Histo-Morphological Study of Full term Placenta and Antioxidant Vitaminlevels of Selected Normotensive and Pre-eclamptic Women in Bangladesh.

A Thesis Submitted In Partial Fulfillment Of The Requirements Of The Degree Of Doctor Of Philosophy In Nutrition And Food Science.

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Dhaka, Bangladesh.

January, 2020.

Dedicated To My Family

CERTIFICATE

This is to certify that the thesis titled “Histo-Morphological Study of the Full term Placenta and Antioxidant Vitamin levels of Selected Normotensive and Pre-eclamptic Women in Bangladesh”Submitted by Dr.Nahid Ahmed Khan, Registration no:139, Session:2014-2015 for the degree of Doctor of Philosophy, University of Dhaka, is a record of original research work.Dr.Nahid Ahmed Khan has carried this research work under our joined supervision and guidance at the Institute of Nutrition and Food Science, University of Dhaka.The results or any part of work used in this thesis has not been submitted elsewhere for the award of any other degree.

This thesis is worthy for the award of the degree of Doctor of Philosophy in accordance with the rules and regulations of Dhaka University.

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I do hereby humbly declare that this Thesis entitled **"Histo-Morphological Study of Full term Placenta and Antioxidant Vitamin levels of Selected Normotensive and Pre-eclamptic Women in Bangladesh"** based on work carried by me. This was carried out in-

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ACKNOWLEDGEMENT

I would like to express my profound gratitude, from the core of my heart to Almighty Allah,the most beneficent and the most merciful for giving me the opportunity, capability, stamina and patience to carry out and complete this thesis work.

I am extremely grateful to my supervisor, Professor Dr.Khaleda Islam, Professor,Institute of Nutrition and Food Science, University of Dhaka for her scholastic guidance,constant supervision and encouragement throughout the period of this research work and in completion of this thesis work.

I would like to express my grateful regards to Co-supervisor Professor Dr.Khursheed Jahan, Professor, Institute of Nutrition and Food Science, University of Dhaka for her expert supervision, invaluable suggestions, strong support and care throughout the course of this research work.

I extend my cordial thank to the Director of Dhaka Medical College Hospital,Mitford Hospital and Holy Family Medical College Hospital for allowing me to undertake the work at their institute.

Finally I would like to thanks my family specially my wife professor Dr. Melia Choudhury and my daughter Dr. Nuzaira Nahid for their prayful concern, support and patience during the completion of this thesis work.

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ABSTRACT

Objective

To compare the plasma levels of antioxidants and the histomorphological variations of placenta in selected pre-eclamptic and normotensive pregnant women.

Methods

220 pregnant women were selected with inclusion and exclusion criteria from 3 different medical colleges and divided into 2 groups – A study group, consisting of 110 pre-eclamptic women and a control group consisting of 110 normotensive pregnant women. Dietary information was collected by 7 days food frequency questionnaire and food score was determined. Anthropometric and biochemical tests were performed. Fresh placenta was obtained from the study group as well as the control group following vaginal deliveries or caesarian section. Histological Examination was performed using samples from the placenta after delivery in the standard laboratory by hemotoxylin and eosin stain method. Biochemical analysis such as serum vitamin C levels were measured by spectrophotometric method, and serum vitamin E levels were measured by HPLC (High Performance Liquid Chromatography) method.

Result

The mean serum levels of Vit. C and Vit. E were found to be significantly lower in the studygroup, compared to the control group. Anthropometric study revealed that the babies born to pre-eclamptic mothers had lower birth weight than those born to normotensive mothers. Moreover, the weight of the placenta, placental diameter and number of cotyledons were also lower in the study group than in the control group. The pre-eclamptic placentas had greater no. of infarcted areas than the normal placenta, and no. of area of syncytial knot

formation, no. of area of cytotrophoblastic cell polyferation, no. of area of fibrinoid necrosis and hyalinised villi were increased in case of pre-eclamptic women compared to normal pregnant women. All the changes in the placenta of case (Study Group A) and control group (Study Group B) were statistically significant ($P<0.05$).

Conclusion

Therefore, low antioxidant levels do play a key role in the development of pre-eclampsia in pregnant women, and histological and morphological changes in the placenta.

ABBREVIATION

- NP = Normal Pregnancy
- PE = Pre-eclampsia
- SBP = Systolic Blood Pressure
- DBP = Diastolic Blood Pressure
- HELLP = Haemolysis elevated liver enzyme and low platelets.
- ROS = Reactive Oxygen Species
- HO = Hydroxyl Radical
- O^{2-} = Superoxide Anion Radical
- RO = Alkoxyl
- ROO = Peroxyl
- H_2O_2 = Hydrogen Peroxide
- HOCl = Hypochlorous Acid
- ONOO- = Peroxynitrite Anion
- RNS = Reactive Nitrogen Species
- NO = Nitric Oxide
- NO_3 = Peroxynitrite
- GSH-Px = Glutathione Peroxidase
- SOD = Superoxide Dismutase
- CAT = Catalase
- GSH = Glutathione
- WHO = World Health Organization
- IgG = Immunoglobin

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Introduction

CHAPTER ONE

INTRODUCTION

The human placenta is an important structure of pregnancy that protects the fetus until birth[1], and supports fetaldevelopment, via its metabolic, immunological requirement and secretory functions[2,3].It is connected to the fetus via the umbilical cord[4].It separates the maternal and fetal blood and it is made up of only one cell layer, that allows efficient nutrients, gas and waste exchange to take place.[5,6].It is of both maternal and fetal origin, and plays an essential role as a barrier and for removal of waste products of metabolism.[7]A full term human placenta is reddish blue, flat, and disc shaped, with a weight of 200-800 gm. The morphology of the placenta e.g. the weight, diameter and the number of cotyledons can be changed in many diseases, including pre-eclampsia .[8]

Pre-eclampsia occurs after the 20th week of pregnancy. It occurs in 2 stages. Firstly, there is decreased blood flow to the placenta, followed by the development of a constellation of symptoms, including hypertension, proteinuria and edema. In developing countries like Bangladesh, it is a major cause of infant and maternal mortality, with approximately 50,000 maternal deaths per year [9].

A number of hypotheses have been proposed regarding the etiology of pre-eclampsia, but the exact cause is still unknown. Dysfunction of vascular endothelium and inadequate trophoblastic invasion, leads to increased resistance and decreased uteroplacental blood flow, resulting in ishchemia of the placenta and decreased oxygenation. Hypoxia causes production of reactive oxygen species or free radicals like superoxide which are capable of damaging proteins, and inducing lipid peroxidation, ultimately resulting in widespread endothelial damage[10]. It has now been suggested that deficiency in antioxidants can lead to development of Pre-eclampsia. Antioxidant vitamins like Vit. C, Vit. E can counteract highly reactive free radicals, and protect against superoxide attack and lipid peroxidation[11].There is no study available in the observing antioxidant deficiency in pre-eclamptic women and relevant histomorphological changes on the placenta in Bangladesh .There for in this study we tried to evaluate the relationship between the plasma antioxidants levels and the changes in

histomorphology of the placenta in both pre-eclamptic and normotensive pregnant women of Bangladesh.

GENERAL OBJECTIVE

The general objective of this study is to assess "the association of serum-vitamin C and vitamin E levels with the histomorphological changes of full term placenta in selected normotensive and pre-eclamptic women of Bangladesh.

SPECIFIC OBJECTIVE

- To observe and compare the macroscopic changes of placenta e.g. the weight, diameter, and number of cotyledons of the placenta in selected normotensive and pre-eclamptic women.
- To observe and compare the histopathological changes of placenta e.g. the numbers of areas of syncitial knot formation, cytotrophoblastic cell proliferation, fibrinoid necrosis and hyalinised villi in selected normotensive and pre-eclamptic women.
- To assess and compare the serum vitamin C and Vitamin E levels in selected normotensive and pre-eclamptic women.
- To assess the food consumption score and Nutritional status of study and control group in selected normontensive and pre-eclamptic women.

RATIONALE

Pre Eclampsia remains a leading cause of mortality and morbidity in developingcountries. The cause of pre-eclampsia is not fully understood. One theory suggeststhat the deficiency of nutrients, like Vit. C and Vit. E can lead to the developmentof pre eclampsia.

Since not much study has been done on it, this data can help usexplore and understand the causes of pre-eclampsia better and in turn educatethe women about the importance of nutritional requirements duringpregnancy. Ultimately, this can reduce the number of pre-eclampticwomen, prevent hospitalization and effectively reduce the morbidity andmortality rates of both the mother and babies.

HYPOTHESIS

Low levels of antioxidants (Vitamin C and Vitamin E) will be associated with pre-eclampsia and histo-morphological changes in placenta of pregnant women.

LITERATURE REVIEW

Pre-eclampsia (Toxemia of pregnancy) is a specific disorder of the second half of pregnancy, consisting of signs of hypertension, proteinuria and oedema and it is an important cause of maternal and prenatal morbidity and mortality[12,13]. In developing countries[14,15] the mortality usually occurs due to renal, haematological, and cerebral complications, including oliguria, haemolysis and eclampsia, as well as thromboembolic manifestations that can further impair the blood flow to vital organs.[16] The cause of pre-eclampsia is still not clear.` The four hypothesis currently accepted are: 1) the placental ischemia hypothesis , 2) genetic hypothesis, 3) the immune maladaption and 4) hypothesis of the imbalance between free oxygen radicals and scavengers in favor of oxidants'[17]. As an important structure of both maternal and fetal origin,the placenta has become a topic of interest in recent times[18].

Pre-eclampsiapredominantly affects the maternal vascular endothelium[19].A key role is played by the endothelium derived relaxing and contracting factors, in the development of pre-eclampsia. Any defect in the production or action of these factors can cause abnormalities in vascular resistance and blood pressure, leading to vasoconstriction, leukocyte adherence, mitogenesis, peroxidation, vascular inflammation[20].

Under normal conditions, peroxidation of lipids peroxidation occur at low levels in all living tissues[21], and a range of mechanisms exist to control this peroxidative process .In diseases like pre-eclampsia, an imbalance is created between lipid peroxidation and antioxidant mechanisms, that disrupts the function of the endothelium, and leads to vascular dysfunction[22,24].Lipid peroxide levels in the blood of pre-eclamptic mothers are significantly raised, and the levels of antioxidants like carotenoids, tocopherols,and ascorbic acids are lowered, compared to normal pregnancy[23,25].

A study was conducted by Segupta Kishwara,Abu Sadat,Shamim Ara[26] and others,in the Department of Anatomy, Dhaka Medical College, in 2010.They observed lower placental weight in pre-eclampsia compared to normal pregnancy .Several other studies assessing the effect of hypertension on pregnancy have demonstrated the weight of the placenta to be moderately or severely reducedmoderate pre-eclamptic and eclamptic pregnancies[27,28,29,30].In his studies, Cibils[31] found that in hypertensive patients, the overall size of the placentas were considerably reduced, which indicated that the growth process was interrupted by high blood pressure, whereas in another study, Shah et al[32]noticed that with increasing severity of pre-eclampsia, the weight of placenta was further reduced.Teasdale[33],Sodhi et al[34] , Barua[35] and Begum[36]also observed a reduction of placental weight in pre-eclampsia .According to Fox[27], Jones[30] and Soma et al[28], the histomorphological abnormalities in hypertensive placenta are due to occlusion or narrowing of the uteroplacental vessels as well as placental ischemia.

In pre-eclampsia the balance between formation of free radicals, and antioxidant mediated defense is disrupted, leading to abnormalities of endothelial function and free radical-mediated endothelial cell injury[37,38].Some studies corroborate that the serum levels of antioxidants such as vitamin C ,Vitamin E ,are reduced in pre-eclamptic women [37,38,39]. Sagol *et al*[40] observed impaired antioxidant activityin women with pre-eclampsia. Agarwal et al., 1983; and others (Menawat *et al*., 1985; and Stewart *et al*., 1989) reported that inadequate supply of nutrients during pregnancy can be responsible for pre-eclampsia. In a study, Mallik, Mirchandani and Chitra[41], Udainia and Jain[42], Majumdar *et*al[43] and Artico *et* al[44] found reduced placental weight in pre-eclampsia.Daminia[45], Fox[46], Kalousek and Langlosis[47] and Majumdar et al[43]observed that the birth weights of babies of pre-eclamptic mothers were significantly lower than normal birth weight of normotensive mothers. In a study, Sanin *et al*[48] found reduced birth weight in pre-eclamptic women and Mirchandani, Malik and Chitra[49], Masodkar, Kalamkar and Patke[50] and Avasthi *et al*[51] also observed increased stillbirth associated with pre-eclampsia. Some studies confirm that levels of antioxidants such as Vitamin C and Vitamin E were reduced

in pre-eclamptic women[52,53,54]. Sagol *et al* also observed the same[55]. There is evidence that decreased concentration of antioxidants such as Vit. C and Vit. E in pre-eclamptic women compared to normal pregnant women[56].Placental infraction was more in case of pre-eclamptic women compared to normal normotensive pregnant women[57]. It was also observed by Mirchandani *et al*[58] and Masodkar *et al*[59].

Segupta kishwara et al., 2010; and several others[26] observed that the mean diameter of placenta was lower in the study group compare to control group, the mean number of cotyledons were lower in the study group compare to control group, whereas the mean number of infarcted areas in the study group was higher compare to control group. M. Akhlag,. 2012; AH Nage, AW Yousuf also observed increased syncitial knot formation in pre-eclamptic women compared to normal pregnant women. Other studies have also found similar results, with increased syncytial knots formation, cytotrophoblastic cell proliferation, fibrinoid necrosis, and hyalinized villi, in pre-eclamptic patient.

Suryakant Nagtilak., 2014[60]; observed that mean serum vit C was lower in pre-eclamptic women compare to normal pregnant women. A study was conducted by Lucy C Chappell, 1999 and others (Paul T Seed, Annette Briley) where they observed reduced levels of Vit C in pre-eclamptic women. Anant S Gupta and TB Sharma also found low level of Vit C in pre-eclamptic women. Suryakant Nagtilak., 2014[60]; observed that meanserum Vit E levels was lower in study group compare to control group. Kharbs Gulati Singh et al., 2000[61], and Yanik FF et al., 1998; also observed lower serum Vit E in pre-eclamptic women compared to normal pregnant women. K Devi Shankar and others observed that there is a reduced placental weight, placental thickness, placental diameter and neonatal weight in pre-eclamptic women compared to normal pregnant women[62]. In another study, it showed that there is an increase in syncytial knot formation in pre-eclamptic women compared to normal pregnant women[63,64,65]. In another study they found increased fibrinoid necrosis in pre-eclamptic women compared to normal pregnant women[66,67].

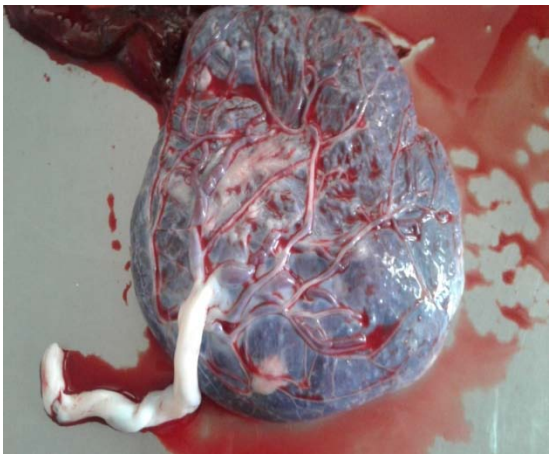
THE PLACENTA

The placenta plays an important role in pregnancy to provide protection to the fetus until birth[1].It obtains its metabolic, immunological requirements and secretory functions to support fetal developments[2,3].The placenta is connected to the uterus on one side, and to the fetus on the other, via the umbilical cord[4].It separates the maternal and fetal blood and it is made up of only one cell layer, that allows efficient nutrients, gas and waste exchange to take place[5,6]. Usually the placenta fixes to the top, sides, front or back of the uterus. However, in rare cases, placenta might be found in the lower region of the uterus[68]. A term placenta measures about 2.0 to 2.6 cm thick and 23 cm in diameter. A term placenta usually weighs approximately 470g to 508g with 500 ml of an average volume[69].

Measurements of placenta differ broadly and substantially in distinct regions[70]. Studies exhibited fetal or maternal illnesses such as acute anaemia, hypertension, and hydrops fetalis effect fetal as well as placental weight[71,72]. Race and socioeconomic position affects the placental weight [73]. In Asia report of placental weight is 588g and 470g in Ukraine [73,74].

SHAPE OF THE PLACENTA

The placental shape is determined by its location, its implanted position in the uterus , interaction with the endometrium and the shape of uterus itself[75].It is usually ovoid, with a 16-20 cm diameter and a 2-3 cm thickness and it grows exponentially during gestation ,from an average of 6 gm at 3 week of gastation to 470 gm at term[76].The placenta implants anywhere in the uterus ,but most commonly ,it is in an anterior or posterior location, much less often it is on thefundus[77].



The Placenta

Cotyledon of the Placenta

STRUCTURE OF THE PLACENTA

By 21 days after fertilization the trophoblasts have begun to sort themselves out into what will become the tree-like structures that make up the placenta[78].

The trophoblasts form an interface between mother and offspring[80], and they are an important constituent of placenta[79].They have some tumour like properties with regards to invasion of the surrounding tissue.However, unlike tumours, theirtissue penetration is precisely controlled in a way that the cells stop penetrating when they reach the inner third of the myometrium, and this invasion occurs only in the early stage of pregnancy[81]. The human trophoblast matures along two pathways[82,83], one of which is the villous trophoblast pathway. Here, the cytotrophoblastic cells differentiate into syncytotrophoblasts, that line the villi. The other pathway is the extravillous trophoblast pathway.

The main component of the placenta are the chorionic villi, which are finger-like structures[78]that are responsible for nutrient absorption, waste elimination and generation of most of the hormones that are produced by the placenta during pregnancy[3].

