

# Histo-morphological study of full term placenta and anti-oxidant vitamin levels of selected normotensive and pre-eclamptic women in Bangladesh

## Abstract

Pre-Eclampsia is a disorder of 2<sup>nd</sup> half of pregnancy which is characterized by a combination of hypertension, proteinuria and edema, secondary to decreased placental perfusion. It is commonly associated with reduced weight, diameter and volume of placenta, along with other microscopic changes. This study evaluated the plasma levels of antioxidants and the histomorphological variations of placenta in selected pre-eclamptic and normotensive pregnant women. A total of 220 pregnant women were selected with inclusion and exclusion criteria from 3 different medical colleges – Dhaka Medical, Mitford and Holy Family Red Crescent Medical College. In this study 56% of Pre-eclamptic women were in the age group of 18-24 years. This study reveals that mean placental weight in pre-eclamptic women was (404.80 kg) compared to normal pregnant women (486.96 kg) ( $p < 0.001$ ). It was observed that the mean diameter of placenta (15.88 cm), the number of cotyledons (16) and the number of infarcted areas (16.02) in case of pre-eclamptic women compared to normal pregnant women were (18.22 cm), (17) and (4.02) respectively. Serum Vit C is below normal in 78% of pre-eclamptic women compared to normal pregnant women, where it was low in only 20%. Serum Vit E is below normal (68%) of pre-eclamptic women compared to normal pregnant women which was (20%). This study therefore indicates an association between deficiency of anti oxidants in blood and development of pre-eclampsia with histological and morphological changes in the placenta.

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Nahid Ahmed Khan,<sup>1</sup> Khaleda Islam<sup>2</sup><sup>1</sup>PhD Research Fellow, Institute of Nutrition and Food Science, University of Dhaka, Bangladesh<sup>2</sup>Professor, Institute of Nutrition and Food Science, University of Dhaka, Bangladesh, Email nuzis@gmail.com**Correspondence:** Nahid Ahmed Khan, PhD Research Fellow, Institute of Nutrition and Food Science, University of Dhaka, Bangladesh, Email nuzis@gmail.com**Received:** November 07, 2019 | **Published:** December 19, 2019

## Background

The human placenta is a unique structure of maternal and fetal origin, the protective role of which, as a barrier for removal of waste products of metabolism is vital, as the fetal hepatic and renal systems have insufficient excretory capacity.<sup>1</sup> At birth the normal placenta is a blue red, flattened, discoid mass, with a weight of 200-800 gm. The architecture of the placenta e.g. the weight, diameter and the number of cotyledons can be changed in many maternal diseases, an important example of which is pre-eclampsia.<sup>2</sup> Pre-eclampsia is a disorder of the second half of pregnancy. It is a 2 stage disease. Stage 1 is decreased placental perfusion. Stage 2 is the maternal syndrome of pre-eclampsia comprising of hypertension, proteinuria and edema.<sup>3</sup> Pre-eclampsia is a leading cause of infant and maternal mortality, with an estimated 50,000 maternal deaths per year in developing countries like Bangladesh.<sup>3</sup> The etiology of pre-eclampsia is still unknown, although a number of hypothesis have been accepted. Dysfunction of vascular endothelium and inadequate trophoblastic invasion, leads to high resistance and low uteroplacental circulation that causes placental ischemia and hypoxia. Hypoxia causes production of reactive oxygen species or free radicals like superoxide which are capable of damaging proteins, DNA and inducing lipid peroxidation, ultimately resulting in widespread endothelial damage.<sup>2</sup> It has now been suggested that deficiency in antioxidants can lead to development of Pre-eclampsia. Antioxidant vitamins like Vit. C, Vit. E with their ability to stabilize highly reactive free radicals, act as the first line of defense against superoxide attack and lipid peroxidation.<sup>4</sup> Therefore in this study we tried to evaluate the plasma levels of antioxidants and

the Histomorphological variations of placenta in pre-eclamptic and normotensive pregnant women of Bangladesh.

## Methods and materials

Type of the study was cross-sectional or observational or analytical in nature. The study was carried out during the period of 3 years after the completion of the registration. A total of 110 preeclamptic women and 110 normal pregnant women between the ages of 18 to 40 years, and in 3rd trimester of pregnancy were selected for the study. Selection of cases was based on strict inclusion and exclusion criteria. 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. A questionnaire was developed to obtain relevant information regarding the socio-economic factors, age, obstetric history, income, living area, family size, education, occupation 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 had been obtained from Ethical review committee of Bangladesh Medical and Research Council. Written consent was taken from both study or group A and control group or Group B. The nature and purpose of the study was explained to each participants and attendants and written consent was taken from both study and control group. Confidentiality of the person and the information was maintained, observed and unauthorized persons did not access to the data. Each subject was assigned a study identification number and these subject identifiers did not release outside the authorized person.

