions were performed and p wave differences of <0.05 were considered as statistically significant.

**Chapter-4: Results**

**Table 5: Socio-demographic information of pregnant women (N-140)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Category** | **Frequency (n)** | **Percent (%)** |
| Religion | Muslim | 93 | 66.4 |
| Hindu | 43 | 30.7 |
| Christian | 3 | 2.1 |
| Buddhist | 1 | .7 |
| Education | Illiterate | 7 | 5.0 |
| Primary | 28 | 20.0 |
| Secondary | 70 | 50.0 |
| Graduation | 34 | 24.3 |
| Family type | Joint | 74 | 52.9 |
| Nuclear | 66 | 47.1 |
| Residence Type | Urban | 117 | 83.6 |
| Rural | 19 | 13.6 |
| Slum | 4 | 2.8 |
| No. of Family member | 140 | 2-10 | 4.77±1.49 |

The table 5 shows the sociodemographic information of the participants. As expected, among the total participants, the Islam found the highest (66%) iterate while the Buddhist found the lowest (0.7%). Fifty percent of the participants were secondary educated followed by graduation (24%), primary (20%) and rest of the participants were illiterate. The proportion of joint family was found higher than nuclear family. Most of the participants were lived in urban area (~84%) and the lowest participant was found in slum area (3%). The number of family ranged from 2 to 10 with an average 4.77.

**Table 6: Life style behavior of pregnant mother (N=140)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  |  |  | | --- | --- | --- | --- | | Variable | Category | Frequency(n) | Percent (%) | | Exposure to sun light | Yes | 47 | 33.6 | | No | 92 | 65.7 | | Exercise | Yes | 1 | .7 | | No | 139 | 99.3 | | Eat additional food during pregnancy | Yes | 63 | 45.0 | | No | 77 | 55.0 | | Avoid eating of food during pregnancy | Yes | 16 | 11.4 | | No | 99 | 70.7 | | Number of meals/day | 3 | 118 | 84.3 | | 4 | 10 | 7.1 | | 5 | 6 | 4.3 | | 6-10 | 5 | 3.5 | | Sleeping time(Hours) | 140 | 1-12 | 7.46±1.88 | | Resting Time(Hours) | 139 | .50-10 | 2.14±1.93 | |

Life style behavior of pregnant mother is depicted in table 6. Two third of the participants did not get exposure of sun light and almost 100% participants did not do any exercise. More than 50% participants were not used to take additional food and almost 70% participants had have tendency to avoid food. Nearly 85% participants take only three times meal/day while 3.5% participants took 6-10 times meal in a day. On an average sleeping time was nearly seven and half an hour and average resting time was 2.14 hours.

**Table 7: Maternal health status (N-140)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Category** | **Frequency (n)** | **Percent (%)** |
| Practice exclusive breast feeding | Yes | 76 | 54.3 |
| No | 63 | 45.0 |
| Age of breast feeding child (months) |  | 6-24 | 19.77±6.56 |
| Number of anti-natal care visit | 1 | 28 | 20.0 |
| 2 | 18 | 12.9 |
| 3 | 42 | 30.0 |
| 4 | 25 | 17.9 |
| 5 | 15 | 10.7 |
| 6 | 10 | 7.1 |
| 7 | 2 | 1.4 |
| Status of pregnancy During Lactation | Yes | 36 | 25.7 |
| No | 104 | 74.3 |
| Parity | 1 | 37 | 26.4 |
| 2 | 61 | 43.6 |
| 3 | 32 | 22.9 |
| 4 | 10 | 7.1 |
| History of miscarriage | Yes | 2 | 1.4 |
| No | 138 | 98.6 |
| Trimester | First | 31 | 22.1 |
| Second | 40 | 28.6 |
| Third | 69 | 49.3 |
| Blood Group | O+ | 61 | 43.6 |
| B+ | 22 | 15.7 |
| A+ | 22 | 15.7 |
| AB+ | 17 | 12.1 |
| B- | 4 | 2.9 |
| O- | 3 | 2.1 |
| A- | 1 | .7 |
| AB- | 1 | .7 |
| Anemia | Yes | 7 | 5.0 |
| No | 133 | 95.0 |
| Oedema | Yes | 11 | 7.9 |
| No | 129 | 92.1 |
| History of Comorbiditis | DM | 12 | 8.6 |
| UTI | 8 | 5.7 |
| HTN | 3 | 2.1 |
| TB | 1 | .7 |
| Asthma | 2 | 1.4 |
| Hyperthyroidism | 3 | 2.1 |

Health status of the pregnant mother is shown in table 7. Almost 55% mother had practiced exclusive breast feeding. And the age of breast feeding child ranged between 6-24 months. One third of the total participants were visited the doctor three times for anti-natal care. Three fourth percent of pregnant mother were in lactation stage. The highest percentage of the pregnant mother (44%) was found in the in the second parity while the lowest percentage of the pregnant mother was found at 4th parity. There is history of miscarriage (1.4%) of pregnant mother. Among the participants almost 50% were found at third trimester. “O” positive group of blood was found the highest percentage (~44%) while the lowest percentage were A- and AB-.