The basic parts of a chorionic villous is revealed when it is cross sectioned.At the end of the first trimester, its components include a central mesenchymal core[84] with embedded fetal capillaries surrounded by a lining of cytotrophoblasts and syncytiotrophoblasts , which are specialized epithelial cells that come in contact with the maternal blood and also line the intervillous space[85,86].

At term, the cross section reveals the same basic structure with some distinct differences.There are more fetal capillaries, and some are located at the edge of the villous to make nutrient exchange easier. It also shows a layer of syncytiotrophoblast, a sheet of flat multinucleated cells which are primarily involved in transport and hormone production[87,88].

BLOOD CIRCULATION OF THE PLACENTA

The placenta's chief role is to facilitate exchange of nutrients and remove waste products between maternal and fetal blood. The weight of the fetus, the size of the placenta, and the uterine and umbilical circulation are all closely related to one another in a normal pregnancy, which indicates the role of placenta in a successful pregnancy [89].

The fetomaternal barrier in the intervillous space separates the maternal blood and the fetal blood, and it is at this layer that the exchange of nutrients, that is, oxygen, water, hormones, and removal of waste products of metabolism takes place[90,91]. It is composed of `fetal vascular endothelial cells and their basement membranes, connective tissue of the villous, the subepithelial basement membrane and its covering of cyto- and syncytiotrophoblasts'.[91]

Oxygen is carried in the blood through the umbilical vein, to the fetus, and the waste products are removed via two umbilical arteries[92]. The maternal arteries drain into placental sinuses containing villi.[93] In the intervillous spaces, exchange between maternal and fetal blood takes place, where oxygen and nutrients travel into the fetal blood , whereas the waste products of fetal blood travel back to the maternal circulation.[94] Blood from the villi then converge into the umbilical vein[93].

Furthermore, most of the blood in the umbilical vein goes directly into fetal liver[95]. In the placenta, the stem villi of the villous trees are thought to control the peripheral villi[96].

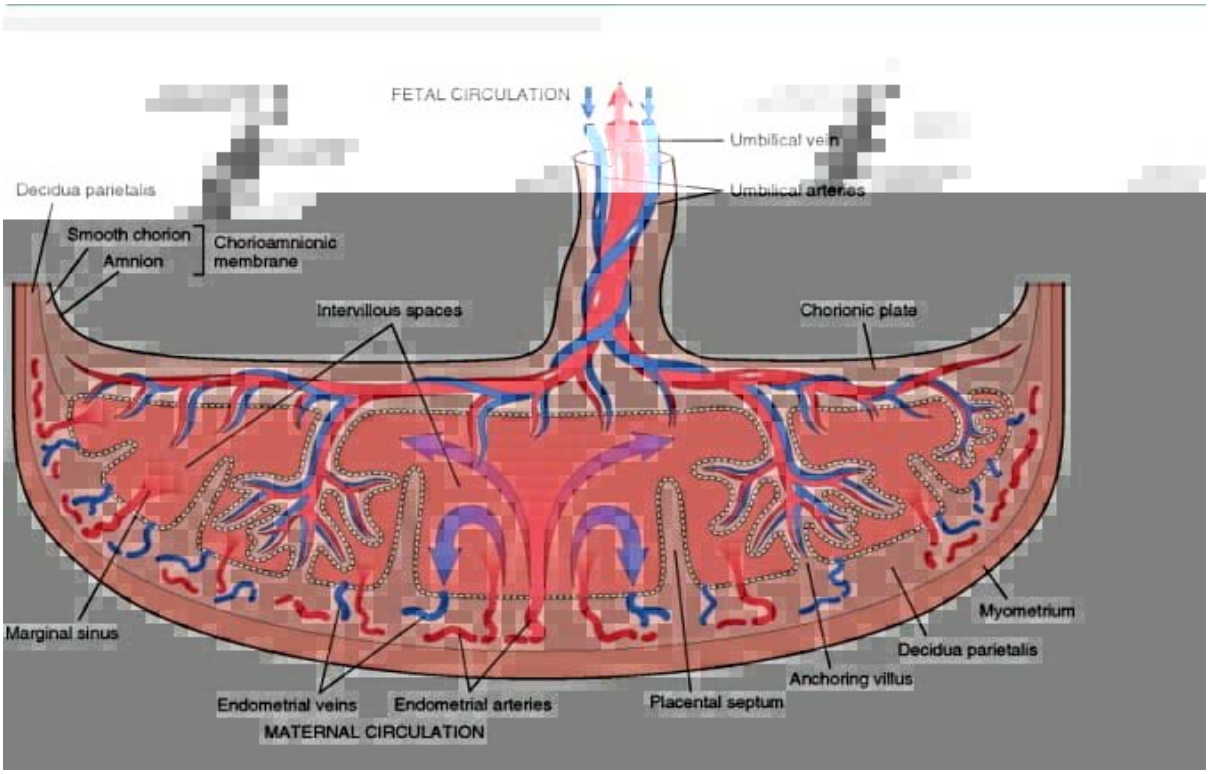


Figure 1: Placenta Biodictionary

PLACENTAL TRANSFER OF SUBSTANCES

The terminal villi of the placenta are the main functional units, which are very important in fetomaternal transfer of substances[97,89]. Oxygen and nutrients from the mother travel through the layers of syncytiotrophoblast, cytotrophoblast, villous basement membrane, and the fetal capillary beds to reach the fetus and carbon dioxide and waste products from the fetus[99] travel back to the maternal circulation.[98]

Hormones[100], cytokines an d substrates[101,102]present in the maternal and fetal bloodbind to specific receptors on the placental surfaces[81], and control the formation and development of placenta.

Fatty acids are an important for the normal development of the fetus, as they are a crucial constituent of cell membranes, they are used as a source of energy, and they are precursors to signaling molecules.[103]

PRE-ECLAMPSIA

Pre-eclampsia is a systemic disorder, the cause of which is still unknown. It manifestsin the form of hypertension, where the blood pressure rises to 140/90 mmHg or more, with proteinuria developing after the 20th week of pregnancy. It is associated with delayed growth of the fetus, preterm delivery, maternal and fetal death[104.]. It occurs in 4-7% of pregnant women worldwide[106,110]. Around 10% women worldwide suffer from hypertensive disorders[107,108] which consists of gestational hypertension, pre-eclampsia , eclampsia and chronic hypertension[108]. Another study shows that 10% pregnant women have high blood pressure, and in 75% of those cases, it is due to pre-eclampsia[111]. If not managed appropriately, pre-eclampsia can lead to a life threatening condition called eclampsia, which is portrayed by seizures in women of pre-eclampsia. The rate of pre-eclampsia in the developing countries is 30 times higher than the developed countries[109].

Widespread endothelial dysfunction in the mothercan lead to development of pre-eclampsia[112,113],anf this, in turn, can lead to a greater risk of dyslipidemia, hypertension and cardiovascular and renal disease[114,103] .

According to WHO, the maternal deaths are usually caused by eitherhemorrhage, infection, eclampsia or obstructed labor. Hypertensive disorders of pregnancy (PE and eclampsia) contribute to 12% of maternal deaths worldwide[115].

MILD PRE-ECLAMPSIA

Usually, when mild pre-eclampsia develops at or near term,it is associated with minimal morbidities, in both the mother and the fetus. In general, women with mild diseases developing at 37 weeks of gestation or afterwards have pregnancy outcomes which are similar to those found in normotensive pregnant women, with a systolic blood pressure (SBP) of 150 mm Hg or less and a urine protein content of 1000 mg or less per 24 hours[116]. The amount of protein lost per day has been thought by some to produce both maternal and fetal outcome[117].

SEVERE PRE-ECLAMPSIA

Severe PE is defined as the presence of PE with any of the following one of symptoms or signs: High Blood Pressure, where the SBP is 160 mm Hg or higher or DBP is 110 mm Hg or more on 2 occasions, at least 6 hours apart, proteinuria of greater than 5 g/24 hour, pulmonary oedema, urine output <400/24 hours, persistent headaches, epigastric pain, impaired liver function, thrombocytopenia, or impaired intrauterine growth.[118,119]

The more profound these aberrations, the more likely are the need for pregnancy termination. Mild disease can rapidly progress to a severe form, which makes the differences between mild and severe disease misleading[120]. Severe PE can often result in life threatening emergencies such as eclampsia, which presents with convulsions, thought to be caused by cerebral vasoconstriction[121]and the HELLP syndrome (haemolysis, elevated liver enzymes and low platelets[122].

PREVALENCE

Pre-eclampsia and other hypertensive disorders are the leading causes of pregnancy related deaths worldwide, causing 46,900 deaths in 2015[123]. Eclampsia is a possibly deadly condition of pregnant women it is one of the predominant source of maternal mortality all over the globe that counts for about 50,000 deaths worldwide[124]. Eclampsia confounds about 1 in every 2000 child births in developed countries[125]. However, the picture is much worse in developing countries, where the incidence of eclampsia fluctuates extensively, from 1 in 1000 to 1 in 1700 deliveries[126,127,128]. Illiteracy, lack of health education and awareness, poverty and fallacies are major cause of high rate of this problem in developing countries and it deprive women from pursuing medical guidance in the course of pregnancy.

In spite of global efforts to decrease inevitable maternal and neonatal mortality, around 5,000 to 6,000 deaths of pregnant mother occur every year in Bangladesh. 20% of these mothersdie due to pre-eclampsia and eclampsia (PE/E) cause. In Bangladesh, after post-partum hemorrhage (PPH), PE/E is the most pre-dominant cause of maternal deaths. However, maternal and newborn deaths owing to PE/E are avoidable taking necessary measures such as early diagnosis[129].

Worldwide approximately 2-8% of all pregnancies are affected by pre-eclampsia[130,131]. The frequency of pre-eclampsia has increased in the U.S. ever since the 1990s and increasing prevalence of predisposing conditons, like chronic hypertension, diabetes, and obesity are being held accountable for this high incidence[132].

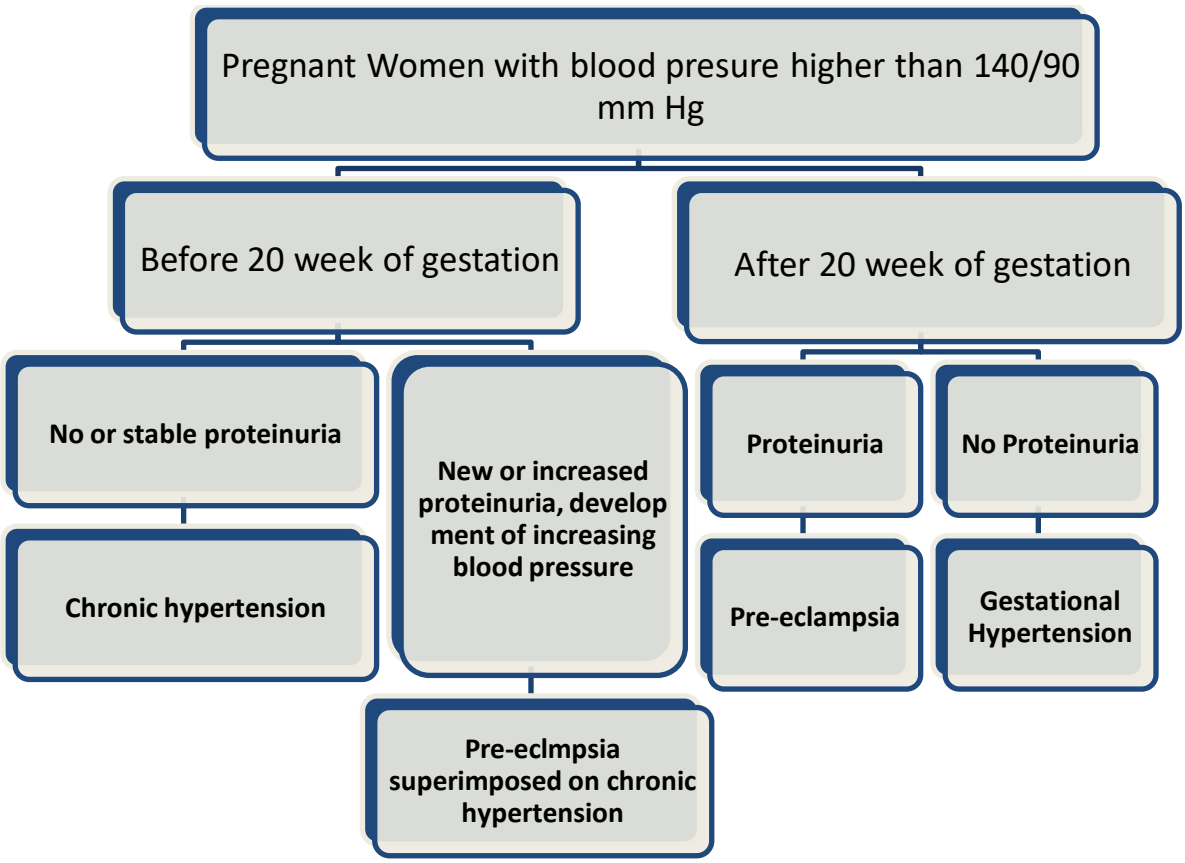
In Africa and Asia and one-quarter in Latin America, approximately one—tenth of all maternal deaths are linked to hypertensive disorders in pregnancy, a category that includes pre-eclampsia[133]. In United Kingdom 6% of maternal deaths are affected by pre-eclampsia[134].

The incidence of pre-eclampsia is seven times higher (2.4% of live birth)in developing countries, compared to developed ones (0.4%), according to the World Health Organization (WHO)[135]. In Bangladesh, the prevalence of pre-eclampsia is worryingly excessive and around 20% of deaths of pregnant mothers are connected with PE and eclampsia[123].

HYPERTENSION IN PREGNANCY

Hypertension can manifest in the following ways in pregnancy:

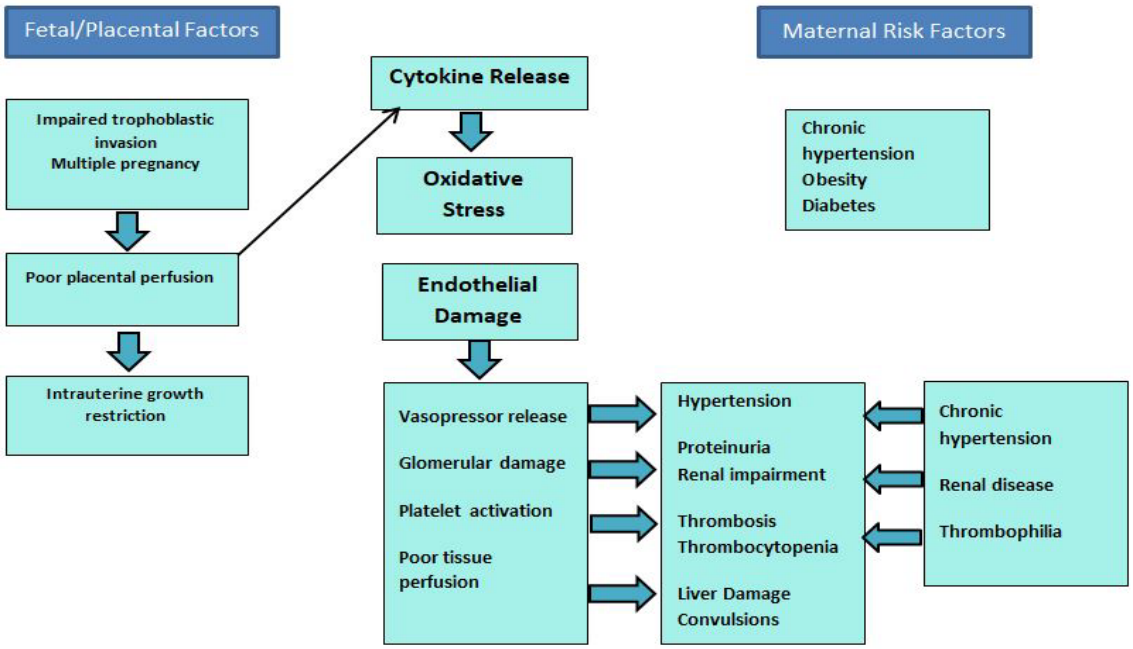
1. Chronic hypertension[136]
   This can be primary or secondary, and is present either before the onset or before 20 weeks of pregnancy.
2. Gestational hypertension[137]
   Hypertension that manifests after 20 weeks of gestation, with no protein in urine; it may or may not be resolved by 12 weeks after delivery.
3. Pre-eclampsia[138]
   Hypertension that develops after 20 weeks of pregnancy; in this case,>300mgof protein is present in 24 hour urine collection.
4. Pre-eclampsia superimposed on chronic hypertension[139]
   Sudden increase in blood pressure after 20 weeks of pregnancy with development or acute worsening of proteinuria.



Pre-eclampsia as a hypertensive disorder of pregnancy[140]

PATHOPHYSIOLOGY

PE is an important disorder of pregnancy, the exact cause of which remains unknown. In recent studies, however, defective attachment of placenta, and defective maturation of placental trophoblasts are thought to play an important role.[140,141] Several pathophysiological changes occur in this condition, which can lead to the symptoms of hypertension and proteinuria. There is development of vasospasm of the systemic arteries, which can cause decreased blood supply to almost all organs[142], ultimately activating the endothelial systems. Impaired spiral artery remodeling does not always lead to PE, however, and can sometimes lead to intrauterine growth restriction only, without any symptoms of PE. This insinuates that genetic predisposition, and several other maternal factors play a role in the development of PE.[143]



Suggested pathophysiological mechanism in pre-eclampsia[143]

DIAGNOSTIC CRITERIA

The PE can diagnosed when a pregnant woman develops high blood pressure of atleast 140/90 or more, taken on two separate occasions, minimum 4 hours apart, and 300 mg of protein (proteinuria) in a 24-hour urine sample[144].