## Hematological and bio chemical assays

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

### Procedure:

- a) Under aseptic conditions 2 ml of blood was collected 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.
- b) 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.
- c) Then blood was given in semiautomated (22 cell counter) Machine, and after one minute the result came out.
- d) A Leishman stain was added to the smear slide for 2 minutes.
- e) For cross checking the slide was examined under the light microscope at 40 magnification.

### ESR Method:

#### Procedure:

- i. ESR was measured by ESR tube by western green method. Normal range for female 0 to 20 ml first hour.

### Fasting Blood Sugar:

#### Procedure:

- i. First 2 ml of blood is collected from the patient median cubical vein, into a clot activator tube and it is labeled with a barcode.
- ii. After the blood clots, the tube is placed in a centrifuge machine and centrifuged at a speed of 3000 RPM.
- iii. During the test Glucose reagent is taken in a plain test tube and 10 µl Serum is mixed with it and kept at room temp for 10 mins.
- iv. Then, the sample is placed in a semi auto Biochemistry machine and the final result is shown.
- v. Urine for Albumin was assessed by heat coagulation test.

### Serum Vitamin C:

#### Procedure:

- i. Vitamin C was analyzed by colorimetric method.
- ii. 3 ml of blood is collected from the patient median cubical vein, placed in a test tube, which is covered by foil paper, then taken to the lab.
- iii. 2.0 ml of freshly prepared meta- phosphoric acid was taken in a test tube, and 0.5 ml of sample or standard was added.
- iv. They were mixed together and centrifuged at 2500 rpm for 15 mins
- v. After that, it was filtered and the supernatant was taken for blank, sample and standard.
- vi. 0.4 ml of DTCS was added in each and kept at 37° C for 3 hours.
- vii. 2 ml of Sulphuric Acid was also added in each
- viii. The absorbance was read at 520 nm against the reagent blank.

### Serum Vitamin E:

#### Procedure:

- i. Vit E was analyzed by HPLC method.
- ii. 100 µl plasma was deproteinized with 100 µl ethanol.
- iii. Hexane was added.
- iv. Shaken for 30 seconds
- v. Centrifuged at 3500 rpm for 4 mins.
- vi. The hexane layer was separated and transferred in a clear glass vial.
- vii. It was evaporated to dry under nitrogen
- viii. It was re dissolved into mobile phase (100 µl methanol) and injected into HPLC.
- ix. Nutritional status was measured by MUAC.
- x. Dietary information was measured by 7 days food frequency and food consumption score.
- xi. Body weight is measured by bathroom scale weight was recorded to the nearest 0.5 kg.
- xii. A wooden height scale was used to record height with bared heels standing upright position, height was measured to nearest 0.1 cm.
- xiii. Blood pressure was measured by sphygmomanometer.
- xiv. 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.)
- xv. Weight of the placenta measured upto nearest gram with weighing machine.
- xvi. Diameter of placenta is measured by taking the average of two maximum diameters of placenta with measuring tape (cm).
- xvii. Cotyledons of placenta were counted from maternal side after removal of deciduas basalis.
- xviii. Number of placental infarcts were counted from fetal side.
- xix. Placental histopathology was done by Hematoxylin and Eosin Stain method.

### Haemotoxylin and Eosin Stain

#### Procedure: Bancroft and Stevens, 1982

- i. Tissue section on slides was dewaxed by xylene.
- ii. The slides were washed in three changes of absolute alcohol, then 95% alcohol and 70% alcohol.
- iii. The slides were washed in water for 5 minutes and the stained with haemotoxylin for 5-10 minutes.
- iv. The slides were washed in water for 5 minutes.
- v. The slides were then placed in eosin for 10-15 minutes.
- vi. The slides were washed in water for 2-3 minutes.

- vii. The sections were then dehydrated in 70, 80, 95% alcohol, for few seconds in each case, and two times in absolute alcohol
- viii. The final step was putting the slides in xylene after drying, and covering by cover slip with Canada balsam.
- ix. Statistical analysis was included in all the slides.

## Results

Table 1 illustrates that 56.36% of the pre-eclamptic women belonged to 18-24 years whereas 56.36% of normal pregnant women aged were 25-30 years.