**Table 8: Anthropometric characteristic of women (N-140)**

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Minimum | Maximum | Mean ± SD |
| Age (Years) | 16 | 38 | 26.33±5.34 |
| BW(Kg) | 36 | 90 | 60.24±10.25 |
| H(Feet) | 4 | 6 | 4.98±.27 |
| WC(cm) | 80 | 140 | 1.09332±11.10 |
| BMI(Kg/m2) | 17 | 39 | 25.91±4.53 |
| MUAC(cm) | 18 | 38 | 25.7±4.03 |
| SBP(mm of Hg) | 80 | 170 | 113.5±13.08 |
| DBP(mm of Hg) | 60 | 100 | 75.07±8 |
| PR(beat/minute) | 60 | 100 | 77.04±9 |
| Age of Spouse (Years) | 22 | 50 | 34.60±7.22 |

**Note:** BW, Body weight’ H, Height; WC, Waist circumference; BMI, Body mass index, MUAC, Mid upper arm circumference; SBP, Systolic blood pressure, DBP, Diastolic blood pressure; PR, Pulse Rate.

Anthropometric parameters of pregnant mothers are shown in table 8. The average age of the pregnant mother was almost 27 years with a range of 16-38 years. The average BMI was nearly 26 with a range between 17 and 39. And the average MUAC was found 25.7. The Systolic and diastolic blood pressure was ranged between 80 to 170 and 60-100, respectively.

**Table 9: Overall hematological and trace elements of pregnant mother (N=33)**

| Variable |  | Min-Max | Mean ± SD | Normal Range |
| --- | --- | --- | --- | --- |
| Hematological parameter | Hb(gm/dl)  ESR(mm/hr) | 8.40-12.70  8-96 | 10.25±1.16  36.36±21.18 | 11.5-16.5 gm/dl |
| 0-15mm/hr |
| Total WBC count(cmm) | 4500-12500 | 8984.8484 | 4000-11000/ cmm |
| Platelet count (cmm) | 180000-350000 | 277272.73 | 150000-450,000/cmm |
| RBC(million/cmm) | 3.42-5.27 | 4.48±.46 | 4.5-6.5 million/cmm |
| PCV (%) | 29-55.50 | 41.65±5.92 | 37-54% |
| MCV (fl) | 67.40-104.90 | 92.57±8.42 | 78-98fl |
| MCH (pg) | 26.90-28.70 | 27.63±.53 | 27-32pg |
| MCHC (dl) | 30.10-50.00 | 32.04±3.28 | 32-36g/dl |
| Neutrophil (%) | 44-78 | 59.73±9.41 | 40-75% |
| Lymphocytes (%) | 4-50 | 32.85±10.30 | 20-45% |
| Monocyte (%) | 1-5 | 3.45±.90 | 01-6% |
| Eosinophil (%) | .00-9 | 2.24±1.56 | 0-2% |
| Trace elements | Calcium (mg/dl) | 8.30-11.10 | 9.85±.64 | 8.6-10.3mg/dl |
| Magnesium (mg/dl) | 1-3.10 | 2.20±.46 | 1.7-2.2mg/dl |
| Phosphorus (mg/dl) | 1.70-3.30 | 2.38±.32 | 2.8-4.5mg/dl |
| Iron (mg/dl) | 32.30-220.50 | 96.49±45.95 | 55.8-26.5mg/dl |
| Inflammatory biomarker | C-reactive protein (mg/L) | .40-107.30 | 9.1±19.25 | <5mg/L |

Note: Hb, haemoglobin; ESR, Erythrocyte sedimentation rate; WBC, White blood cell count; RBC, Red blood cell count; PCV, Packed cell volume; MCV, Mean corpuscular volume, MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration;

Hematological, trace elements and inflammatory biomarkers of pregnant mother is shown in table 9. Among the complete blood counts, the total WBC, RBC and platelet counts were ranged between 4500-12500, 3.42-5.27, and 180000-350000, respectively. In case of trace elements, the level of calcium, magnesium, phosphorus and iron was ranged between 8.3-11.10, 1-3.1, 1.7-3.3, and 32.3-220.5, consecutively. And the C-reactive protein was ranged between 0.40-107.3.