Proteinuria is defined as the presence of 300mg/L or more protein in a 24-hour urine collection , which is around 1+ or greater on a urine dipstick test[145,146]. A rise in systolic BP of 30mm Hg , or diastolic BP of 15 mmHg from the baseline is considered important to note but is not considered diagnostic[147], when it does not meet the absolute criteria of 140/90.

Only hypertension and proteinuria are now required for the diagnosis of PE, but previously swelling or oedema of the hands and face, was considered an important sign [148]. Abnormal weight gain and oedema occur early and reflect an expansion of extravascular fluid compartments; this expansion is related to the capillary permeability that allows the fluid to diffuse from the intravascular to the extravascular space[149].

CAUSES OF PRE-ECLAMPSIA

The etiology of PE and the cause of its advancement to eclampsia or late postpartum eclampsia is unknown[150]. It has been labeled the “disease of theories” because of the multiple conjecturesthat have been proposed to explain its occurance. It is recognized that abnormal placentation and placental vascular insufficiency are core features of PE, but why these and associated systemic abnormalities occur remains uncertain[151].

The most widely acknowledged hypothesis revolves around a discrepancy between the uteroplacental circulation and the oxygen demands of the fetus. This results in production of excess reactive oxygen species, that cause peroxidation of membrane lipids, and damage the endothelial cells[152]. As a result, inappropriatevasoconstriction and platelet aggregation occurs, which in turn contributes to early signs of atherosclerosis, hypertension and coronary vasospasm[153].

The cause of PE is still unknown, but several theories are present which involves: ‘Endothelial cell injury, immune rejection of the placenta, compromised placental perfusion, altered vascular reactivity, Imbalance between prostacyclin and thromboxane, decreased glomerular filtration rate with retention of salt and water, decreased intravascular volume, increased central nervous system irritability, disseminated intravascular coagulation, uterine muscle stretch (ischemia), dietary factors, including vitamin deficiency, genetic factors, air pollution,obesity’[155]

In PE, the physiological hemodynamic and vascular changes which occur during pregnancy get disturbed. Of the few studies conducted, one study shows that women with increased cardiac output throughout pregnancy go on to develop PE. However, after the development of PE, it can bedecreased, normal or significantly increased.The late hemodynamic changes in PE include increased blood pressure, reduced plasma volume, increased peripheral vascular resistance and vasoconstriction[156].

Risk factors for development of PE involve offsprings of mothers with PE, having a sister PE or being pregnant from a partner who fathered a PE

pregnancy[157]. Thus genetic predispositions to PE seem to be both maternally and paternally transmitted[158,159]. Environmental factors may also play a part indevelopment of PE. For instance, the high incidence of PE in many developing countries indicates that dietary deficiencies, including deficiency of calcium, zinc, vitamin C and vitamin E may have a role in the pathogenesis.[160]

PATHOGENESIS

Some pathological features of PE include: Small placentas with decidual arteriopathy, infarcts in central portions of the placenta,abruption placenta.intervillous thrombosis.[161]

Intrauterine growth retardation and arteriopathy are common in PE, but the other findings are nonspecific[162]. It is postulated that PE, comprising of hypertension, proteinuria, and oedema results from a generalized inflammatory reaction, that results in dysfunction of maternal endothelium[163].This may be a final pathway leading to metabolic disturbances and eventually clinical manifestations, and it is responsible for activation of coagulation cascades, producing procoagulants, ultimately formingmicrothrombi and free oxidative radicals, causing maternal vascular dysfunction and leucocytes activation[164] especially neutrophils which releases superoxides and various cytokines[165].

Inadequate uteroplacental oxygenation in PE is believed to be the cause of the pathological changes, that lead to the clinical manifestations of this disease[166]. The changes that occur in placental villi in pre-eclampsia, particularly the syncytiotrophoblast, includes: increased cell death and loss of syncytiotrophoblasts and increased density of syncytial knots[167,168].

The exact etiology of increased apoptosis in PE is currently unknown. The increased death of syncytiotrophoblasts cause increased[169]amount of syncytiotrophoblast debris, syncytial knots to travel into the mothers blood, causing activation of the endothelial cells[170,171,172].

Decreased blood supply to the placenta is a possible cause of PE. This occurs due to structural defects and occlusion of the spiral arteries, as a result of impaired invasion and maturation of the trophoblasts[173,174,175].

The PE is a multisystem disorder that can affect most organs of the body[176] including the liver, lungs, kidney, brain and cardiovascular system[177]. In addition, oedema that accompanies a normal pregnancy is more severe during PE pregnancies[178].

Alanine aminotransferase and aspartate aminotransferase levels in serum can be used to evaluate theliver function in PE;[179] elevated levelsare a feature of HELLP syndrome, where coagulopathies occur due to impaired liver function and not disseminated intravascular coagulation. The kidney functions are usually intact in PE:[181] however, rise in creatinine in case of severe diseases are a sign of poorer outcomes.[180]

Because PE resolves postpartum, premature delivery of the baby may be necessary to save the mother's life. NICU or special care units are required by many neonates born to PE mothers[143]. Apparently, a interplay of multiple maternal components (genetics, environmental, and behavioral) and failure of physiological adaptation are able to increase a women susceptibility to develop the clinical syndrome of PE[136].

COMPLICATIONS OF PRE-ECLAMPSIA

Maternal complications of PE include[77]:

- Hypertensive crisis
- Renal impairment and infarction
- Neurological complications (including seizures and cerebrovascular accidents)
- Impaired liver function
- Placental abruption

Fetal complications include[182,183]:

- Intrauterine growth retardation
- Prematurity
- Stillbirth in severe cases
- Oligohydramnios

RISK FACTORS OF PRE-ECLAMPSIA

Medical conditions like diabetes mellitus, chronic hypertension, vascular disorders, connective tissue disorders, etc that can cause microvascular diseases, antiphospholipid antibody syndrome, and nephropathy, are risk factors for PE. Other risk factors include pregnancy associated risk factors (chromosome abnormalities, hydrops fetalis, multiple pregnancies, urinary tract infections, etc)[184,185], maternal risk factors (age less than 20 or more than 35 years, black race, history of pre-eclampsia in the family, PE in a previous pregnancy, nulliparous female, diabetes, gestational diabetes, chronic hypertension, dietary insufficiencies etc)[186,187,188], and paternal risk factors (father for the first time, previously fathered a pregnancy with PE).

FREE RADICALS

A free radical is an unstable atom, molecule, or compound, with free electrons in their outer shell, which react with other atoms or molecules to attain stability.[189]The free radicals formed may damage lipids, proteins, carbohydrates and nucleic acids, which are the basic molecules of the cells[190].

Most free radicals in biology fit within the broader category of:

Reactive oxygen species (ROS), which include :

- Hydroxil radical (HO.)
- Superoxide anion radical (O2.-)
- Alkoxyl (RO.)
- Peroxyl (ROO.)

Also, reactive molecules without unpaired electrons may include[191]:

- Hydrogen peroxide (H2O2)
- Hypochlorous acid (HOCl)
- Peroxynitrite anion (ONOO-)
- Lipid peroxides

Reactive nitrogen species (RNS) include free radicals, such as[192]:

- Nitric Oxide (NO.)
- Nitrogen dioxide (NO.2)
- Peroxynitrite (NO3-)
- Nitroxyl anion (HNO)
- Peroxynitrous acid (HNO3)

Increased free radicals mediate tissue injury and result in a wide range of human disease[193] such as cancer, heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, inflammation, and cerebrovascular disease through multiple mechanisms[3].

Many free radicals also enter the body constantly from exogenous sources such as: [194]:

- Air pollution
- Cigarette smoke
- Drugs
- Pesticides
- Exposure to ionizing radiation
- Heavy metal exposure, including mercury, cadmium and lead
- Alcohol consumption
- Exposure to a variety of environmental chemicals

Other sources are endogenous sources, such as[195]:

- Inflammation
- The respiratory burst
- Xenobiotic killing

Free radicals seem to be primarily produced by the placenta but maternal leukocytes and the maternal endothelium are also likely sources[196]. Recent studies indicate that neutrophils can cause endothelial damage in women with PE because of their ability to produce ROS [197]. According to one study, in PE subjects the neutrophils produced significantly more ROS than the neutrophils in non pregnant and normotensive pregnant women[198]. ROS are intermediateswhich are normally produced during metabolism. The balance between their production and breakdown, as well as the level of ROS in the body is monitored by enzymatic and non-enzymatic defense mechanisms[199].

ANTIOXIDANTS

The body is protected from the ROS induced damage by antioxidants, which react with free radicals and terminate the oxidative process by which cell components are damaged.[200].

The natural defense mechanisms against free radicals consist of[201,199,202]antioxidant enzymes like:[201,199,202]

- Glutathione peroxidase (GSH-Px)
- Superoxide dismutase (SOD)
- Catalase (CAT)
- Glutathione reductase

Antioxidant non-enzymes like:

- Glutathione (GSH)
- Ascorbate
- Vitamin A, C and E
- Beta carotene
- Arginine, citrulline, taurine and creatine
- Selenium
- Zinc
- Tea polyphenols

Antioxidants can be obtained from exogenous sources like fruits, vegetables, seeds, nuts, meats, oils, or from endogenous sources, e.g, produced inside the

body. Under normal physiological conditions, an equilibrium is maintained between the ROS and antioxidant production in the body[203,200]. The antioxidants in fruits and vegetables may contribute to the beneficial effects[204].

In women at risk for PE, lipid peroxides production surpasses the scavenging ability of the antioxidants, creating a state of oxidative stress. Blood levels of lipid peroxides are increased and antioxidant levels are reduced in pre-eclamptic women, which supports this fact.[205].

ANTIOXIDANT ENZYMES

The activity of enzymatic antioxidants protects living organisms against ROS. Uncontrolled increase of ROS may cause damage of cells, tissues and alter some metabolic pathways leading to different disturbances[206].Antioxidant enzymes are important for intracellular defense like catalase[207].

LIPID PEROXIDATION

Lipid peroxides and other intermediates are produced by the lipid peroxidation of unsaturated fatty acids by ROS[207].

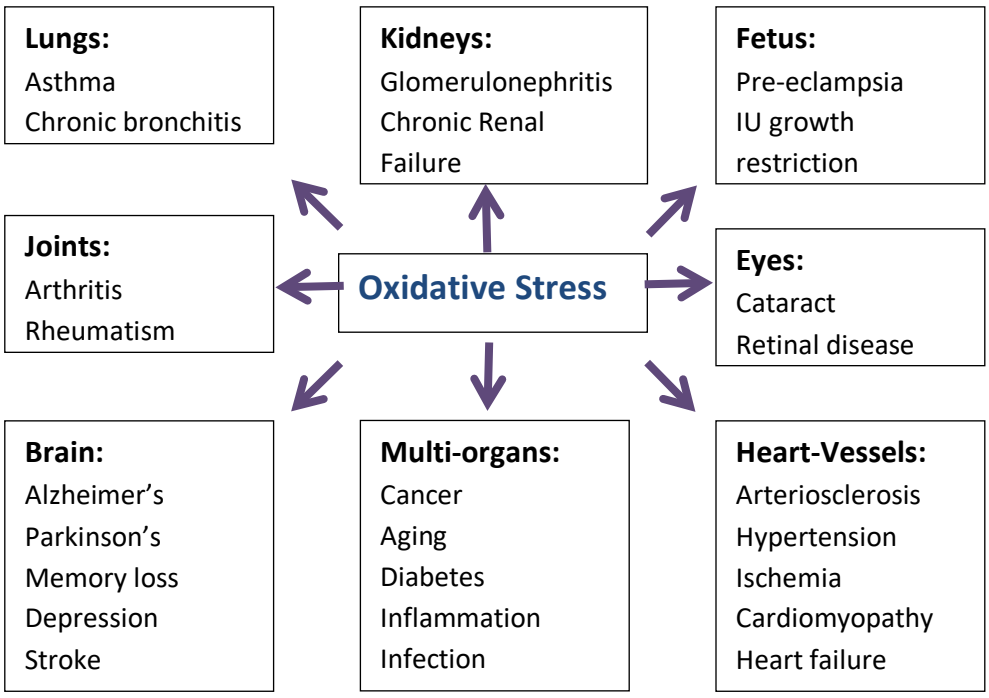
Decreased perfusion of the placenta causesrelease of free radicals which damage polyunsaturated fatty acids in cell membranes[208], initiating lipid peroxidation, and convert them to lipid peroxides and a variety of other intermediates[209].

Lipid peroxidation is a process by which cell components are damaged. It corresponds to oxidative stress in the cells, and increased levels are found in a wide range of diseases in both humans and animals[210,21]. Lipid peroxides are unstable compounds, that can break to form a range of complex intermediates,[211] like carbonyl compounds, the most abundant malondialdehyde.

Many studies showthese intermediates, primarily thiobarbituric acid reactive substances, including malondialdehyde are increased in plasma of pre-eclamptic women[23,212].

OXIDATIVE STRESS

Oxidative stress is defined as a disparity between ROS synthesis and antioxidant protection[213], caused by the presence of free radicals or radical-generating agents[214]. Biological substances such as lipids, DNA and proteins[215,216]are affected by oxidative damage, and it is also involved in the pathogenesis of aging as well as many other diseases[217] such as: ischemia-reperfusion injury, hyperoxia, hypoxia, iron overload and intoxication[218]. This process generates a variety of metabolites, including oxidized thiols, lipid peroxides and isoprostane, which have been employed as biomarkers of increased oxidative stress[219].



Oxidative stress-induced diseases in humans[220]

OXIDATIVE STRESS IN NORMAL PREGNANCY

There is at present much curiosity about the part played by oxidative stress in the development of complications of human pregnancy[221]. Majority of complications of pregnancy have been related to defective formation of the uterine spiral arteries, suggesting that hypoperfusion of the placenta plays a key

role[222].In pregnancy, increased metabolic activity of the mitochondria produces elevated levels of free radicals, which exceeds the capacity of the antioxidants to neutralize them[223]. Mounting evidence suggests that the placenta has a major role in the production of oxidative stress during pregnancy[224].

OXIDATIVE STRESS IN PRE-ECLAMPSIA

The pathogenesis of PE and several other complications of human pregnancy involve oxidative stress[225,226]. More and more data supports the theory that oxidative stress is responsible for PE[227,228], and it is currently thought to be the mechanism behind the pathogenesis of PE[229].

In PE, there is defective trophoblastic invasion and the remodeling of spiral arteries is hampered. The presence of endothelium and the muscle layer reduces theamount of blood reaching the intervillous space, causing hypoperfusion of the placenta and the fetus,and creating a state of oxidative stress[230].There is increased production of lipid peroxides, reactive oxygen species and superoxide anion radicals. These causeendothelial dysfunction, platelet and neutrophil activation[229,231]. There is evidence that oxidative stress occurs in PE,but it remains unclear whether the impairedperfusion increases ROS or whether it is the other way around, and oxidative stress leads to the impairment of uterine perfusion[232].

Oxidative stress may be responsible not only for PE but also for endothelial cell dysfunctions[110], leading to atherosclerosis and cardiovascular disorders[233]. Evidence for oxidative stress in PE includes elevated lipid hydroperoxides and their metabolites, with reduced plasma antioxidants [234]. Placental oxidative stress appears to play a pivotal role in the development of PE. Therefore, any increase in the levels of endogenous antioxidants may prevent the development of PE[235].

Chapter Two

Methods and Materials

CHAPTER TWO

METHODS AND MATERIALS

Type of the study

Comparative Cross-sectional study.

The study period

Three years.

The duration of study

01.06.2015 to 31.05.18

Selection of Population

Study Groups were selected from three major tertiary hospitals located in Dhaka City: Dhaka Medical College Hospital, Mitford Medical College and Hospital and Holy Family Medical College and Hospital.

The sample size

To calculate prevalence or proportion of pre-eclampsia we followed the procedure. A total number of 10,800 delivery patients, admitted in Gynae and Obs Dept. of Dhaka Medical College, Mitford Medical College and Holy Family Red Crescent Medical College from 01.06.2015 to 31.05.2018, among them a total of 1800 complicated by pre-eclampsia.

So, Proportion of pre-eclampsia, P=(1800/10800)=0.166…=0.17

The sample size was calculated using the following standards formula:

$$n = \frac{P(1-P)\,Z^2\,\alpha}{d}$$

where,

n= Sample size for a specified population.

P= Proportion of Pre-eclampsia

Z= Z score (95% confidence interval) = 1.96

d= Permissible error = 5% = 0.05

α= level of significance

So, Sample size:

$$n = \frac{0.17 \times 0.83 \times (1.96)^2}{(0.05)^2}$$

$$= \frac{0.54205}{0.0025}$$

$$= 216.82$$

$$= 217$$

Selection Criteria of the Population

Selection of cases was based on strict inclusion and exclusion criteria.

Inclusion Criteria

In case of Pre-eclamptic women,

- Age groups: 18-40 years
- Pregnancy status: third trimester of pregnancy.
- Blood pressure: Diastolic Blood Pressure above 90 mm Hg.
- Clinically oedema of legs: present.
- Proteinuria: Confirmed by biochemical test.

Exclusion Criteria

- Age: Less than18, greater than 40
- No oedema.
- No proteinuria.
- Normal Blood pressure. (Diastolic< 90 mm Hg).

Questionnaire

A questionnaire was developed to obtain relevant information regarding the socio-economic status, age, obstetric history, monthly income, living area, family size, education, type of jobs and usual habits of food before their admission to hospital.

The questionnaire was pretested before finalization and they were excluded from the study.

Ethical Permission

Ethical permission had been obtained from Ethical review committee of Bangladesh Medical and Research Council (B.M.R.C.).

Written Consent

Written consent was taken from both pre eclamptic and normal pregnant women.

Hematological and Bio Chemical Assays

(CBC, HB%, ESR and Fasting Blood Sugar).

