RESULTS

Of those who carried male fetuses and had blood sampled, sixteen subjects subsequently developed preeclampsia, as the only antenatal complication.

The mean gestational age at onset of PE was 34.5 ± 1.6 (32 – 36) weeks. Preeclampsia was defined essentially as described previously by new onset blood pressure of at least 140/90 mmHg in 2 determinations 4 hour apart or by a diastolic blood pressure > 110 mmHg as well as associated proteinuria of at least 300 mg/24h or 100 mg/dl or more in at least 2 random urine specimen collected 6 or more hour a part after 20 weeks of gestation.

Another 36 subjects who carried male fetuses and had no antenatal complication were randomly selected as a positive control group. 9 pregnant subjects carrying female fetuses and had no antenatal complication were selected as a negative control group. The number of all controls = 45 pregnant women.

The maternal clinical characteristic and the gestational age at onset of preeclampsia are listed in table (4).

Table (4): Mean values ± SD of clinical characteristics of the study cohorts

Groups / Data	Mean ± SD of PET patients (Range)	Mean ± SD of controls (Rang)
	n= 16	n= 45
Age (year)	24.2±5.5 (19-39)	24.0 ± 4 (18-35)
Weight (Kg)	76 ± 11.5 (50-94)	75 ± 12.7 (50-100)
Blood pressure (mmHg)		
Systolic	126 ± 9.5 (100-140)	117 ± 7.5 (100 – 130)
Diastolic	85 ± 9.5 (60- 100)	76.6 ± 7.6 (60-90)
Parity		
Primagravida	7/16 43.75 %	23/45 51 %
Multigravida	9/16 56.25%	22/45 49%
Previous history of PE	3/16 (18.75%)	0/45 (0%)
Edema lower limb and proteinuria at sampling	No in all cases 0/16 (0%)	No in all cases 0/45 (0%)
Gestational age at onset of PE (week)	34.5 ± 1.6 early PE ≤ 34 week (8/16) Late PE > 34 week (8/16)	-
Type of labor	12/16 CS (75%) 4/16 NVD (25%)	0/45 CS (0%) 45/45 NVD (100%)
Sex of babies after birth	16/16 male (100%)	9/45 female (20%) 36/45 male (80%)

n= number PE- preeclampsia
CS= cesarean section NVD = Normal vaginal delivery

Table (5): Mean values ± SD of total double stranded DNA concentrations μg/ml plasma and gestational ages in the study cohorts (range)

Groups / Data	PET patients (n= 16)	Controls (n = 45)	t	P
Gestational age at sampling (weeks)	22.8 ± 4.5 (14 – 29)	21.5 ± 4.5 (14 – 31)	0.96	P > 0.05
Gestation age at delivery (weeks)	36.8 ± 0.9 (36 – 38)	40 ± 1.8 (37 – 42)	9.1	P < 0.001
Total ds DNA concentrations μg/ml.	155.2 ± 64.4 (77 – 247.5)	22.3 ± 9.2 (8.5 – 42.5)	8.2	P < 0.001
260/280 ratio	1.7 ± 0.2			

n. = number

P > 0.05 non significant

P < 0.001 significant

Table (6): **Mean values ± SD of total double stranded DNA concentrations μg/ml plasma in patients**

Group / Data	Early onset PE (n=8)	Late onset PE (n=8)	t	p
Total dsDNA concentrations μg/ml	189.8 ± 78.6	101.6±31.1	2.95	P < 0.01

The mean gestational ages at sampling of the preeclamptic and control subjects were 22.8 ± 4.5 and 21.5 ± 4.5 respectively.

There was no statistically significant difference in the gestational ages at blood sampling between the preeclamptic and control groups (P > 0.05).

There was a statistically significant decrease in the gestational age at delivery in the preeclamptic patients as compared to the controls $p < 0.001$.

With regards to total double stranded DNA concentrations, there was a statistically highly significant increase in the preeclamptic patients as compared to controls ($P < 0.001$), and this increase is significantly higher in early onset PE than late onset one as shown in table (6).

Mean value of OD_{260}/OD_{280} ratio of all samples was 1.7 ± 0.2 indicating that there was no contamination with protein or RNA in the extracted samples.

Table (7): Correlation coefficient (r) for total ds DNA concentration (μg/ml) comparing other clinical characteristic data in the study cohorts.

Groups / Data	Total ds DNA concentrations μg/ml in patient group. (n = 16)	Total ds DNA concentrations μg/ml in controls groups (n = 45)
Age (year)	r = 0.22 p > 0.05	r= 0.21 p > 0.05
Weight (kg)	r= - 0.432 p > 0.05	r= 0.24 p > 0.05
Systolic blood pressure (mmHg)	r= 0.26 p > 0.05	r= 0.23 p > 0.05
Diastolic blood pressure mmHg	r= 0.23 p > 0.05	r= 0.13 p > 0.05
Gestational age at sampling (weeks)	r= - 0.1 p > 0.05	r= 0.9 p < 0.001*
Gestational age at delivery (weeks)	r= - 0.87 p < 0.001*	r= - 0.56 p < 0.001*
Gestational ages at onset of PE	r= - 0.94 p < 0.001*	

n= number
PE = preeclampsia
P > 0.05 non significant
P < 0.001 highly significant

Table (7) shows correlation coefficient (r) for total ds DNA concentrations comparing other clinical data, in different groups.

Our data revealed that in patients group, there was a statistically significant negative correlation between total ds DNA concentrations and both, gestational ages at delivery and gestational ages at onset of

preeclampsia ($P < 0.001$) respectively; but no significant correlation was detected between total dsDNA concentrations and both of maternal age, systolic and diastolic blood pressure in both patients prior PE and controls.