**Table 1** Distribution of age of selected pre-eclamptic women and normal pregnancy

Age group (yrs)	Pre-eclamptic Women (Study Group A)	Normal Pregnancy (Control Group B)
18-24	62(56.36)	38(34.54)
25-30	42(38.18)	62(56.36)
≥31	6(5.45)	10(9.10)
<b>Total</b>	<b>110(100)</b>	<b>110(100)</b>

Table 2 illustrates that 56.36% of the pre-eclamptic women were housewives whereas 47.27% of normal pregnant women were housewives.

**Table 2** Occupation of selected pre-eclamptic women (Study Group A) and Normal Pregnancy (Control Group B)

Occupation	Pre-eclamptic Women (Study Group A)	Normal Pregnancy (Control Group B)
Day labor	18(16.36)	14(12.73)
Skill labor	12(10.91)	20(18.18)
Agriculture labor	4(3.64)	2(1.82)
Housewife	62(56.36)	52(47.27)
Others	14(12.73)	22(20.0)
<b>Total</b>	<b>110(100)</b>	<b>110(100)</b>

Table 3 is observed from table-3 68.18% of pre-eclamptic women never consumed citrus fruits whereas 53.64% of normal pregnant women consumed citrus fruit irregularly (1-3 days/week).

**Table 3** Consumption of citrus fruits of selected pre-eclamptic women (Study Group A) and Normal Pregnancy (Control Group B)

Consumption of citrus fruits	Pre-eclamptic Women (Study Group A)	Normal Pregnancy (Control Group B)
Daily/week	2(1.82)	4(3.64)
Regularly (4-6 days/week)	3(2.73)	6(5.45)
Irregularly (1-3 days/week)	30(27.27)	59(53.64)
Never	75(68.18)	41(37.27)
<b>Total</b>	<b>110(100)</b>	<b>110(100)</b>

Table 4 shows that 78% of pre-eclamptic women had below Normal (<0.60 mg/dl) serum vitamin C and 80% of normal pregnant women had normal (0.60–2 mg/dl) serum vitamin C.

**Table 4** Distribution of serum vitamin C of selected pre-eclamptic women (Study Group A) and Normal Pregnancy (Control Group B)

Serum Vitamin C	Pre-eclamptic Women n=50	Normal Pregnant Women n=50
Normal (0.60 – 2 mg/dl)	11(22)	40(80)
Below Normal(<0.60 mg/dl)	39(78)	10(20)
<b>Total</b>	<b>50(100)</b>	<b>50(100)</b>

From Table 5, Mean serum Vitamin C Level of Pre-eclamptic Women was (0.49±0.12)mg/dl whereas Mean serum Vitamin C Level of normal pregnant Women was (1.24±0.39)mg/dl.

**Table 5** Serum vitamin C of selected pre-eclamptic women (Study Group A) and Normal Pregnancy (Control Group B)

Group	Vitamin C n = 50 (Mean±SD)	p value
Pre-eclamptic Women (Study Group A)	0.49±0.12	0.001
Normal Pregnant Women (Control Group B)	1.24±0.39	0.001

Table 6 shows that 68% of pre-eclamptic women had below Normal (<500 ug/dl) serum vitamin E and 80% of normal pregnant women had normal (500-1800 ug/dl) serum vitamin E.

**Table 6** Distribution of serum vitamin E of selected pre-eclamptic women (Study Group A) and Normal Pregnancy (Control Group B)

Serum vitamin E	Pre-eclamptic Women n=50	Normal Pregnant Women n=50
Normal (500-1800 ug/dl)	16(32)	40(80)
Below Normal (<500 ug/dl)	34(68)	10(20)
<b>Total</b>	<b>50(100)</b>	<b>50(100)</b>

From Table 7, Mean serum Vitamin E Level of Pre-eclamptic Women was (359.95±139.27) ug/dl whereas Mean serum Vitamin E Level of normal pregnant Women was (815.64±281.17)ug/dl.

**Table 7** Serum vitamin E of selected pre-eclamptic women (Study Group A) and Normal Pregnancy (Control Group B)

Group	Vitamin E n= 50 (Mean±SD)	p value
Pre-eclamptic Women (Study Group A)	359.95±139.27	0.001
Normal Pregnant Women (Control Group B)	815.64±281.17	

Table 8 shows that mean placental weight in pre-eclamptic women was (404.80 kg) compared to normal pregnant women (486.96 kg)

( $p < 0.001$ ) and the mean diameter of placenta (15.88 cm), the number of cotyledons (16) and the number of infarcted areas (16.02) in case of pre-eclamptic women compared to normal pregnant women were (18.22cm), (17) and (4.02) respectively. The mean number of

cotyledons were (14.30+2.47) in the study group and (15.77+2.80) in the control group ( $p < 0.002$ ), whereas the mean number of infarcted areas in the study group was 15 and 4 in the control group ( $p < 0.001$ ).