Measurement of CBC Procedure

Under aseptic conditions, total 14 ml of blood was collected from median cubital vein by a disposable syringe.

For complete blood count (CBC), 2ml blood was placed in a CBC or EDTA tube with a disposable syringe. An ID number was allocated to the sample and it was brought to the laboratory.

In the laboratory one drop of blood was placed in a slide and a smear was made. The CBC tube was kept in the roller machine for 10 minutes.

Then blood was given in semi-automated (22 cell counter) Machine, and after one minute the printed result came out.

A Leishman stain was added to the smear slide for 2 minutes.

For cross checking the slide was examined under the light microscope at 40 magnification.

ESR

ESR was measured by Westergren method.

Procedure

0.4 ml of 3.8% sodium citrate was taken by a 1 ml pipette into a test tube

1.6 ml blood was taken by a 2 ml pipette into the same test tube

Then they were mixed gently.

The westergren tube was filled with this mixture up to the zero mark and placed in the ESR holder.

After one hour the ESR reading was taken in mm.

Ref: Normal range for female is 0 to 20 mm in first hour.

Fasting Blood Sugar

Procedure

2 ml of fasting blood was collected from the patient's median cubital vein, into a clot activator tube and it was labeled with a barcode and taken to the lab.

After the blood clots, the tube was centrifuged at a speed of 3000 RPM for 5 mins.

The serum was then separated from the blood cells.

During the test 1 ml Glucose reagent was taken in a plain test tube and 10 µl Serum was mixed with it and kept at room temp for 10 mins.

Then, the sample was placed in a semi auto Biochemistry machine and the final result was shown within 30 seconds.

Fasting Blood Sugar 3.5 to 5.5 mmol/L

Urine for Albumin

Assessed by heat coagulation test.

Procedure

At first, freshly voided mid-stream urine was collected in a plain test tube. Two third of the test tube was filled with urine.

Then the test tube was held with a test tube holder and the upper one third of the test tube was heated. On heating the urine turns cloudy.

A few drops of 5% acetic acid was added, and reheated.

After reheating, the precipitate increases/remains the same, which was an indicator of the presence of albumin in urine.

Gradation of result

According to turbidity/cloudiness,

- No turbidity (nil)
- Trace
- Distinct turbidity (1+)
- Heavy turbidity (2+ or more)

Serum Vitamin C:was measured by Spectrophotometric Method[239]

Procedure

3 ml of blood was collected from the patient median cubital vein, placed in a test tube, labeled with a bar code, which was covered by foil paper, placed in an ice box, and then immediately taken to the lab because it oxidises rapidly

2.0 ml of freshlprepared meta- phosphoric acid was taken in a test tube, and 0.5 ml of sample or control was added.

They were mixed together and centrifuged at 2500 RPM for 15 mins.

After that, it was filtered and the supernatant was taken for sample and control.

0.4 ml of DTCS was added in each and kept at 37° C for 3 hours.

2 ml of Sulphuric Acid was also added in each.

The absorbance was read at 520 nm against the reagent blank.

Serum Vitamin E

Assessed by High-performance Liquid Chromatography (HPLC) method.[240]

Procedure

3 ml of blood was collected from the patient's median cubital vein, labeled with a bar code and taken to the laboratory in an ice-box.

100 µl plasma was deproteinized with 100 µl ethanol.

Hexane was added.

Shaken for 30 seconds.

Centrifuged at 3500 RPM for 4 mins.

The hexane layer was separated and transferred in a clear glass vial.

It was evaporated to dry under liquid nitrogen. It was re dissolved into mobile phase (100 µl methanol) and injected into High-performance liquid chromatography machine (HPLC).

Nutritional status

Measured by Mid upper Arm Circumference (MUAC), using a measuring tape.(In cm).

Normal Range:> 23.9 cm in healthy female

Dietary information

Dietary information was measured by 7 days food frequency questionnaire.

Measurement of weight

Body weight was measured by bathroom scale, and weight was recorded to the nearest 0.5 kg.

Measurement of height

A wooden height scale was used to record height with bared heels standing upright position, height was measured to nearest 0.1 cm.

Blood pressure measurement

The blood pressure was measured by sphygmomanometer machine and stethoscope.

Birth weights of new born babies

Birth weights of new born babies were recorded to the nearest 20 gram after delivery without cloths on a beam balance (Dedecto medic, Delecto scale inc., U.S.A.).

MORPHOLOGICAL STUDY OF PLACENTA

A.Weight of the placenta

At first the decidual part of the placenta was removed.

Then umbilical cord was cut, nearest to the placenta, to drain the blood from the placental vessels.

After that, the weight of the placenta was measured upto nearest gram with weighing machine.

B.Diameter of placenta

Measured by taking the average of two maximum diameters of placenta with measuring tape (cm).

C.Cotyledons of placenta

Were counted from maternal side after removal of deciduas basalis.

D.Number of placental infarcts

Were counted from fetal side.

Placental Histopathology

Was done by Haemotoxylin and Eosin stain method.

Procedure

Tissue samples were collected from the placenta after delivery, and were prepared for histopathological studies.

In the first step the samples werecut into small segments, about 2 to 3 cm long.

The segments were placed in 4% formaldehyde solution and brought into the laboratory, and kept for 24 hours.

The water was removed from the tissue samples by immersing them in increasing concentrations of ethyl alcohol (70%, 90%, then absolute alcohol) for 30 to 60 mins.

Then tissues were treated with xylene for 2 to 3 hours to replace the alcohol.

After that, molten liquid paraffin, typically at 58 to 60° C, was poured into the block containing the tissues.

The molten paraffin wax was allowed to cool, and the block was prepared.

The block was then cut into 5 µm thick sections with a microtome.

The sections were floated on hot water and transferred to glass slides to be stained by haematoxylin and eosin.

The tissue sections were then dewaxed by xylene.

The slides were hydrated by passing throught decreasing concentrations of alcohol (absolute alcohol, then 90% then 70%).

They were then washed with water for 5 mins, and then stained with haematoxylin for 5 to 7 mins.

Next, they were washed in running water until the sections turn blue.

The slides were then stained with 1% eosin for 1 minute, the washed with water for 2 to 3 minutes.

Afterwards, the stained sections were dehydrated with 70%, 90% then absolute alcohol.

Lastly, the slides were cleared in xylene, after drying, and covered with cover slip.

Chapter Three

Results

CHAPTER THREE

RESULTS

GENERAL DESCRIPTION OF THE STUDY PARTICIPANTS

Table 1: Name of the Hospitals and number and percentage distribution of Respondents of Selected Pre-eclamptic Women (Study Group–A) and Normal Pregnancy (Control Group – B)

Hospitals	Pre–eclamptic Women (Study Group A) N=110		Normal Pregnancy (Control Group – B) N = 110	
DMCH	70	63.64%	70	63.64%
Mitford	30	27.27%	30	27.27%
HFRCMH	10	9.09%	10	9.09%
Total	110	100%	110	100%

The table-1 shows, 63.64% of the respondents were from DMCH, 27.27% were from Mitford & 9.09% were from HFRCMH.

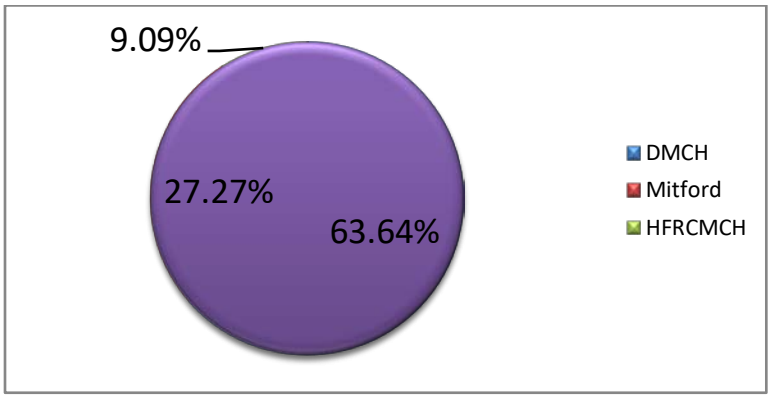


Figure 1: Distribution of respondents according to their hospital

Table 2: Number and percentage distribution of Geographical Area of Selected Pre-eclamptic Women (Study Group–A) and Normal Pregnancy (Control Group – B)

District	Pre–eclamptic Women (Study Group A) N=110		Normal Pregnancy (Control Group – B) N = 110	
Dhaka	68	61.82%	94	85.45%
Manikganj	10	9.09%	3	2.73%
Munshiganj	8	7.27%	4	3.64%
Narayanganj	10	9.09%	3	2.73%
Barisal	6	5.45%	2	1.81%
Others	8	7.27%	4	3.64%
Total	110	100%	110	100%

Table 2 shows that 61.82% of the pre-eclamptic patients were from Dhaka district, compared to normal pregnancy it was 85.45%.

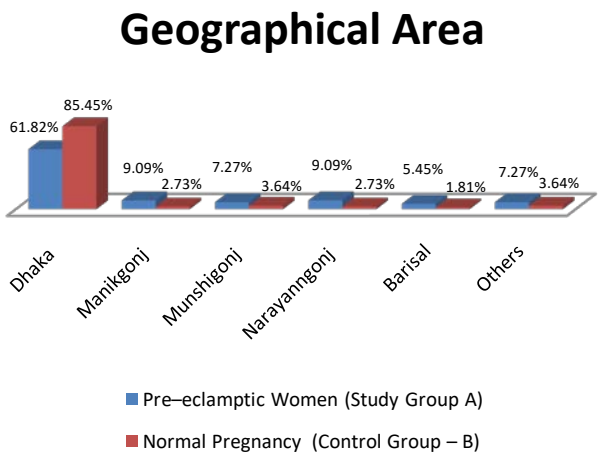


Figure 2: Distribution of respondents according to their geographical area

Table 3: Number and Percentage distribution of Age of Selected Pre-eclamptic Women (Study Group–A) and Normal Pregnancy (Control Group – B)

Age Group	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
18 – 24 Years	62	56.36%	38	34.54%
25 – 30 Years	42	38.18%	62	56.36%
31 and above	6	5.45%	10	9.10%
Total	110	100%	110	100%

Table 3 shows that 56.36% of pre-eclamptic women were in the age group of 18-24 years compared to normal pregnancy where it was 34.54% only.

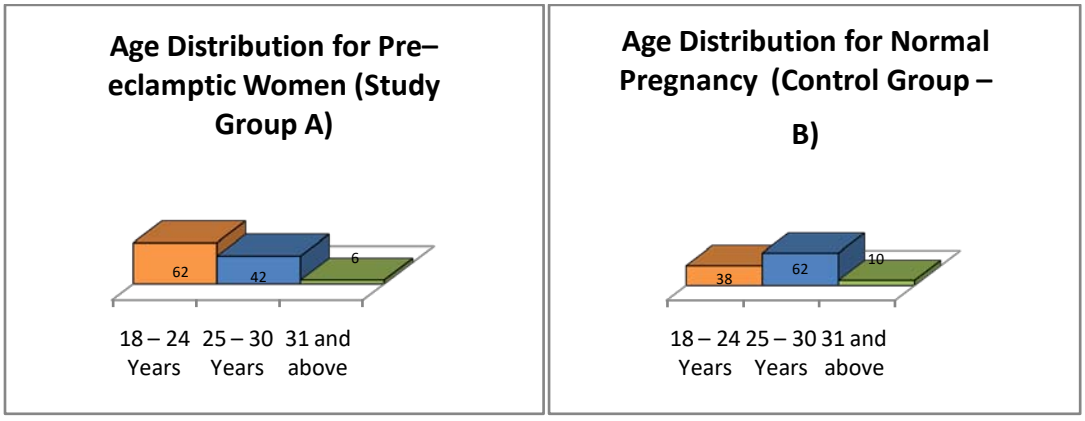


Figure 3: Distribution of Respondents according to their ages

Table 4: Distribution of Respondents according to their religion

Religion	Pre–eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Muslim	100	90.91%	98	89.09%
Hindu	7	6.36%	11	10%
Christian	3	2.73%	1	0.91%
Total	110	100%	110	100%

Table 4 shows that 90.91% of pre-eclamptic women were muslims compared to normal pregnancy where it was 89.09%.

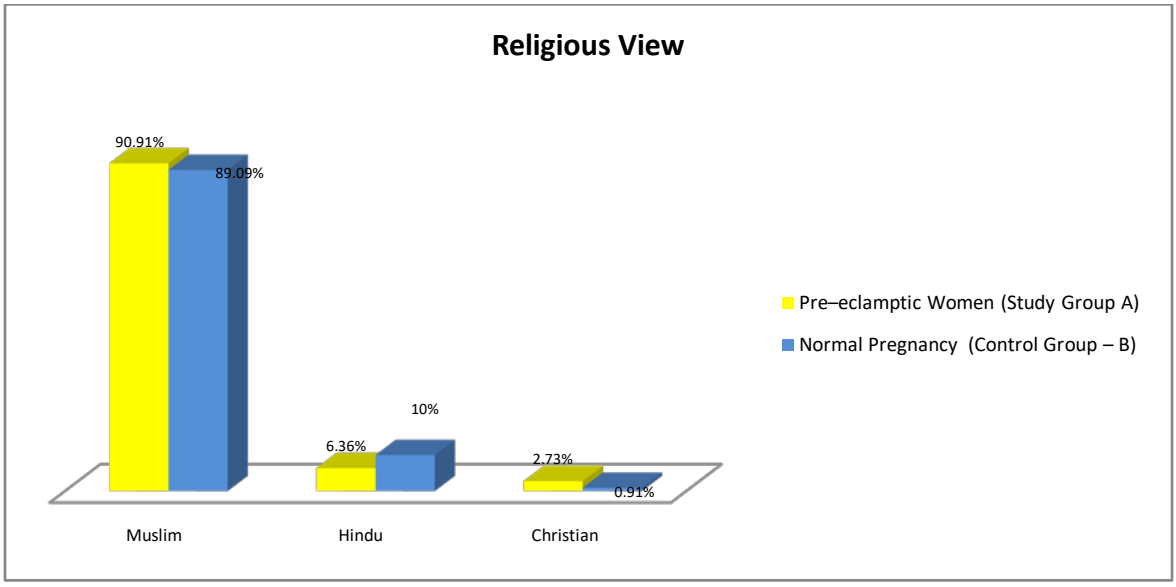


Figure 4: Distribution of Respondents according to their religion

Table 5: Distribution of Respondents according to Residential Status

Residential Status	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Urban	12	10.91%	20	18.18%
Semi – Urban	78	70.91%	80	72.73%
Rural	2	1.82%	6	5.45%
Slum	18	16.36%	4	3.64%
Total	110	100%	110	100%

Table 5 shows that 16.36% of pre-eclamptic women lived in slum area compared to normal pregnancy where it was 3.64% only.

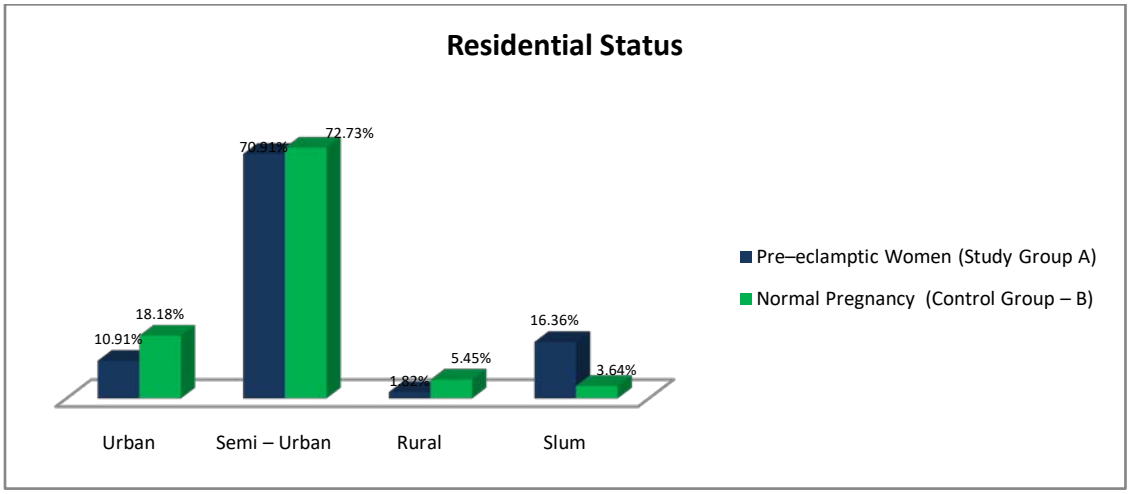


Figure 5: Distribution of Respondents according to Residential Status

Table 6: Distribution of Respondents according to educational qualifications

Education Level	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Illiterate (Sign Only)	50	45.45%	28	25.45%
Less than SSC	26	23.64%	30	27.27%
SSC to HSC	22	20%	32	29.10%
Higher than HSC	12	10.91%	20	18.18%
Total	110	100%	110	100%

Table 6 shows that 45.45% of pre-eclamptic women were illiterate compared to normal pregnancy where it was 25.45% only.