**Table 10: Baseline characteristics according to sun light exposure (N=47)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes (n=47) | No(n=92) |  |
| **Variable** | **Mean ± SD** | **Mean ± SD** | **P-value** |
| Sleeping Time | 8.19±2.12 | 7.11±1.63 | **.001** |
| Resting Time | 2.72±2.41 | 1.82±1.57 | **.009** |
| Family size | 4.76±1.22 | 4.43±.97 | .079 |
| Age of breast feeding child | 19.19±7.05 | 20.21±6.26 | .455 |
| BW (kg) | 60.47±10.04 | 20.21±6.26 | .828 |
| Height (Feet) | 5.03±.33 | 20.21±6.26 | .081 |
| WC (cm) | 1.0919 | 1.09369 | .929 |
| BMI (kg/m2) | 25.73±3.97 | 25.89±4.72 | .841 |
| SBP (mm of Hg) | 116.06 | 112.119 | .094 |
| DBP (mm of Hg) | 76.81±6.63 | 74.24±8.55 | .074 |
| Pulse rate (beat/min) | 78.02±9.53 | 76.50±8.78 | .349 |
| MUAC (cm) | 25.28±3.87 | 25.91±4.13 | .382 |

Note: BW, Body weight’ WC, Waist circumference; BMI, Body mass index, SBP, Systolic blood pressure, DBP, Diastolic blood pressure; MUAC, Mid upper arm circumference. P value <0.05 indicates level of significance.

According to exposure to sun light the baseline characteristics of the participants is shown in table 10. The people participants who got sun light exposure sleeping time (p=.001), and resting time (p=.009) found significantly higher as compared to those, who did not exposure to sun light.

**Table 11: Baseline characteristics of pregnant women between first and third trimester of visit (T-Test) (N=30)**

| **Variables** | **First trimester (n-10)** | **Third trimester (n=20)** |  |
| --- | --- | --- | --- |
| **Mean ± SD** | **Mean ± SD** | **p-value** |
| Age (Year) | 29.6±5.69 | 29.35±4.85 | .606 |
| Sleeping time (hour) | 7.20±1.03 | 7.50±.89 | .887 |
| BW(kg) | 62.10±11.15 | 63.65±10.68 | .887 |
| WC (cm) | 110.645±12.72 | 109.23±10.79 | .804 |
| BMI (kg/m2) | 25.20±4.39 | 26.35±4.74 | .824 |
| SBP (mm of Hg) | 112.419±11.609 | 115.65±14.32 | .768 |
| DBP (mm of Hg) | 73.0±9.19 | 74.0±7.71 | .701 |
| PR (b/min) | 81.5±11.08 | 70.95±7.05 | **.012** |
| MUAC (cm) | 25.70±4.90 | 26.65±4.88 | .875 |
| Cal (mg/dl) | 9.75±.83 | 9.89±.58 | .218 |
| Mg (mg/dl) | 2.13±.67 | 2.25±.35 | **.034** |
| P (mg/dl) | 2.44±.22 | 2.37±.36 | .133 |
| Fe (mg/dl) | 90.50±48.06 | 104.88±45.67 | .949 |
| CRP (mg/L) | 18.29±32.44 | 5.41±7.79 | **.012** |
| Hb (mg/dl) | 10.66±0.887 | 10.2402±1.35 | .440 |
| ESR(mm/hr) | 44.50±27.62 | 29.60±15.85 | .123 |
| Platelet (cmm) | 269500±48100.01 | 274250±4243493 | .712 |
| RBC(million/cmm) | 4.32±.53 | 4.62±.41 | .342 |
| PCV (%) | 40.77±7.72 | 42.17±5.46 | .207 |
| MCV (fl) | 93.73±8.91 | 90.96±8.38 | .807 |
| MCH (pg) | 27.44±.39 | 27.71±.57 | .076 |
| MCHC (g/dl) | 31.20±.47 | 32.54±4.16 | .236 |
| Neutrophils (%) | 63.50±8.69 | 57.55±9.67 | .602 |
| Lymphocytes (%) | 31.0±8.11 | 33.75±11.74 | .267 |
| Monocyte (%) | 3.20±1.03 | 33.75±11.74 | .463 |
| Eosinophil (%) | 2.30±.48 | 2.30±1.98 | .057 |

**Note:** BW, Body weight’ WC, Waist circumference; BMI, Body mass index, SBP, Systolic blood pressure, DBP, Diastolic blood pressure; MUAC, Mid upper arm circumference’ Cal, Calcium, Mg, Magnesium, P, Phosphorus, Fe, iron; Hb, Haemoglobin, ESR, Erythrocyte sedimentation rate; RBC, red blood cell count, PCV, Packed cell volume, MCV, Mean corpuscular volume, MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration. P value <0.05 indicates level of significance

Table 11 shows the baseline characteristics between first and third trimester of the pregnant mother. The level of magnesium found lower in first trimester as compared to third trimester (p<.05), the pulse rate found higher in first trimester than third trimester (p<.05) and CRP level found higher in first trimester than third trimester (p<0.05).