**Table 8** Placental Weight (kg), Placenta Diameter (cm), No. of infarcted area in placenta, Number of cotyledons, Number of Syncytial Knot Formation, number of Cytotrophoblastic cell proliferation, number of areas of Fibrinoid Necrosis and number of Areas of Hyalinised Villi. of Pre-eclamptic Women (Study Group A) and Normal Pregnancy (Control Group B)

Characteristics	Pre-eclamptic Women (Group A) n=50	Normal Pregnant Women Control (Group B) n=50	p value
	(Mean±SD)	(Mean±SD)	
Wt. of placenta (gm)	404.80±4.04	486.96±1.62	0.001
Placenta diameter (cm)	15.88±0.13	18.22±0.79	0.001
No. of infarcted area in placenta	16.02±0.80	4.02±0.80	0.001
Number of cotyledons	16±0.78	17.10±0.89	0.002
Number of Syncytial Knot formation	26.76±3.86	9.60±1.46	0.001
Number of Cytotrophoblastic cell proliferation	21.52±5.03	7.16±2.06	0.001
Number of areas of Fibrinoid necrosis	10.68 ±3.33	2.24 ±0.69	0.001
Number of areas of Hyalinised Villi.	9.46±4.10	2.32±0.59	0.001

## Discussion

The present study was conducted amongst 220 pregnant women to see the effect of pre-eclampsia on the placenta and to determine the serum levels of Vit C and Vit E in pre-eclamptic women. 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. But so far, the nutritional status of pre-eclamptic women has never been properly evaluated. In this study 56% of pre-eclamptic women were in the age group of 18-24 years. This study reveals that mean placental weight in pre-eclamptic women was (404.80 kg) compared to normal pregnant women (486.96 kg) ( $p < 0.001$ ). In another study Shah et al., 1985; and others (Cibil LA, Teasdale, Barua R., and Begum) also observed reduced placental weight (390.82 gm) in Pre-eclamptic women in compared to normal pregnant women (496.56 gm) ( $p < 0.001$ ). It was observed that the mean diameter of placenta (15.88 cm), the number of cotyledons (16) and the number of infarcted areas (16.02) in case of pre-eclamptic women compared to normal pregnant women were (18.22cm), (17) and (4.02) respectively. Seguptakishwara et al.,<sup>4</sup> and several others (Abu Sadat Mohammad Nurunnabi, Mahamuda Begum, Abu Rayhan, Shamim Ara) observed that the mean diameter of placenta was (16.08+2.08 cm) in the study group and (18.80+2.32cm) in the control group ( $p < 0.001$ ). The mean number of cotyledons were (14.30+2.47) in the study group and (15.77+2.80) in the control group ( $p < 0.002$ ), whereas the mean number of infarcted areas in the study group was 15 and 4 in the control group ( $p < 0.001$ ). This study also reveals that mean number of areas of syncytial knot formation (26.76) cytotrophoblastic cell proliferation (21.52) and Area of Fibrinoid necrosis (10.68), and Hyalinised villi were (9.46) in case of pre-eclamptic women, compared to normal pregnancy which were (9.60), (7.16) (2.24) and (2.32)

respectively. M. Akhlag, AH Nage, AW Yousuf (2012) also observed increased syncytial knot formation (25.23+1.23) in pre-eclamptic 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 necrosis (9+2.96) and hyalinized villi (8.96+2.42), in pre-eclamptic patient (Hum palhol 1997; 28: 353). Serum Vit C is below normal in 78% of pre-eclamptic women compared to normal pregnant women, where it was low in only 20%. In this study, mean serum vit C was (0.49+0.12) mg/dl in the study group, and (1.24+0.39) mg/dl in the control group ( $p < 0.001$ ). Suryakant Nagtilak., 2014; 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 ( $p < 0.001$ ). 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<sup>5</sup> also found low level of Vit C in pre-eclamptic women. In this study, serum Vit E is below normal (68%) of pre-eclamptic women compared to normal pregnant women which was (20%). Mean Vit E levels was (359.95µg/dl) in study group and (815.64µg/dl) in control group ( $p < 0.001$ ). Suryakant Nagtilak (2014) observed that mean serum Vit E levels was (0.66+0.26) mg/dl in study group and (0.82+0.26) mg/dl in control group ( $p < 0.001$ ). Kharbs Gulati Singh, et al.<sup>6</sup> also observed lower serum Vit E in pre-eclamptic women compared to normal pregnant women.<sup>7-11</sup>

## 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 vill., 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, CytotrophoblasticCell Proliferation, Fibrinoid Necrosis and Hyalinised Villi on microscopic examination.

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