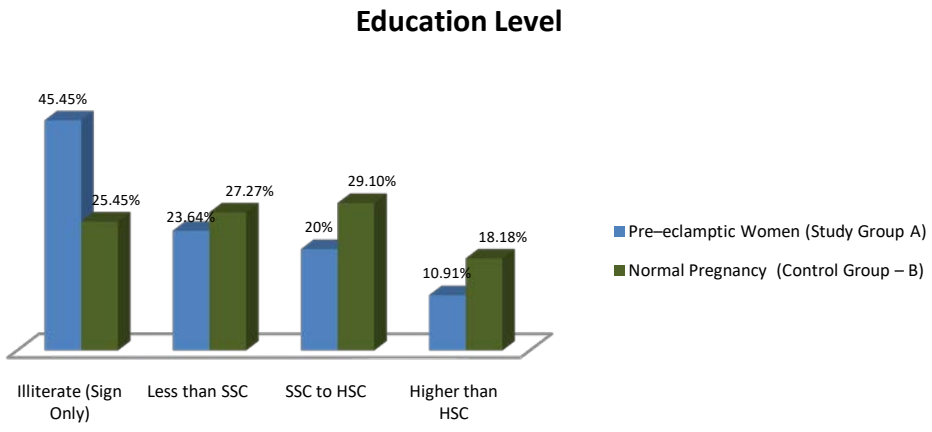


Figure 6: Distribution of Respondents according to educational qualifications

Table 7: Distribution of Respondents according to occupation

Occupation	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Day Labour	18	16.36%	14	12.73%
Skill Labour	12	10.91%	20	18.18%
Agriculture Labor	4	3.64%	2	1.82%
House Wife	62	56.36%	52	47.27%
Others	14	12.73%	22	20%
Total	110	100%	110	100%

Table 7 shows that 56.36% of preeclamptic women were housewives compared to normal pregnancy where it was 47.27% only.

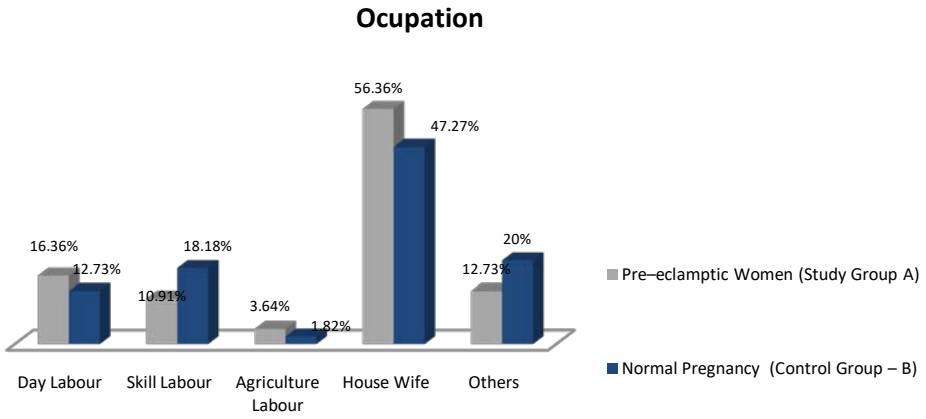


Figure 7: Distribution of Respondents according to occupation

Table 8: Distribution of Respondents according to family size

Family Size	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
2 – 4	40	36.36%	68	61.82%
5 – 6	64	58.18%	39	35.45%
7 – 8	6	5.45%	3	2.73%
Total	110	100%	110	100%

Table 8 shows that 58.18% of pre-eclamptic women family size consists of 5-6 members compared to normal pregnancy where it was 35.45% only.

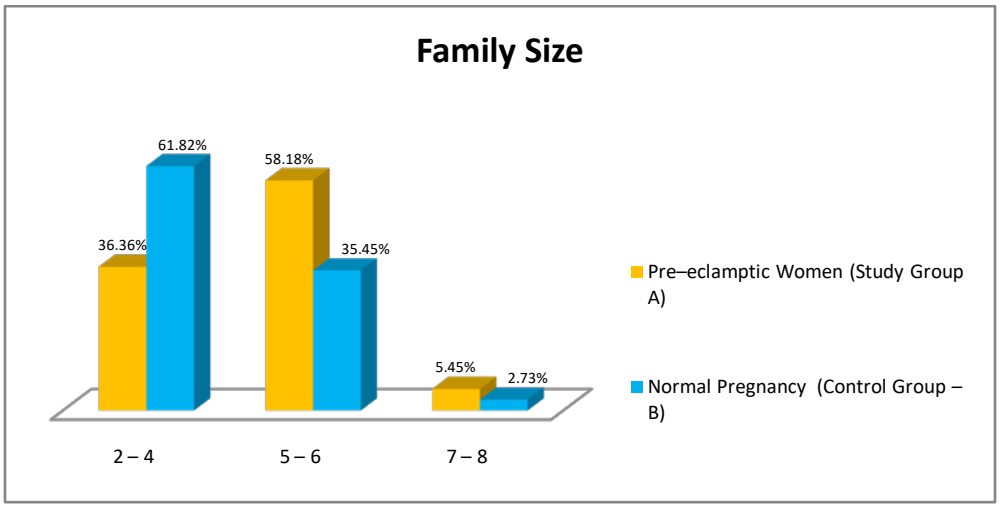


Figure 8: Distribution of Respondents according to family size

Table 9: Distribution of Respondents according to Monthly Income

Monthly Income (Tk.)	Pre – eclamptic Women(Study Group A)		Normal Pregnancy (Control Group – B)	
9000 – 24999 Tk.	40	36.36%	12	10.91%
25000 – 29999 Tk.	54	49.09%	30	27.27%
30000 – 34999 Tk.	12	10.91%	48	43.64%
35000 and above Tk.	4	3.64%	20	18.18%
Total	110	100%	110	100%

Table 9 shows that 36.36% of pre-eclamptic women's monthly income was less than 25000TK compared to normal pregnancy where it was 10.91% only.

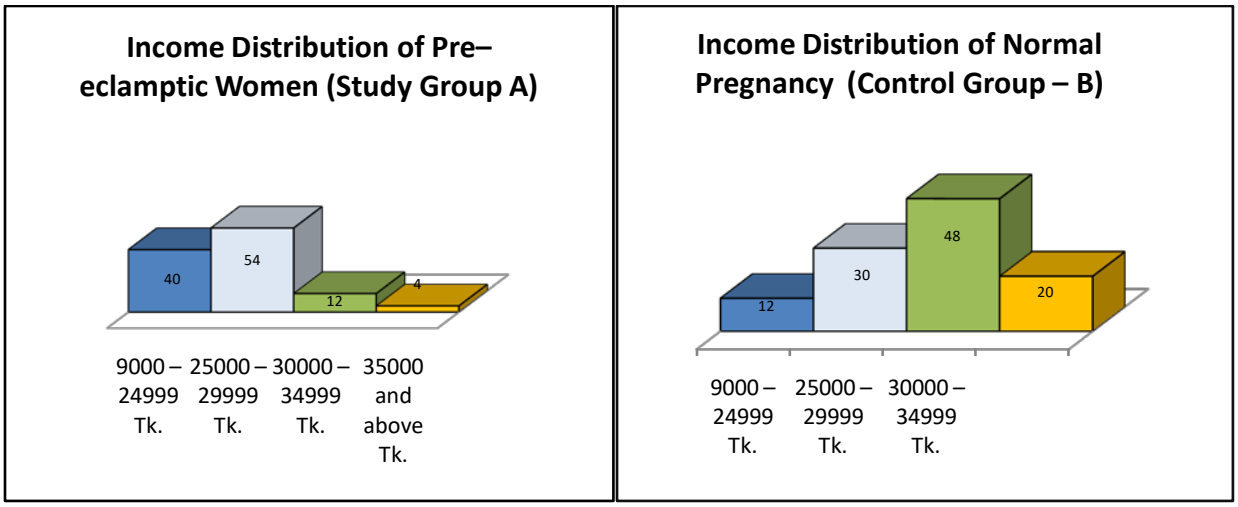


Figure 9: Distribution of Respondents according to Monthly Income

Table 10: Distribution of Respondents according to Age of Marriage

Age of Marriage	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
>15	6	5.45%	2	1.82%
15 – 19	65	59.10%	40	36.36%
20 – 25	35	31.82%	64	58.18%
>25	4	3.63%	4	3.64%
Total	110	100%	110	100%

Table 10 shows that 59.10% of pre-eclamptic women married at the age of 15-19 years compared to normal pregnancy where it was 36.36% only.

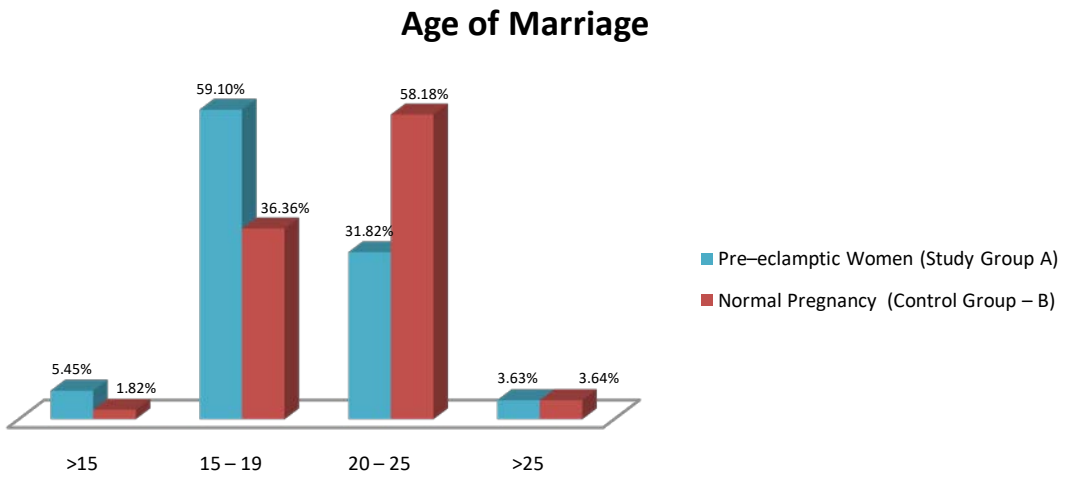


Figure 10: Distribution of Respondents according to Age of Marriage

Table 11: Distribution of respondents according to number of live birth

Live Birth	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
0	34	30.91%	26	23.64%
1	26	23.64%	42	38.18%
2	22	20%	28	25.45%
3 or more	28	25.45%	14	12.73%
Total	110	100%	110	100%

Table 11 shows that 30.91% of pre-eclamptic women were primiparous compared to normal pregnancy where it was 23.64% only.

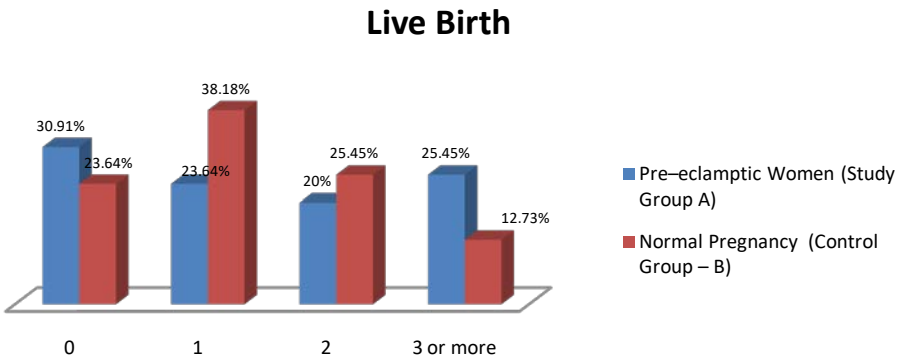


Figure 11: Distribution of Respondents According to Number of Live Births

Table 12 : Distribution of Respondents according to number of abortion

No. of Abortion	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
0	38	34.55%	68	61.82%
1	64	58.18%	36	32.73%
2 or more	8	7.27%	6	5.45%
Total	110	100%	110	100%

Table 12 shows that 58.18% of pre-eclamptic women had one abortion compared to normal pregnancy where it was 32.73% only.

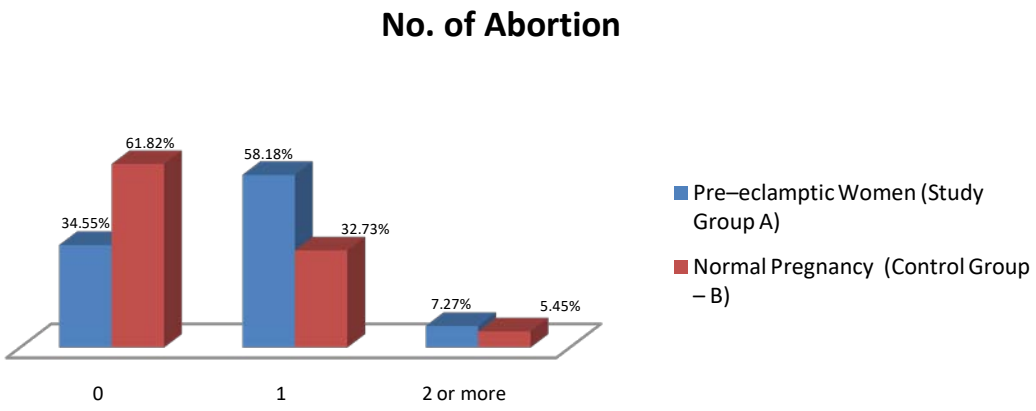


Figure 12: Distribution of Respondents according to number of abortion

Table 13: Distribution of Respondents according to pregnancy number

Total Pregnancy	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
1	44	40%	30	27.27%
2	36	32.73%	52	47.27%
3 and more	30	27.27%	28	25.45%
Total	110	100%	110	100%

Table 13 shows that 40% of pre-eclamptic women had one pregnancy compared to normal pregnancy where it was 27.27% only.

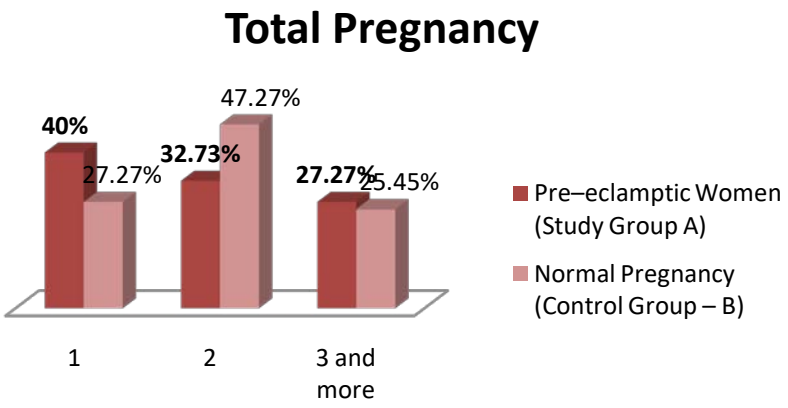


Figure 13: Distribution of Respondents according to pregnancy number

DIETARY INFORMATION & FOOD CONSUMPTION SCORE

CARBOHYDRATE GROUP

Table 14: Distribution of the respondents by their consumption of Rice/Ruti/Bread of selected pre-eclamptic women& Normal pregnancy

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy(Control Group – B)	
Daily (7 Days) / Week	59	53.64%	34	30.91%
Regularly (4 – 6 Days) / Week	45	40.91%	67	60.91%
Irregularly (1 – 3 Days) / Week	6	5.45%	9	8.18%
Never	0	0%	0	0%
Total	110	100%	110	100%

Table 14 shows that 40.91% of pre-eclamptic women consumed carbohydrate regularly compared to normal pregnancy where it was 60.91%.

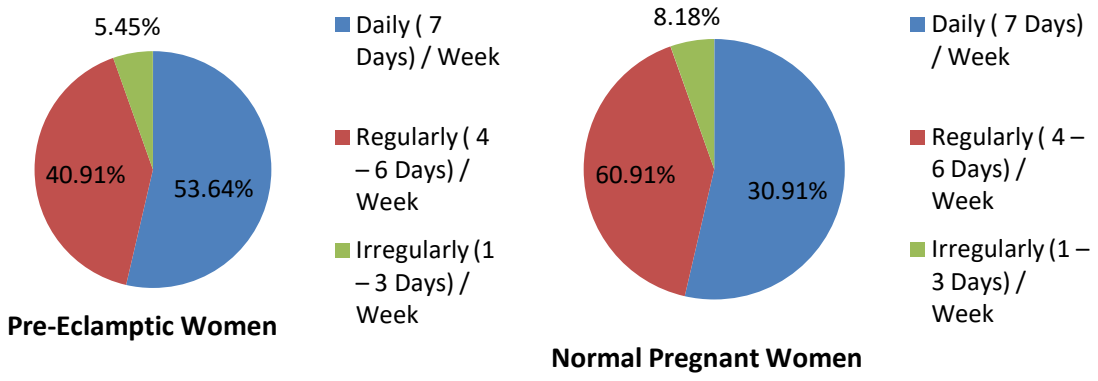


Figure 14: Distribution of the respondents by their consumption of carbohydrate.

Table 15: Distribution of the respondents by their consumption of Chira/Muri of selected pre-eclamptic women (Study Group-A) & Normal pregnancy (Control Group-B)

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	1	0.91%	2	1.82%
Regularly (4 – 6 Days) / Week	3	2.73%	6	5.45%
Irregularly (1 – 3 Days) / Week	94	85.45%	92	83.64%
Never	12	10.91%	10	9.09%
Total	110	100%	110	100%

Table 15 shows that 85.45% of pre-eclamptic women consumed chira-muriirregularly compared to normal pregnancy where it was 83.64%.