**Table 12: Linear Correlation analyses between the indices of anthropometric, health status, trace elements and inflammatory biomarkers**

|  | **Age** | **RT** | **WC** | **BMI** | **SBP** | **DBP** | **PR** | **MUAC** | **Cal** | **Mg** | **P** | **Fe** | **CRP** | **ESR** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Age |  | .001 | .105 | .133 | .247\*\* | .109 | -.171\* | .215\* | .160 | -.028 | .101 | -.102 | -.050 | .183 |
| ST | -.042 | -.054 | -.091 | -.130 | .152 | .158 | -.079 | .050 | -.254 | .280 | .050 | .041 | .024 | -.360\* |
| RT | .001 | 1 | .067 | -.109 | -.162 | -.123 | .066 | -.163 | -.217 | -.291 | -.210 | -.020 | .782\*\* | .461\*\* |
| WC | .105 | .067 | 1 | .237\*\* | *.111* | .072 | -.247\*\* | .318\*\* | .257 | -.070 | .199 | .100 | .026 | -.057 |
| BMI | .133 | -.109 | .237\*\* | 1 | .062 | .221\*\* | -.156 | .552\*\* | .216 | .080 | -.237 | .117 | .013 | .374\* |
| SBP | .247\*\* | -.162 | .111 | .062 | 1 | .508\*\* | .001 | .193\* | -.097 | .028 | -.066 | .039 | -.094 | -.013 |
| DBP | .109 | -.123 | .072 | .221\*\* | .508\*\* | 1 | .034 | .327\*\* | .300 | -.040 | .017 | .318 | .231 | .334 |
| PR | -.171\* | .066 | -.247\*\* | -.156 | .001 | .034 | 1 | -.085 | -.288 | -.298 | .109 | -.035 | .358\* | .367\* |
| MUAC | .215\* | -.163 | .318\*\* | .552\*\* | .193\* | .327\*\* | -.085 | 1 | .190 | .192 | -.063 | .263 | -.053 | .282 |
| Cal | .160 | -.217 | .257 | .216 | -.097 | .300 | -.288 | .190 | 1 | .334 | -.187 | .416\* | -.268 | .127 |
| Mg | -.028 | -.291 | -.070 | .080 | .028 | -.040 | -.298 | .192 | .334 | 1 | -.223 | .030 | -.549\*\* | -.208 |
| P | .101 | -.210 | .199 | -.237 | -.066 | .017 | .109 | -.063 | -.187 | -.223 | 1 | -.184 | .084 | -.107 |
| Fe | -.102 | -.020 | .100 | .117 | .039 | .318 | -.035 | .263 | .416\* | .030 | -.184 | 1 | -.066 | -.001 |
| CRP | -.050 | .782\*\* | .026 | .013 | -.094 | .231 | .358\* | -.053 | -.268 | -.549\*\* | .084 | -.066 | 1 | .355\* |
| Trimester | .044 | -.093 | -.038 | -.064 | .125 | .098 | -.021 | .033 | .104 | .121 | -.080 | .169 | -.293 | -.348\* |

Note: ST, Sleeping time; BW, Body weight’ WC, Waist circumference; BMI, Body mass index, SBP, Systolic blood pressure, DBP, Diastolic blood pressure; MUAC, Mid upper arm circumference’ Cal, Calcium, Mg, Magnesium, P, Phosphorus, Fe, iron; Hb, Haemoglobin, ESR, Erythrocyte sedimentation rate; RBC, red blood cell count, PCV, Packed cell volume, MCV, Mean corpuscular volume, MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration. P value <0.05 indicates level of significance

The table 12 depicted the correlations between the different indices of anthropometric, health status, trace elements, and inflammatory biomarkers. Systolic blood pressure and MUAC highly positively correlated with age and negatively correlated with pulse rate respectively(-.085). The BMI is significantly positively correlated with diastolic blood pressure, waist circumference and MUAC. Waist circumference highly significantly correlated with age, waist circumference, BMI, SBP and DBP. The level of calcium significantly highly positively correlated with iron. Magnesium significantly negatively correlated with the C-reactive protein.