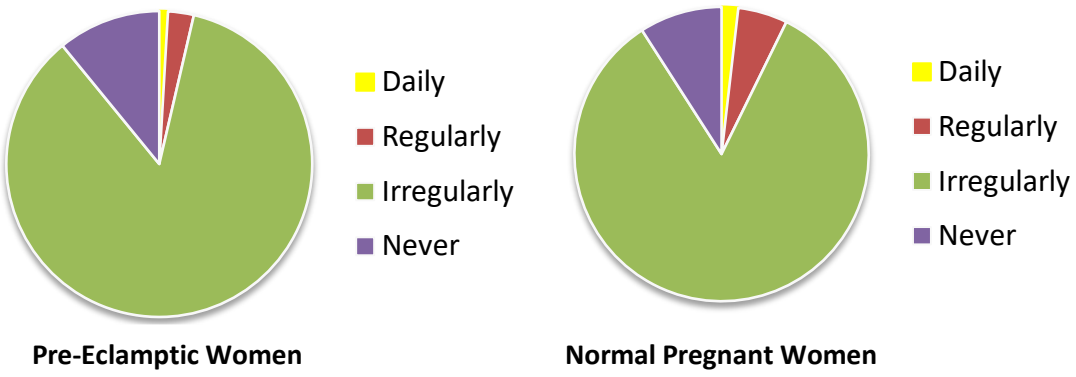


Figure 15: Distribution of the respondents by their consumption of Chira-muri of Pre-eclamptic women and Normotensive Pregnant Women

PROTEIN GROUP

Table 16: Distribution of respondents by their Consumption of Chicken/Beef/Mutton

	Pre-eclamptic Women		Normal Pregnancy	
Daily (7 Days)/Week	2	1.82%	6	5.45%
Regularly (4-6 Days)/Week	8	7.27%	10	9.10%
Irregularly (1-3 Days)/Week	86	78.18%	88	80%
Never	14	12.73%	6	5.45%
Total	110	100%	110	100%

Table 16 shows that 12.73% of pre-eclamptic women never consumed chicken/beef/mutton in a week compared to normal pregnancy where it was 5.45%.

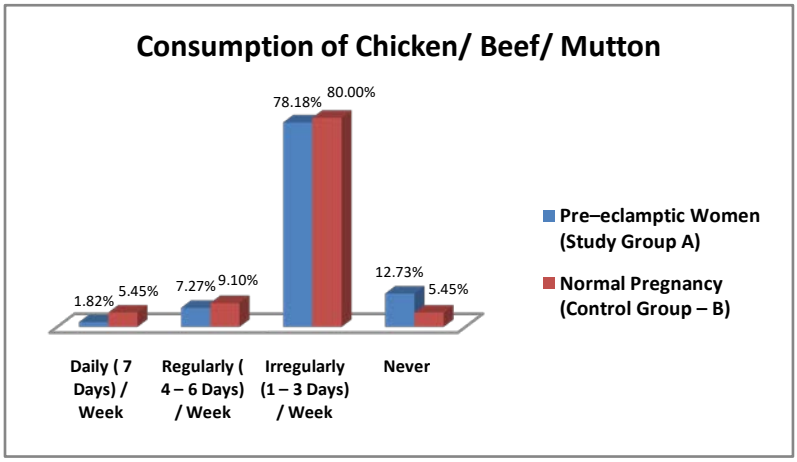


Figure 16: Distribution of respondents by their Consumption of chicken/beef/mutton

Table 17: Distribution of respondents by their Consumption of fish of selected pre-eclamptic women and normal pregnancy

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	0	0%	2	1.82%
Regularly (4 – 6 Days) / Week	4	3.64%	26	23.63%
Irregularly (1 – 3 Days) / Week	89	80.91%	80	72.73%
Never	17	15.45%	2	1.82%
Total	110	100%	110	100%

Table 17 shows that 3.64% pre-eclamptic women consume fish regularly compared to normal pregnant women where it was much higher, 23.63%.

Table 18: Distribution of respondents by their Consumption of Milk & Milk products of selected pre-eclamptic women and normal pregnancy

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	0	0%	4	3.64%
Regularly (4 – 6 Days) / Week	18	16.36%	28	25.45%
Irregularly (1 – 3 Days) / Week	88	80%	76	69.10%
Never	4	3.64%	2	1.82%
Total	110	100%	110	100%

Table 18 shows that 16.36% pre-eclamptic women consume milk regularly compared to normal pregnant women where it was much higher, 25.45%.

Table 19: Distribution of respondents by their Consumption of Lentils/Mug/Coli/Peas of selected pre-eclamptic women and normal pregnancy

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	26	23.63%	28	25.45%
Regularly (4 – 6 Days) / Week	23	20.91%	31	28.18%
Irregularly (1 – 3 Days) / Week	47	42.73%	45	40.91%
Never	14	12.73%	6	5.45%
Total	110	100%	110	100%

Table 19 shows that 42.73% pre-eclamptic women consume lentis/mug/coli/peas irregularly compared to normal pregnant women where it was 40.91%.

FAT GROUP

Table 20: Distribution of respondents by their Consumption of Fats

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	62	56.36%	68	61.82%
Regularly (4 – 6 Days) / Week	31	28.18%	30	27.27%
Irregularly (1 – 3 Days) / Week	9	8.18%	8	7.27%
Never	8	7.27%	4	3.64%
Total	110	100%	110	100%

Table 20 shows that 56.36% pre-eclamptic women consume fat daily compared to normal pregnant women where it was 61.82%.

Table 21: Distribution of respondents by their Consumption of Cooking oil/Butter/Margarine of selected pre-eclamptic women and normal pregnancy

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	62	56.36%	68	61.82%
Regularly (4 – 6 Days) / Week	31	28.18%	30	27.27%
Irregularly (1 – 3 Days) / Week	9	8.18%	8	7.27%
Never	8	7.27%	4	3.64%
Total	110	100%	110	100%

Table 21 shows that 56.36% pre-eclamptic women consume cooking oil/butter/margarinedaily compared to normal pregnant women where it was 61.82%.

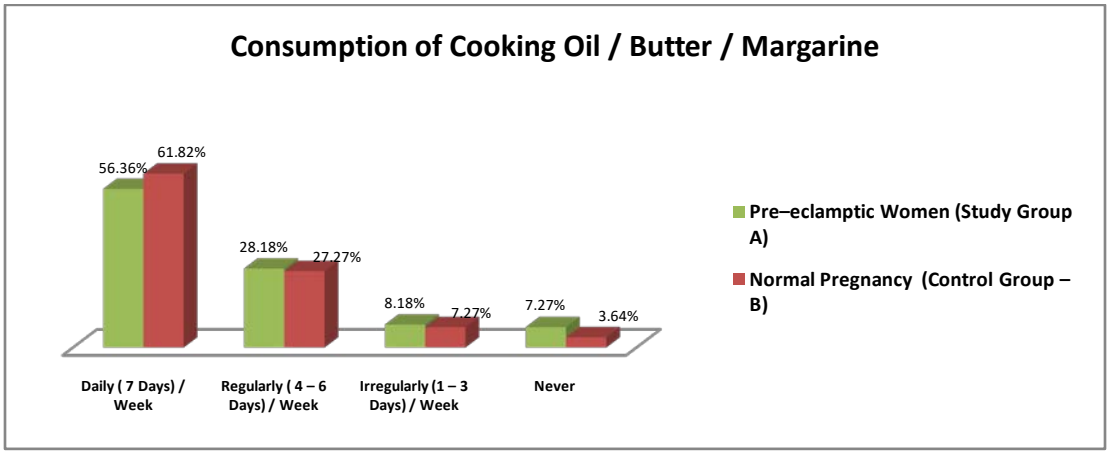


Figure 17: Distribution of respondents by their consumption of cooking oil/butter/margarine

VEGETABLE GROUP

Table 22: Distribution of respondents by their Consumption of Potato/Carrot/Mula/Kochu of selected pre-eclamptic women and normal pregnancy

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	16	14.54%	20	18.18%
Regularly (4 – 6 Days) / Week	20	18.18%	34	30.91%
Irregularly (1 – 3 Days) / Week	62	56.36%	48	43.63%
Never	12	10.90%	8	7.27%
Total	110	100%	110	100%

Table 22 shows that 18.18% pre-eclamptic women consume potato/carrot/mula/kochu regularly compared to normal pregnant women where it was 30.91%.

Table 23: Distribution of respondents by their Consumption of Lal Shak/Pui Shak/Palong Shak of selected pre-eclamptic women and normal pregnancy

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	18	16.36%	12	10.91%
Regularly (4 – 6 Days) / Week	54	49.09%	64	58.18%
Irregularly (1 – 3 Days) / Week	32	29.09%	30	27.27%
Never	6	5.45%	4	3.64%
Total	110	100%	110	100%

Table 23 shows that 49.09% pre-eclamptic women consume lal shak/pui shak/palong shak regularly compared to normal pregnant women where it was 58.18%.

Table 24: Distribution of respondents by their Consumption of Citrus fruits

	Pre – eclamptic Women (Study Group A)		Normal Pregnancy (Control Group – B)	
Daily (7 Days) / Week	2	1.82%	4	3.64%
Regularly (4 – 6 Days) / Week	3	2.73%	26	23.64%
Irregularly (1 – 3 Days) / Week	30	27.27%	39	35.45%
Never	75	68.18%	41	37.27%
Total	110	100%	110	100%

Table 24 shows that 68.18% of pre-eclamptic women never consume citrus fruits compared to normal pregnant women where it was 37.27%.

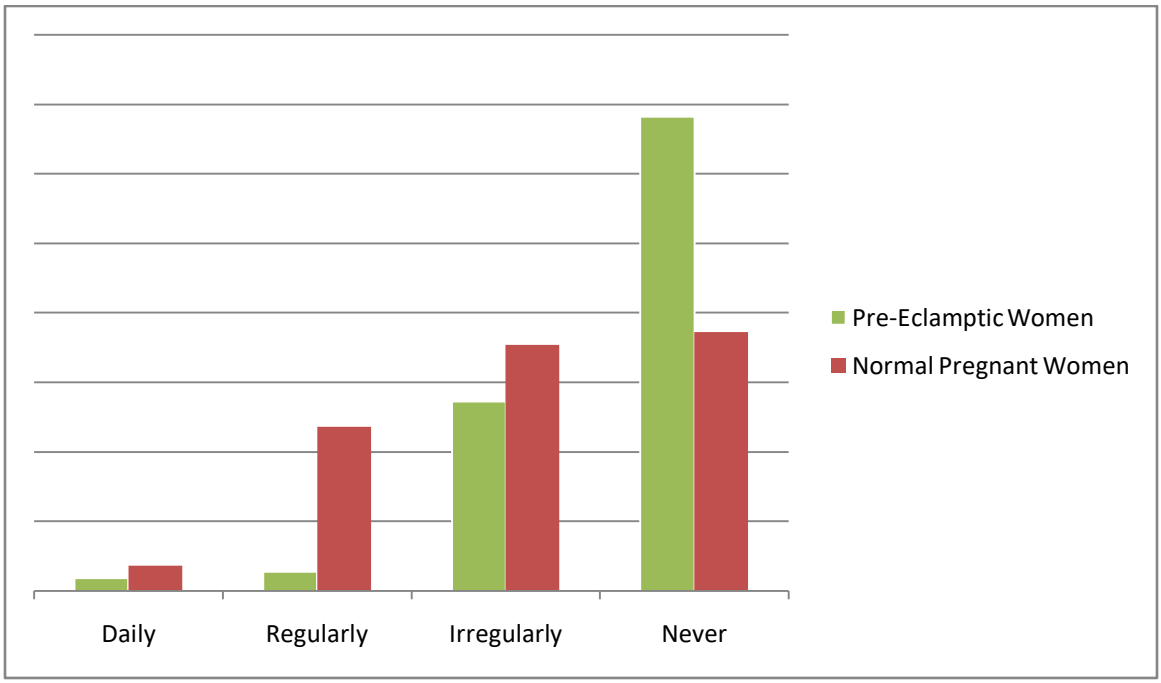


Figure 18: Distribution of respondents by their Consumption of Citrus Fruits

Table 25: Distribution of respondents by Food consumption Score

Food Consumption Score	Pre-eclamptic women (Study Group A) n=110		Normal Pregnancy (Control Group B) n=110	
Poor Food Consumption	13	11.82%	4	3.64%
Borderline Food Consumption	26	23.64%	38	34.54%
Low Acceptable Food Consumption	53	48.18%	42	38.18%
Highly Acceptable Food Consumption	18	16.36%	26	23.64%
Total	110	100%	110	100%

Table 25 shows that 11.82% of pre-eclamptic women were in the poor food consumption group, compared to normal pregnancy where it was 3.64% only.

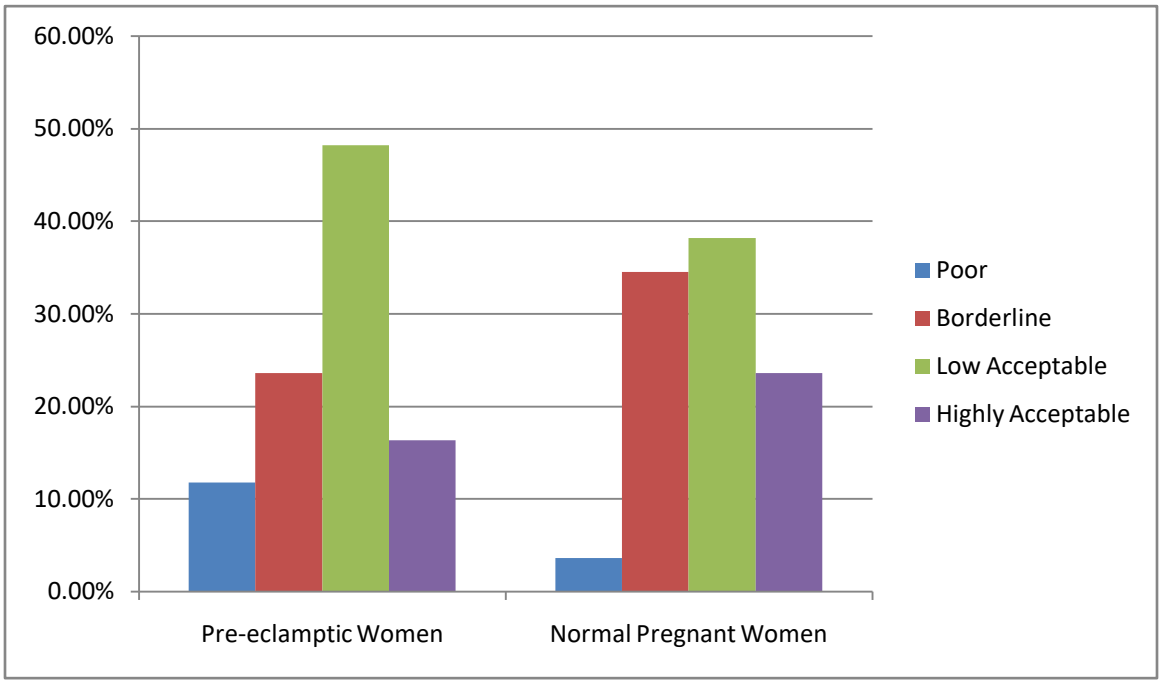


Figure 19: Distribution of respondents by food consumption score

HAEMATOLOGICAL & BIO-CHEMICAL PARAMETERS

Table 26: Value of serum vitamin C in pre-eclamptic and normal pregnant women

	Pre-eclamptic Women N=110		Normal Pregnant Women N=110	
Normal (0.60 – 2 mg/dl)	24	21.82%	88	80%
Below Normal (<0.60 mg/dl)	86	78.18%	22	20%
Total	110	100%	110	100%

Table 26 shows that 78.18% of pre-eclamptic women had serum vitamin C level was below normal, compared to normal pregnancy where it was 20% only.

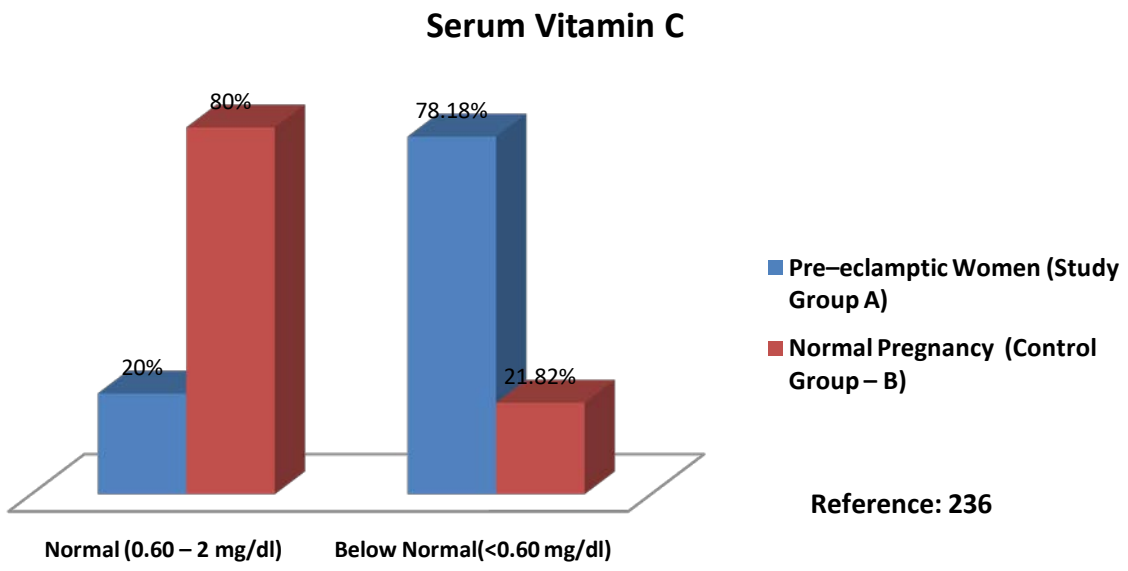


Figure 20: Distribution of respondents by serum vitamin C

Table 27: Mean Value of serum Vitamin C in Pre-eclamptic and normal pregnant Women

N=220	Vit. C (Mean±SD)	P. Value
Preeclamptic Women mg/dl. n=110	0.49±.12	0.001
Normal Pregnancy mg/dl. n=110.	1.24±.39	

Table 27 shows that Mean value of serum vitamin C in case of pre-eclamptic women was0.49±.12 compared to normal pregnancy where it was1.24±.39.

Table 28: Value of serum vitamin E in pre-eclamptic and normal pregnant women

	Pre-eclamptic Women N=110		Normal Pregnant Women N=110	
Normal (500-1800µg/dl)	34	30.91%	88	80%
Below Normal (<500µg/dl)	76	69.09%	22	20%
Total	110	100%	110	100%

Table 28 shows that 69.09% of pre-eclamptic women had vitamin E level below normal compared to normal pregnant women where it was 20% only.

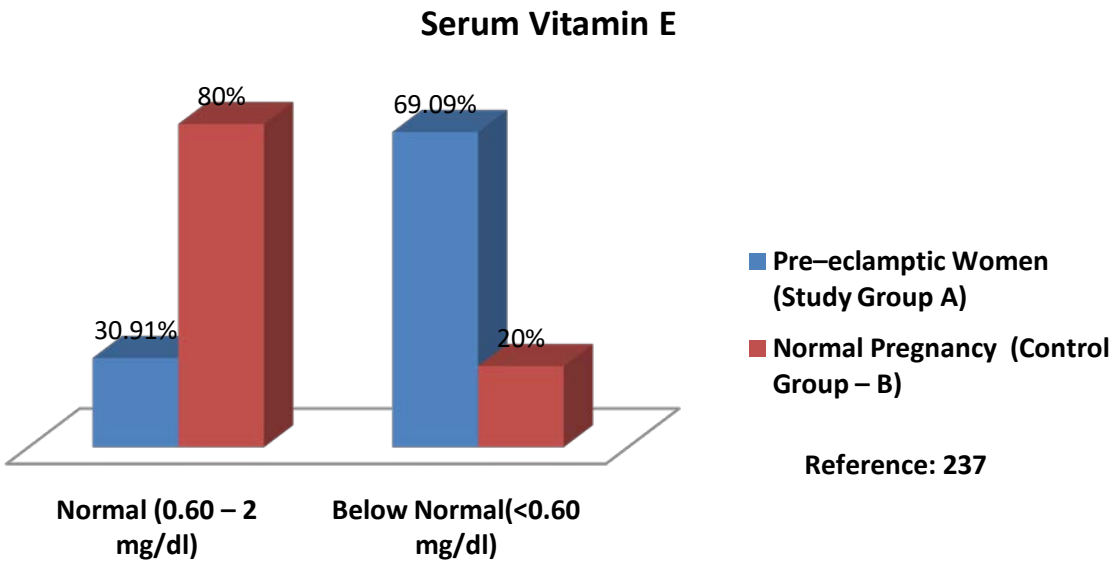


Figure 21: Distribution of respondents by serum vitamin E

Table 29: Mean Value of Serum Vitamin E in Pre-eclamptic and normal pregnant women

N=220	Vit. E (Mean±SD)	P. Value
Preeclamptic Women µg/dl. n=110	359.95±139.27	0.001
Normal Pregnancy µg/dl. n=110.	815.64±281.17	

Table 29 shows that Mean value of serum vitamin E in case of pre-eclamptic women was359.95±139.27 compared to normal pregnancy where it was815.64±281.17.

Table 30: Value of Hemoglobin level in preeclamptic and normal pregnant women.

	Pre-eclamptic Women N = 110			Normal Pregnant Women N=110			
Hb (gm/dl)	(Mean ±SD)	Median	Range	(Mean ±SD)	Median	Range	P. Value
	11.76±0.61	11.60	3.40	12.65±0.37	12.60	2.20	0.002

Reference: 238

Table 30shows that mean hemoglobin level of pre-eclamptic women (11.76 ± 0.61) was significantly lower than the mean hemoglobin level of normal pregnant women (12.65 ± 0.37).

Table 31: Distribution of Urine Albumin Level of the respondents

Urine Albumin	Pre-eclamptic Women N=110		Normal Pregnant Women N=110
(Nil) no cloudiness	0	0%	Nil
Trace	16	14.55%	Nil
Moderate (1+) / Definitive cloud	34	30.91%	Nil
2+ / Heavy and granular cloud	60	54.54%	Nil
Total	110	100%	

Table 31 shows that 54.54% of pre-eclamptic women's urine albumin was 2+/heavy and granular cloud.

Table 32: Anthropometric and clinical indices

	Pre - Eclamptic (Group - A) N = 110			Normal Pregnant Women (Control - B) N = 110			P. Value
	(Mean±STD.)	Median	Range	(Mean±STD.)	Median	Range	
Weight (Kg.) of the Patient	66.65±5.34	67.00	45.00	66.9±2.05	67.00	10.00	0.65
Height (cm.) of the Patient	154.06±3.58	152.40	20.32	156.003±3.36	157.48	15.24	0.62
MUAC (CM)	28.1±2.24	28.23	16.47	27.5±1.06	27.42	5.03	0.004
Systolic Blood Pressure (mm/Hg)	125.14±28.34	105.00	80.00	117.27±4.47	120.00	10.00	0.001
Diastolic Blood Pressure (mm/Hg)	98±5.55	95.00	20.00	79±3.51	80.00	20.00	0.001
Weight of Babies (Kg)	2.09±0.13			1.80±0.12			0.001

Table 32 shows that there was significant difference between mean weight of newborn babies of pre-eclamptic and normal pregnant women.

Table 33: Relation Between Age Group, Vitamin C (Ascorbic Acid mg/dl),Vitamin E (Alpha-Tocopherol ug/dl)

Age Group	(Mean ± STD.)	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P. Value
			Lower Bound	Upper Bound			
Vit. C 18 - 24 Years	0.48+0.1	0.20	0.44	0.52	0.28	0.72	0.85
25 – 30 Years	0.5+0.15	0.04	0.42	0.59	0.26	0.73	
31 above	0.5+0.12	0.05	0.39	0.62	0.36	0.63	
Vit. E 18 - 24 Years	331.12+130.95	24.75	280.34	381.9	223.1	612.14	0.11
25 - 30 Years	422.39+142.18	36.71	343.65	501.12	237.20	570.24	
31 and above Years	341.48+141.61	53.52	210.51	472.45	238.60	570.64	

Table 33 shows that there was no significant difference between age group and Vitamin C and Vitamin E.

Table 34: Relation between Hemoglobin and Age Group of Selective pre-eclamptic women

Age Group	(Mean±SD)	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P. Value
			Lower Bound	Upper Bound			
Hb % 18 - 24 years	11.87±.62	0.09	11.69	12.05	10.8	13.2	0.14
25 - 30 years	11.74±.62	0.11	11.52	11.97	10.8	13.2	
31 and above years	11.59±.56	0.10	11.38	11.8	9.8	12.6	

Table 34 shows that there was no significant difference between mean hemoglobin levels among different age group of pre-eclamptic women.

Table 35: Relation between Hemoglobin and Age Group of Selected normal pregnant women

Age Group	(Mean±SD)	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P. Value
			Lower Bound	Upper Bound			
Hb 18 - 24 Years	12.72±.32	0.05	12.61	12.83	12.2	13.4	0.32
25 - 30 Years	12.63±.39	0.05	12.52	12.73	11.2	13.2	
31 and above Years	12.57±.33	0.09	12.36	12.77	12.2	13.2	

Table 35 shows that there was no significant difference between mean hemoglobin levels among different age group of normal pregnant women.

Table 36: Relation between Hemoglobin and Parity of Selected pre-eclamptic women

Parity	(Mean±SD)	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P. Value
			Lower Bound	Upper Bound			
Hb% Parity 1	11.82±.54	0.09	11.64	12	11.00	13.20	0.43
Hb Parity 2 or more	11.73±.65	0.07	11.58	11.88	9.80	13.20	

Table 36shows that there was no significant difference in mean Hb% level among different parity of pre-eclamptic women.

Table 37: Relation between Hemoglobin and Parity of Selected normal pregnant women

Parity	(Mean±SD)	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P. Value
			Lower Bound	Upper Bound			
Hb% Parity 1	12.84±.42	0.10	12.61	13.02	12.00	13.40	0.32
Hb% Parity 2 or more	12.62±.35	0.03	12.55	12.70	11.20	13.20	

Table 37shows that there was no significant difference in mean Hb% level among different parity of normal pregnant women.

MORPHOLOGICAL STUDY OF PLACENTA

Table 38: Placental Weight (gm) of pre-eclamptic and normal pregnant women

	Pre-eclamptic Women (Group-A N=110	Normal Pregnant Women Control Group – B N=110	P. Value
	(Mean±SD)	(Mean±SD)	
Wt. of Placenta (gm.)	404.80±4.04	486.96±1.62	0.001

Table 38 shows that mean placental weight of pre-eclamptic women (404.80 ± 4.04) was significantly lower than the mean placental weight of normal pregnancy women (486.96 ± 1.62).

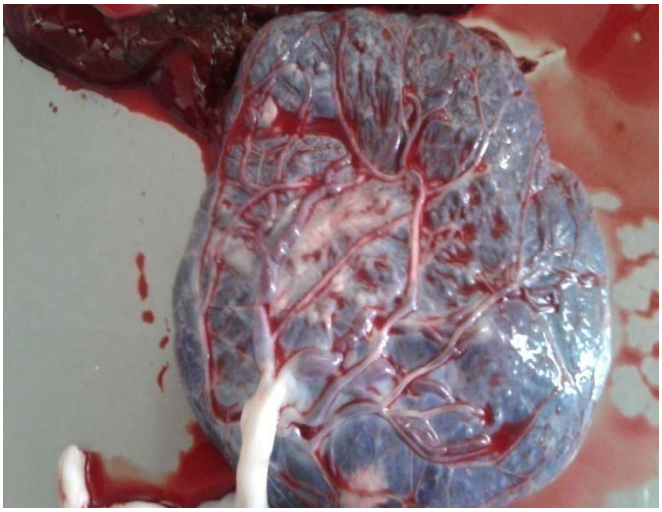
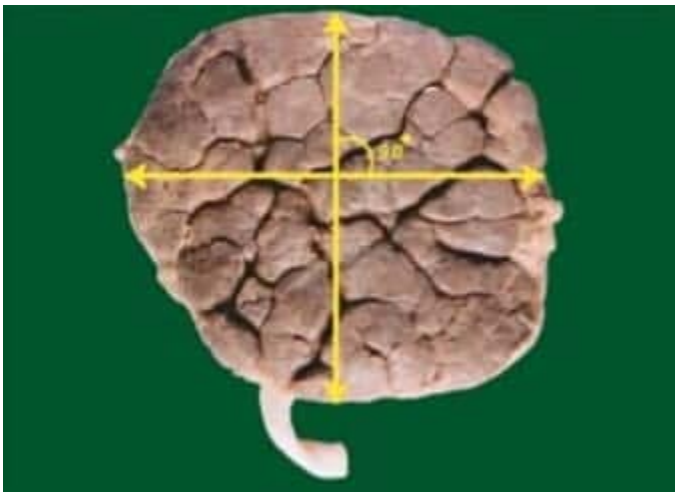


Table 39: Diameter (cm) of Placenta of pre-eclamptic and normal pregnant women

	Pre-eclamptic Women (Group-A N=110	Normal Pregnant Women Control Group – B N=110	P. Value
	(Mean ±SD)	(Mean ±SD)	
Placenta Diameter (cm.)	15.88 ± 0.13	18.22 ± 0.79	0.001

Table 39 shows that mean placenta diameter of pre-eclamptic women (15.88 ± 0.13) was significantly (p-value .001) lower than the mean placental diameter of normal pregnancy women (18.22 ± 0.79).

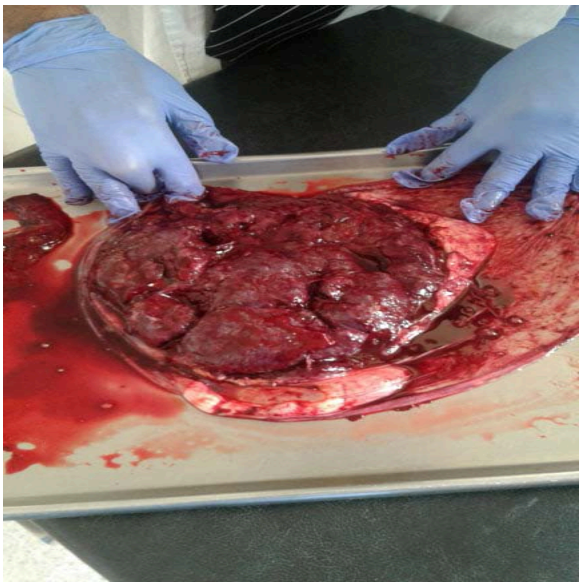


Diameter of placenta

Table 40: Number of Cotyledons of of pre-eclamptic and normal pregnant women

	Pre-eclamptic Women (Group-A N=110	Normal Pregnant Women Control Group – B N=110	P. Value
	(Mean ±SD)	(Mean ±SD)	
No of Cotyledon (Nos.)	16 ± 0.78	17.10 ± 0.89	0.002

Table 40 shows that mean no. of cotyledon (Nos.) of pre-eclamptic women (16 ± 0.78) was significantly lower than the mean no. of cotyledon (Nos.) of normal pregnant women (17.10 ± 0.89).



Cotyledons of placenta

Table 41: Number of Infarcted Areas of pre-eclamptic and normal pregnant women

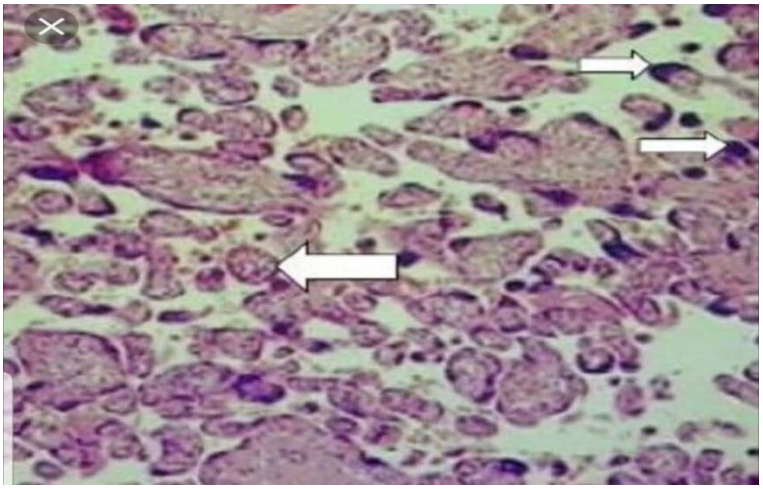
	Pre-eclamptic Women (Group-A N=110	Normal Pregnant Women Control Group – B N=110	P. Value
	(Mean ±SD)	(Mean ±SD)	
No. of Infracted Area in Placenta	16.02 ± 0.80	4.02 ± 0.80	0.001

Table 41shows that mean no. of infracted area in placenta of pre-eclamptic women (16.02 ± 0.80) was significantly (p-value .001) higher than the mean no. of infracted area in placenta of normal pregnancy women (4.02 ±0.80).

Table 42: Mean No. Areas of Syncytial Knot Formation in pre-eclamptic and normal pregnant women

	Pre-eclamptic Women (Group-A) N=110	Normal Pregnant Women Control Group – B N=110	P. Value
	(Mean ±SD)	(Mean ±SD)	
Mean No. Areas of Syncytial Knot Formation	26.76 ± 3.86	9.60 ± 1.46	0.001

Table 42 shows that mean no. areas of syncytial knot formation of pre-eclamptic women (26.76 ± 3.86) was significantly (p-value .001) higher than the mean no. areas of syncytial knot formation of normal pregnant women (9.60 ± 1.46).

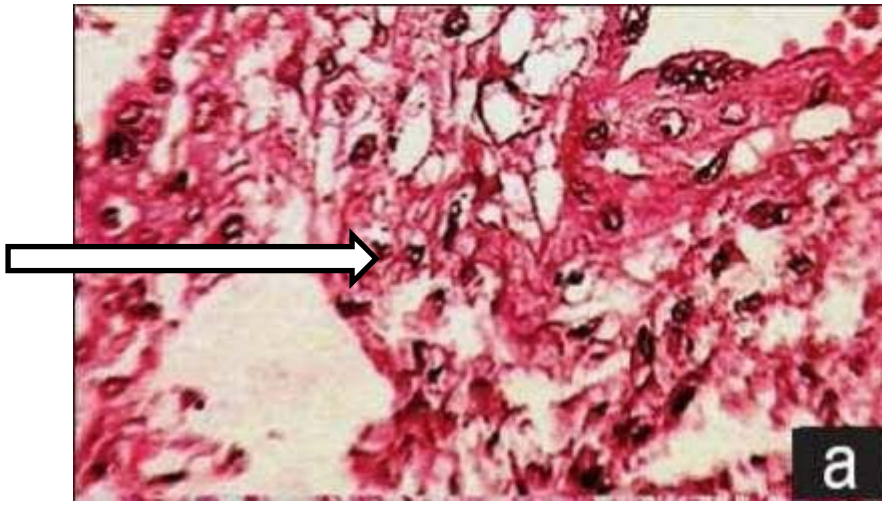


Syncytial knot formation

Table 43: Mean No. Areas of Cytotrophoblastic Cell Proliferation in pre-eclamptic and normal pregnant women

	Pre-eclamptic Women (Group-A) N=110	Normal Pregnant Women Control Group – B N=110	P. Value
	(Mean ±SD)	(Mean ±SD)	
Mean No. of Areas of Cytotrophoblastic Cell Proliferation	21.52 ± 5.03	7.16 ±.2.06	0.001

Table 43 shows that mean no. of areas of cytotrophoblastic cell proliferation of pre-eclamptic women (21.52 ± 5.03) was significantly (p-value .001) higher than the mean no. of areas of cytotrophoblastic cell proliferation of normal pregnancy women (7.16 ±.2.06).