**Table 13: Regression analysis if the correlation of C-reactive protein with magnesium and resting time in pregnant women**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **C-Reactive protein** | | | | | |
|  |  | **Standardized Coefficients** | **95% Confidence Interval** | |  |
| **Beta** | **Lower Bound** | **Upper Bound** | ***p-value*** |
| **Resting Time** | | | | | |
| Model 1 | RT | .800 | 7.457 | 13.782 | .000 |
| Model 2 | RT | .692 | 6.324 | 12.032 | .000 |
|  | Mg | -.350 | -23.665 | -5.803 | .002 |
| Model 3 | RT | .688 | 6.076 | 12.174 | .000 |
|  | Mg | -.347 | -23.941 | -5.299 | .003 |
| **Magnesium** |  |  |  |  |  |
| Model 1 | Mg | -.559 | -36.848 | -10.209 | .001 |
| Model 2 | Mg | -.494 | -34.608 | -7.025 | .004 |
| Model 3 | RT | .688 | 6.076 | 12.174 | .000 |
|  | Mg | -.347 | -23.941 | -5.299 | .003 |

For resting time; Model-1 Age, resting time, BMI; Model-2 adjusted for Age, resting time, BMI and Magnesium Model-3 adjusted for Age, resting time, BMI, pulse rate and Magnesium.

For serum magnesium; Model-1 Age, BMI and magnesium; Model-2 adjusted for Age, pulse rate, BMI and Magnesium; Model-3 adjusted for age, resting time, BMI, pulse rate and Magnesium.

Magnesium is negatively related with CRP (B-0.347, 95%CI-23.9,p=.003) and RT is positively related with CRP(B-0.688,95%CI6.076,p=.000)

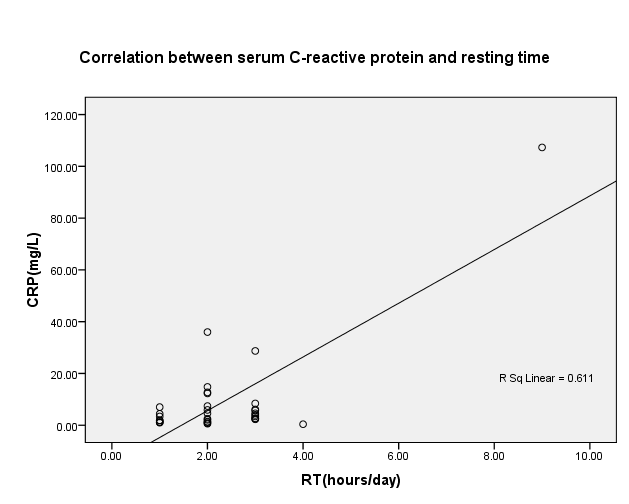


Figure 1: Correlation between serum C- reactive protein and resting time (hours/day) in pregnant women.

CRP is positively correlated with resting time.

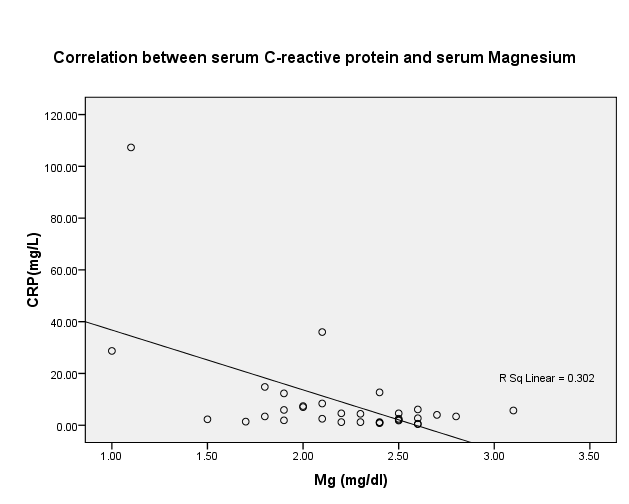


Figure 2: Correlation between C-reactive protein and serum magnesium level in pregnant women.

Magnesium significantly negatively correlated with the CRP.

**Chapter-5: Discussion**

Pregnancy is a period of physiological changes, which is more dynamic, that necessitates to assess the lifestyle behavior and health status of pregnant women to save the life of both mother and incumbent child. During this period micronutrients play essential roles along with other macronutrients. The principle finding of the present study is that inflammatory biomarkers C-reactive protein is proportionately associated with resting time of pregnant women and inversely associated with serum magnesium levels, which has impacts on maternal and incumbent child health.

Elevated serum CRP level during pregnancy is associated with increased risks of fetal growth restriction and neonatal complications, such as preterm birth, low birth weight, and small size for gestational age (Grgic, 2010; Pitiphat, 2005; Tjoa, 2003). Dysfunction of endothelium from low grade inflammation leading to vascular malfunction and suboptimal placental development. Maternal systemic inflammation might also be a response to ischemia of the placenta due to sub optimal placental development (Lam, 2005; Redman, 2004), which might predispose mothers to increased risks for various pregnancy complications (Redman, 2009; Roberts, 2010).