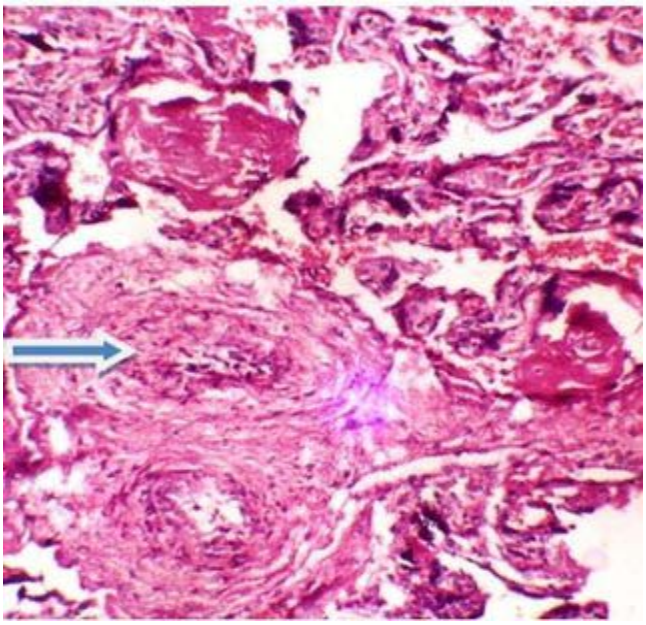


Cytotrophoblastic cell proliferation

Table 44: Mean No. of Area of Fibrinoid Necrosis in pre-eclamptic and normal pregnant women

	Pre-eclamptic Women (Group A) N=110	Normal Pregnant Women Control Group – B N=110	P. Value
	(Mean ±SD)	(Mean ±SD)	
Mean No. of Area of Fibrinoid Necrosis	10.68 ± 3.33	2.24 ± 0.69	0.001

Table 44 shows that mean no. of areas of fibrinoid necrosis of pre-eclamptic women (10.68 ± 3.33) is significantly (p-value .001) higher than the mean no. of areas of fibrinoid necrosis of normal pregnancy women (2.24 ± 0.69).



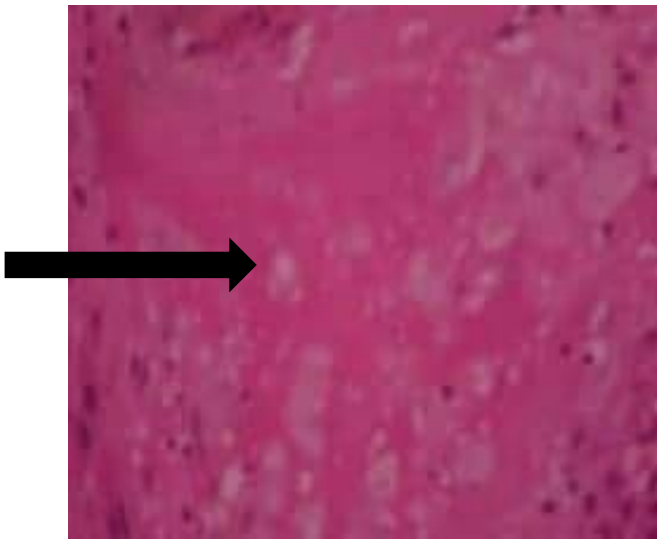
Fibrinoid Necrosis

Table 45: Mean number of Areas of Hyalinised Villi. Of Pre-eclamptic and normal pregnant women

	Pre-eclamptic Women (Group-A) N=110	Normal Pregnant Women Control Group – B N=110	P. Value
	(Mean ±SD)	(Mean ±SD)	
Mean No. of Area of Hyalinised Villi	9.46 ± 4.10	2.32 ± 0.59	0.001

Table 45 shows that mean no. of areas of hyalinised villi of pre-eclamptic women (9.46 ± 4.10) was significantly (p-value .001) higher than the mean no. of areas of hyalinised villi of normal pregnancy women (2.32 ± 0.59).

Area of hyalinization



Chapter Four

Discussion

CHAPTER FOUR

DISCUSSION

The present study was conducted amongst 220 pregnant women to see the effect of antioxidants (vitamin C and E) on pre-eclampsia and histo-morphological changes in placenta of pregnant women,and also investigate the relationship between pre-eclampsia with various socioeconomic factors, environmental factors and nutritional status in selected population of Bangladesh. But so far, the nutritional statuses of pre-eclamptic women have never been properly evaluated.

In this study 56% of Pre- eclamptic women were in the age group of 18-24 years. It was observed that 45% of pre- eclamptic patients were illiterate, 36% had a low income, and 29.09% was malnourished. Comb-Orme et al., 1993; and several others observed that risk factors associated with pre-eclampsia are poor housing, low income, poor nutrition and cultural deprivation.

Reddy et al., 1984 and several others (Joyti and Meenawat., 1984) reported in their studies, a significant relationship between parity and toxemic condition during pregnancy. Their research showed that 61% of toxemic mothers were primiparous, while this study also showed that 30% of pre- eclamptic women were primiparous.

This study shows that the mean placental weight in pre-eclamptic women was lower(404.80 gm) than normal pregnant women (486.96 gm).Other studies indicated similar findings. A study was conducted by Segupta Kishwara, Abu Sadat, Shamim Ara[26] and others, in the Department of Anatomy, Dhaka Medical College, in 2010. They observed lower placental weight in pre-eclampsia compared to normal pregnancy .Several other studies assesing the effect of hypertension on pregnancy have demonstrated the weight of the placenta to be moderately or severely reduced moderate pre-eclamptic and eclamptic pregnancies[27,28,29,30]. In his studies, Cibils[31] found that in hypertensive patients, the overall size of the placentas were considerably reduced, which indicated that the growth process was interrupted by high blood pressure, whereas in another study, Shah et al[32] noticed that with increasing severity of pre-eclampsia, the

weight of placenta was further reduced. Teasdale[33] ,Sodhi et[34] , Barua[35] and Begum[36] also observed a reduction of placental weight in pre-eclampsia . According to Fox[27], Jones[30]and Soma et al[28], the histomorphological abnormalities in hypertensive placenta are due to occlusion or narrowing of the uteroplacental vessels as well as placental ischemia.

In pre-eclampsia the balance between formation of free radicals, and antioxidant mediated defense is disrupted, leading to abnormalities of endothelial function and free radical-mediated endothelial cell injury[37,38] .Some studies corroborate that the serum levels of antioxidants such as vitamin C ,Vitamin E ,are reduced in pre-eclamptic women [37,38,39] .

In a study, Mallik, Mirchandani and Chitra[41], Udainia and Jain[42], Majumdar *et*al[43] and Artico *et* al[44] found reduced placental weight in pre-eclampsia. In pre-eclampsia, birth weights of newborn babies were significantly lower than normal birth weight of normotensive mothers observed by Daminia[45], Fox[46], Kalousek and Langlosis[47] and Majumdar *et al*[43]. In a study, Sanin *et al*[48] found reduced birth weight in pre-eclamptic women and Mirchandani, Malik and Chitra[49], Masodkar, Kalamkar and Patke[50] and Avasthi *et al*[51] also observed increased stillbirth associated with pre-eclampsia. Many studies confirm that levels of antioxidants such as Vitamin C and Vitamin E were reduced in pre-eclamptic women[52,53,54]. Sagol *et al* also observed the same[55]. There is evidence that decreased concentration of antioxidants such as Vit. C and Vit. E in pre-eclamptic women compared to normal pregnant women[56].

Placental infraction was more in case of pre-eclamptic women compared to normal normotensive pregnant women[57]. It was also observed by Mirchandani *et al*[58] and Masodkar *et al*[59].

It was observed that the mean diameter of placenta (15.88 cm), the number of cotyledons (16) was less and the number of infarcted areas was increased (16.02) in case of pre-eclamptic women compared to normal pregnant women were (18.22 cm), (17) and (4.02) respectively. Segupta kishwara et al., (2010); and several others (Abu Sadat Mohammad Nurunnabi, Mahamuda Begum, Abu Rayhan, Shamim Ara)[26] observed that the mean diameter of placenta

, (16.08 ± 2.08 cm), mean number of cotyledons were (14.30 ± 2.47) was less in the study group ,compared to the control group (18.80 ± 2.32cm) and (15.77 ± 2.80) respectively. They also observed increased number of infarcted areas, 15, in the placenta of study group, compared to the control group,which were only 4.

This study also reveals that mean number of areas of syncytial knot formation (26.76),cytotrophoblastic cell proliferation (21.52), area of fibrinoid necrosis(10.68), and hyalinised villi (9.46) were increased in case of pre-eclamptic women, compared to normal pregnancy which were (9.60), (7.16) (2.24) and (2.32) respectively. M. Akhlag,. 2012; and others (AH Nage, AW Yousuf) also observed increased syncitial knot formation (25.23±1.23) in pre-elemptic women compared to normal pregnant women. Other studies have also found similar results, with increased syncytial knots formation(26.31±2.72); cytotrophoblastic cell proliferation (22.53±1.74) fibrinoid necosis (9±2.96) and hyalinized villi (8.96±2.42), in pre-eclamptic patient.

Serum Vit C was below normal in 78.18% of pre-eclamptic women compared to normal pregnant women, where it was below normal in only 20% cases. In this study, mean serum vit C was (0.49 ± .12) mg/dl in the study group, and (1.24+.39) mg/dl in the control group. Suryakant Nagtilak., (2014)[60]; observed that mean serum vit C was (0.49±0.23) mg/dl in pre eclamptic women and (0.82 ± 0.41) mg/dl in normal pregnant women. A study was conducted by Lucy C Chappell, 1999 and others (Paul T seed, Annette Briley) where they observed low levels of Vit C in pre –eclamptic women. Anant S Gupta and TB Sharma also found low level of Vit C in pre-eclamptic women. Suryakant Nagtilak., 2014[60]; observed that mean serum vit C was lower in pre-eclamptic women compare to normal pregnant women. A study was conducted by lucy C Chappell, 1999 and others (Paul T seed, Annette Briley) where they observed low levels of Vit C in pre-eclamptic women. Anant S Gupta and TB Sharma also found low level of Vit C in pre-eclamptic women. Suryakant Nagtilak., 2014[60]; observed that meanserum Vit E levels was lower in study group compare to control group. Kharbs Gulati singh, and Yanik FF et al., 1998; also observed lower serum Vit E in pre-eclamptic women compared to normal pregnant women. K Devi Shankar and others observed that there is a reduced placental weight, placental thickness, placental

diameter and neonatal weight in pre-eclamptic women compared to normal pregnant women[62]. In another study, it showed that there is an increase in syncytial knot formation in pre-eclamptic women compared to normal pregnant women[63,64,65]. In another study they found increased fibrinoid necrosis in pre-eclamptic women compared to normal pregnant women[66,67,57,59].

In this study, serum Vit E was below normal in 69.09% of pre-eclamptic women compared to normal pregnant women which was only 25.45%. Mean Vit E levels was (359.95µg/dl) in study group and (815.64µg/dl) in control group.

Suryakant Nagtilak., (2014)[60]; observed that mean serum Vit E levels was (660±260) µg/dl in study group and (820±260) µg/dl in control group. Kharbs Gulati singh[61], and Yanik FF et al., (1998); also observed lower serum Vit E in pre-eclamptic women compared to normal pregnant women.

In this study mean haemoglobin level of pre-eclamptic women was 11.76 gm/dl compared to normal pregnant women which was 12.65 gm/dl. Shah et all,. (2010); and others (Cibil LA, Teasdale, Barua R. and Begum) observed reduced haemoglobin levels (9.5±2.57)g/dl in pre eclamptic women, compared to normal pregnant women. In this study, majority of pre- eclamptic women were anaemic.

CONCLUSION

This was a comparative study to evaluate the effects of pre-eclampsia on the placenta and the corresponding serum levels of antioxidants C and E. Pre-eclampsia is thought to be caused by build-up of oxidative stress in the chorionic villi, which leads to endothelial dysfunction.

It was revealed that most of the pre-eclamptic women did not consume sufficient amount of food rich in ascorbic acid (Vit C) and alfa tocopherol (Vit. E) and their corresponding blood levels of Vit C and E were also lower.

Moreover changes were observed in the pre-eclamptic placenta. The placentas were smaller in size, less weight had fewer numbers of cotyledons and

there were increased number of Cyncitial Knot Formation, Cytotrophoblastic Cell Proliferation, Fibrinoid Necrosis and Hyalinised Villi on microscopic examination.

This study therefore indicates a positive co-relation between deficiency of antioxidants in blood and development of pre-eclampsia with histological and morphological changes in the placenta.

LIMITATIONS OF THE STUDY

This study had the following limitations:

- We used Food Frequency Questionnaire for dietary assessment, which may have underreporting and over reporting biases as the information are dependent on the memory of the respondents.
- We did not determine the validity and reproducibility of the Food Frequency Questionnaire.
- Most of the pre- eclamptic and normotensive pregnant women were from one district (Dhaka), and they may not have been a complete representation of the entire population of Bangladesh.
- A larger sample size would have been more conclusive.

RECOMMENDATIONS

Based on the study the following recommendations were put forward:

- Women should be properly educated about the importance of ante-natal check-up.
- Women and men should be educated about pre-eclampsia the preventive measures, as well as the consequences.
- Special emphasis should be given on the importance of anti-oxidants containing food like citrus fruits, small and big fish, in the diet of pregnant women.

- Improvement of nutritional status by proper diet and nutritional education.
- They should be aware of their health and daily needs.
- To reduce low birth weight, pre-eclamptic women should be treated timely and properly.
- Future study with larger sample size, longer follow up should be conducted to ascertain the results.

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Appendix

Questionnaire

QUESTIONNAIRE

Date:

Name of the Hospital: [1] DMCH [2]Mittford Hospital

[3]HFRCMH

A.Identification

1.Name of the Patient:

2.Sex:

3.Age of the Patient:

4.Religion: []Muslim []Hindu []Christian []Buddhist []Others

5. Residential Status: []Rural []Urban []Semi Urban []Slum

6. Present Address:

7.Permanent Address:

8.Educational Qualification: []Illiterate []Can read and write

[]Primary High School []Graduate and above

9.Occupation: []Day Labour []Skill Labour

[]Agriculture Labour []House wife [] Others

10.Smoking habit: [] Yes []No

11. Chewing of betel leaf: []Yes []No

B. Socio Economic History

1. Total Family members:

2.Monthly Family Income:

3.No of earning family members:

4. Sources of earning:

[]Agriculture []Service []Pension

[]Business []House Rent []Contribution from son&daughter

Expenditure:

Monthly Expenditure on food:

Monthly Expenditure on education:

Monthly Expenditure on Health:

C.Marital Status &Contraceptive history

1.Age at Marriage: []<15 []15-19 []20-25 []>25

2.Number of Still birth:

3.Number of Abortion:

4.Total pregnancy:

5.Age of last child:

6.Child died within 1-12 month after birth: []Yes []No

7.Did you face any problems in previous pregnancy? If yes mention it……
……..

8.Have you ever used the contraceptive: [] Yes []No

If yes please cheek following:

a.Injection [] Monthly [ ] Yearly

b.Oral contraceptive pills(OCP) [] Monthly [ ] Yearly

c.Barrier methods (Condom/Diaphragm)[] Yes []No

d.Others.

D.Antenatal Care:

- Have you visited a doctor during pregnancy? []Yes []No
- If yes how many times? _____
- Have you taken any injection during pregnancy []Yes []No
- Are you taking any medicine?[]Yes []No If yes mention it

  ……
  ……
  ……..
- Do you have any other complain during pregnancy such as:

  []Swelling of the legs

  []Seizure

  []Lower abdominal pain

  []Vomiting

  []Blood in the urine

  []Others

E.Anthropometric and Clinical Data:

Patients name……..

ID No:

Age:_____Years

Sex: _____

Pulse: _____ /Minute

Blood pressure(BP): _____ mmHg

Height: _____cm Weight: _____kg

BMI= Weight in Kg/Height in Meter^2=

MUAC: _____mm

General Examination: (Tick or Encircle the Correct Answer)

Hair a.Sparse

b.Discoloured

Eye a.pallor

b.Night Blindness

c.Bitots Spot

d. Corneal xerosis

e.Keratomalacia

f.Corneal opacity

Lips a.Angular scars

b.Angular stomatitis

c.Cheilosis

Gums a.Swollen red papillae

b.Marginal redness

c.Bleeding gums

Teeth	a.Dental caries
	b.Dental decay
Tongue	a.Raw and red
	b.Papillary atrophy
Glands	a.Enlarged thyroid
	b.Enlarged parotid
	c.Enlarged toncil
Skin	a.Follicular hyperkeratosis
	b.Dermatosis
Nail	a.Kolionychia
	b. Clubing
Others	a.Marasmus
	b.Kwashiorkor
	c.Mild PEM

Systemic examination

- Heart
- Lungs
- Liver
- Spleen
- Kidney

F. Bio Chemical estimation

- Complete Blood Count
- Determination of Serum Glucose (fasting)
- Measurement of Urinary protein
- Estimation of Serum Anti-oxidant, Vit C,Vit E
- Histopathology of placenta

Food Consumption pattern:

Food Group	Food Items	Frequency 1.Daily(7days/week) 2.Regularly (4-6days/week) 3. Irregularly(1-3days/week) 4.Never	Quantity 1 .Sufficient 2.Moderately Sufficient 3.Not Sufficient (feels hungry)
Cereal	Rice/Ruti/Bread		
	Chira/Muri		
Roots &Tuber	Potato/Carrot/ Mula/Kachu		
Pulses	Lentils/Mug/ Coli/Peas		
Leafy Vegetables	Lalshak/Puishak/ Palong shak		
Non-Leafy Vegetables	Cabbage/Cucumber/ Potato/Tomato		
Fishes	Big Fish		
	Small Fish		
	Dry Fish		

Meat and Egg	Red Meat(Beef/Mutton)		
	Chicken/Duck		
	Egg		

Milk and Milk Product	Milk/Cheese/Butter		

Fruits	Citrus Fruits		
	Other Fruits		

Oil and Fat	Cooking oil/Butter/Margarine		

Sugar	Sugar/Artificial Sweetener/Gur		

FastFood/Snacks	Burger/Pizza/ Samusa/Biscuit		