As expected, among the total participants, the Muslims found the highest (66%) followed by the Hindu (30.7%), the Christian (2.1%) and the Buddhist (0.7%), which is found dissimilar with the population census that showed the Muslim comes in with 89.1% of the population, Hindu with 10%, and other religions make up the remaining 0.9% (includes Buddhist, Christian) of the population (https://worldpopulationreview.com/countries/bangladesh-population) and this discrepancy might be due to location of the study place. Fifty percent of the participants were secondary educated followed by graduation (24%), primary (20%) and rest of the participants were illiterate, which is coincided with the earlier study with slight differences (Rahman et al., 2019) that indicate the education for all program in Bangladesh is going to be successful. However, the majority of participants were secondary school educated that could be due to the economic constraints, early marriage and social barriers. The proportion of joint family was found higher than nuclear family which indicates the social bonding and especiallyboth the elderly people and the child get social security from joint family. Most of the participants were from urban area that could be the study was conducted in the hospital located in urban area.

Life style behavior shows that two third of the participants did not get exposure of sun light and almost 100% participants did not do any exercise because of limited or lack of open space for walking and exercise. More than 50% participants were not used to take additional food that could be due to lack of knowledge on nutrition or financial crisis and almost 70% participants had tendency to avoid food that could be due to changes in appetite and feelings of nausea or morning sickness are well-known features of pregnancy. Sometimes women will have food cravings, and some women will experience a food aversion-a strong dislike of certain foods. (https://www.pregnancybirthbaby.org.au/appetite-changes-and-food-aversions-during-pregnancy).

In the current study nearly 55% pregnant mother had practiced exclusive breast feeding and the age of breast-feeding child ranged between 6-24 months, who needs special care to maintain the maternal and incumbent child health. One third of the total participants were visited the doctor three times for ante-natal care. Every pregnant woman needs to visit a doctor regularly. According to the Bangladesh Demographic and Health Survey (BDHS) 2007, 52% of mothers received at least one antenatal check-up, and 21% of mothers received at least one postnatal check-up from a trained care provider, which indicates that the maternal health care facilities have been grown up than the previous decades. The highest percentage of the pregnant mother (44%) was found in the in the second parity while the lowest percentage of the pregnant mother was found at 4th parity which does not corroborated with the earlier study who reported almost 59% of the participants were in first parity (Rahman et al., 2019). . There is history of miscarriage (1.4%) of pregnant mother which is quiet lower than the previous report who found that 7% pregnancies were spontaneous miscarriages and 3% were stillbirths (Gipson and Hindin, 2007).

Pregnancy health status is commonly assessed by the evaluation of haemoglobin percentage, which is an important biomarker that reflect the nutritional status pregnant women. The prevalence of anaemia in the current study population of the pregnant women is almost 5%, which does not corroborated with the previous study who reported that 49% of the pregnant mother suffers from anaemia (Ahmed, 2000). This discrepancy might be due to the study on small number of population and the study location in the City area, where usually the pregnant women easily get access to antenatal health care facilities.

The average age of the pregnant mother was almost 27 years with a range of 16-38 years, which is close agreement with the earlier study (Chowdhury, 2015). The average BMI was nearly 26 kg/m2, which indicates over weight reported by Goon (2013). Our findings reveal that majority of the pregnant women suffered from chronic energy deficiency as indicated by low weight even during pregnant and the average MUAC was found 25.7.Despite great progress over the last 20 years, poor nutrition is still hurting Bangladesh, its children and its future. In case of trace elements, the level of calcium, magnesium, phosphorus and iron was ranged between 8.3-11.10, 1-3.1, 1.7-3.3, and 32.3-220.5, consecutively. Calcium supplementation, balanced energy protein supplementation and iron folate supplementation offer substantial economic benefits relative to cost and have an opportunity to improve the health and nutrition of pregnant women.(Bangladesh Nutrition Priorities). The C-reactive protein was ranged between 0.40-107.3.In addition, a cross-sectional study also found an inverse association between dietary Mg intake and serum CRP levels. However, another recent cross-sectional study found no association between dietary Mg intake and CRP levels. Therefore, we summarized the quantitatively to estimate the overall association of Mg and CRP levels by conducting a meta-analysis as well as systematic review.

When analyzed the correlations between the different indices of anthropometric, health status, trace elements, and inflammatory biomarkers. Systolic blood pressure and MUAC highly positively correlated with age and negatively correlated with pulse rate. The BMI is significantly positively correlated with diastolic blood pressure, waist circumference and MUAC. Waist circumference highly significantly correlated with age, waist circumference, BMI, SBP and DBP. The level of calcium significantly highly positively correlated with iron. Magnesium significantly negatively correlated with the C-reactive protein.

**Chapter-6: Conclusions**

This study revealed a negative association of CRP with magnesium in pregnant women. Magnesium intake was inversely associated with plasma CRP concentration. This valuable data can help to establish the reference values of 14 serum trace elements and provide clear evidence that all of the selected elements are visibly altered when classified by age interval, residence, anthropometric status, and duration of pregnancy.

**Chapter-7: Strength & Limitation**

The strengths of the present study are we have collected the data own self by producing a pre-structured questionnaire from two hospitals, where the pregnant women comes for antenatal care. We assessed the socio-economic status, demographic information, general health characteristics of 140 participants but we could not do hematological and biochemical parameters with large samples size due to the limited funds. The Limitations of this study are inability to assess the status of all micronutrients, although this would have been cost prohibitive. Strengths include the small sample size, sample collected at narrowly defined time period including immediately after pregnancy ascertainment and the number of micronutrients assessed.

This study may contribute to limited evidence regarding maternal nutrition education strategies to improve birth weight of infants.

**Chapter-8: Recommendations**

A comprehensive study is warranted with large sample size, different study locations covering the whole country consisting of urban, slums and country sides. Socio-economic status, nutrient consumptions and life style behaviors pregnant women mothers, biochemical parameters those reflects the general health status and other trace elements are necessary to analysis with large sample size. Association between serum fat soluble vitamins such as vitamins D and exposure to sun light of pregnant women is highly appreciated in further study in the context of Bangladesh.

Furthermore, these findings shall be useful for future research to assess the trace element nutritional status and health risks of environmental maternal exposure, and to protect population in Bangladesh at greater risk.

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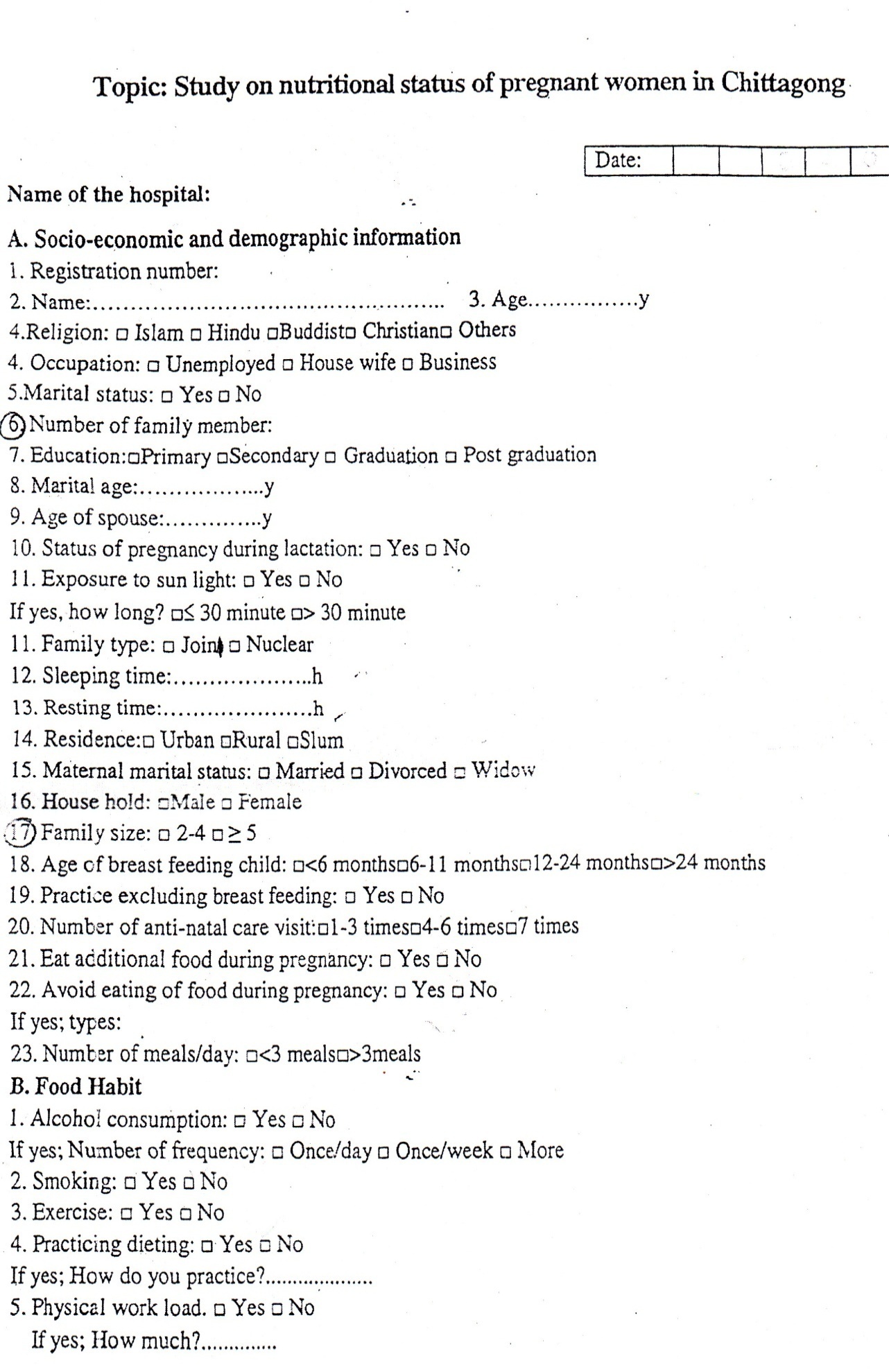
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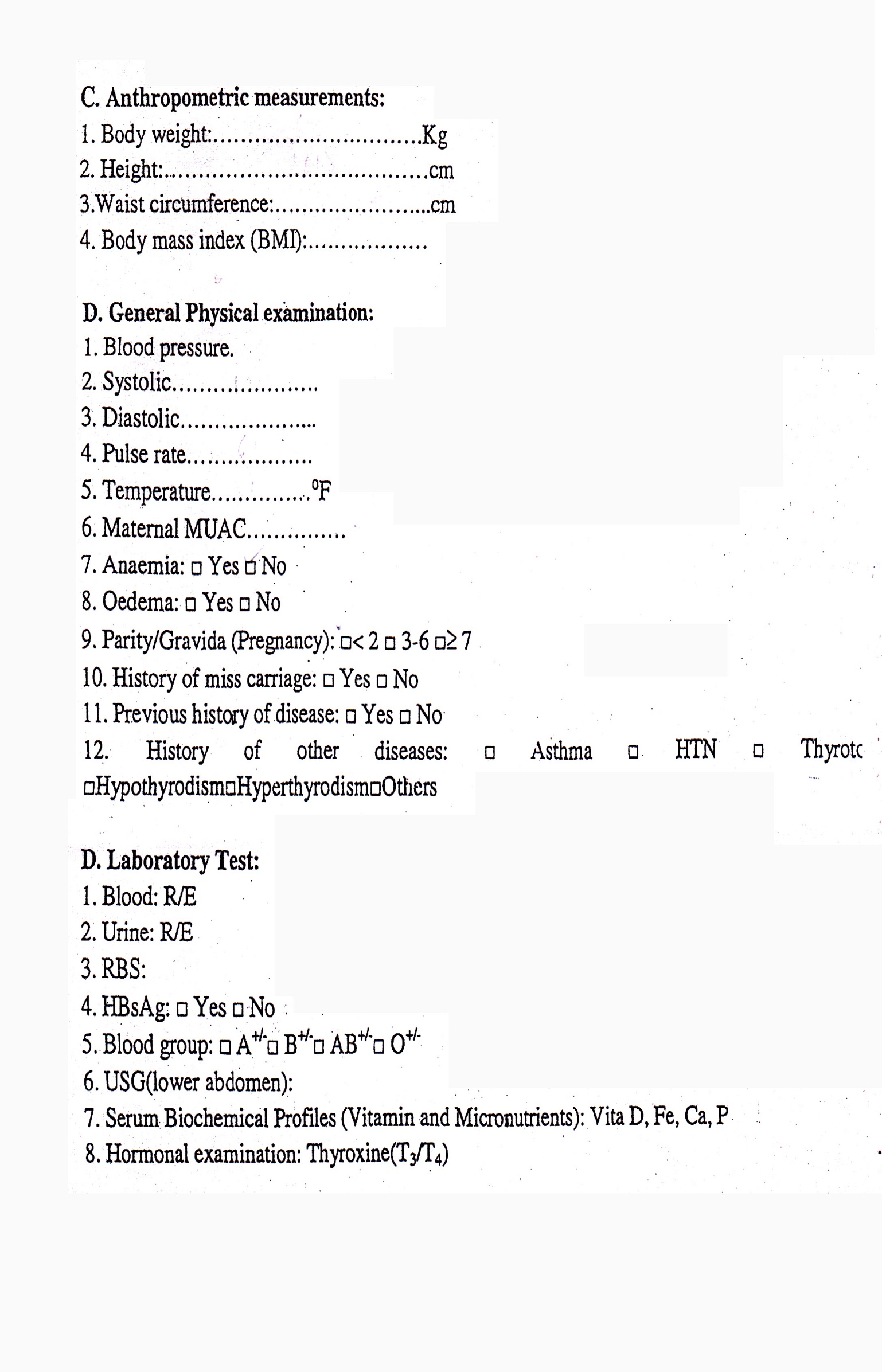
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**Annex**

**Questionnaire**

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**Study on socio-demographic, anthropometric, life style behavior and trace elements of pregnant women**

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