But in normal pregnancies, the total dsDNA concentrations had a significant positive correlation with gestational age at sampling and a significant negative correlation with gestation age at delivery.

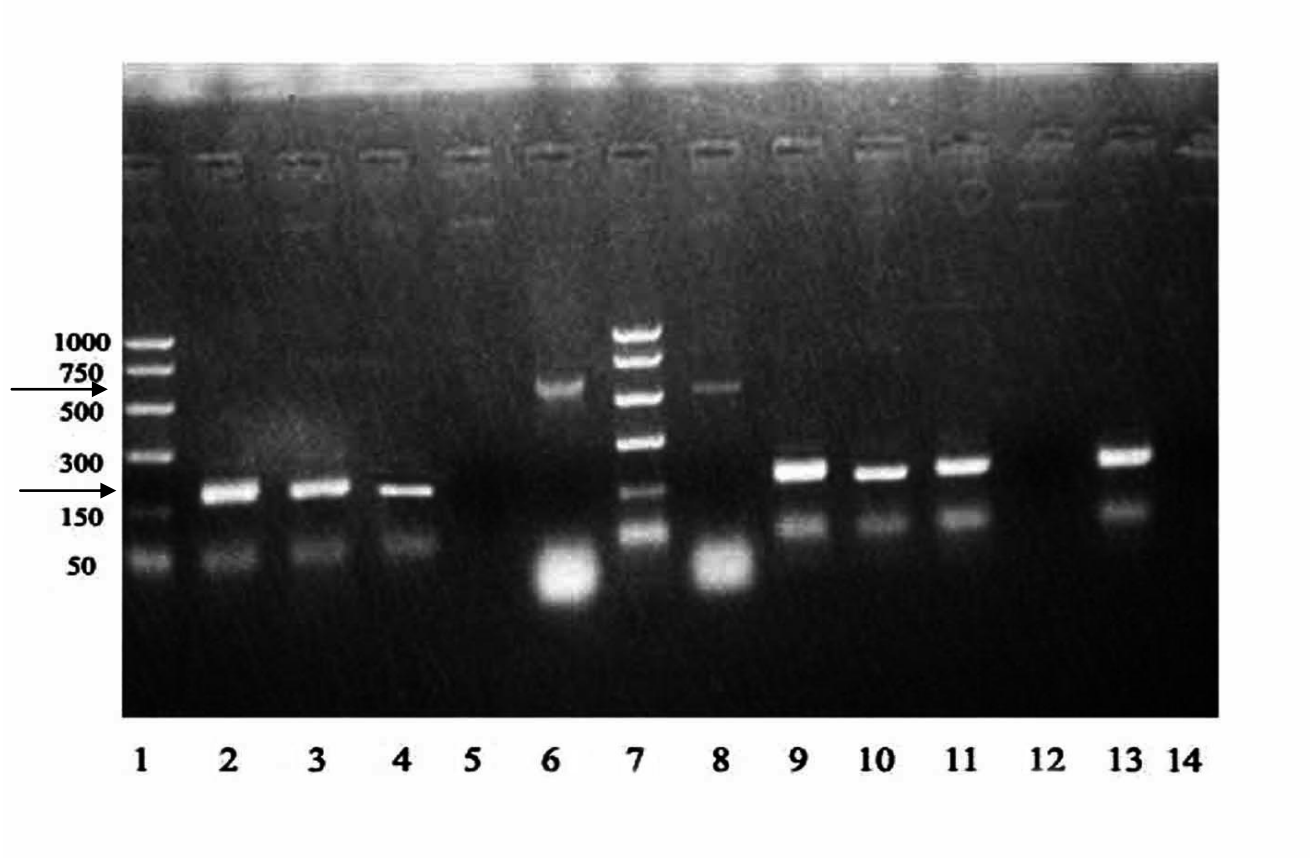

Fig. (1): Shows polymerase chain reaction (PCR) analysis of fetal Y chromosome (SRY) sequence and B globin gene in maternal plasma lanes 6 and 8 represents the B globin locus from a patient and a control respectively. Lanes 2, 3, 4, 5, 9, 10, 11, 12 and 13 represent the SRY locus. Lanes 2, 3, 9, 11, and 13 indicate male fetus from preeclamptic patients; ID (44, 68, 4, 39 and 28) respectively lane 4 and 10 indicate male fetus from positive controls (ID 65, 43) . Lanes 5 and 12 indicate a female, fetus ID (56, 57) as a negative control lane 14 is water blank control (without DNA) in PCR. The faster migrating bands represent primer dimer formation.

Positive amplification bands from the β globin gene were detected in all tested samples at (538 bp PCR amplification product) confirming the quality of DNA samples and indicate that the PCR was functional. An example is shown in Fig. 1: (lane 6 & 8).

All PET patients (n=16), and the selected positive control samples (n = 36) contained a 196 bp amplification product, indicating, the presence of SRY sequence and hence, a male fetus, example in Fig. 1 (Lane, 2, 3, 4, 9, 10, 11 and 13). The selected negative control samples did not contain a SRY amplification product and hence, a female fetus (Lane, 5, 12) in Fig. (1).

There was an increase by 2 to 3 folds in SRY amplification bands, (fluorescence yielded) of PET patient samples as in (lane 2, 3, 9, 11, 13) when compared with bands of positive control samples as in (lane 4, 10) Fig. (1).

As SRY amplification represent the fetal DNA in the samples and as the amount of UV induced fluorescence emitted by ethidium bromide represent the quantity of DNA in the sample. So, we can say that there was an increase in circulating free fetal DNA concentrations in PET patients prior the onset of clinical disease than in those of positive controls with matched gestational ages.

Fig. (2): Gestational age at sampling (weeks) in patients and controls

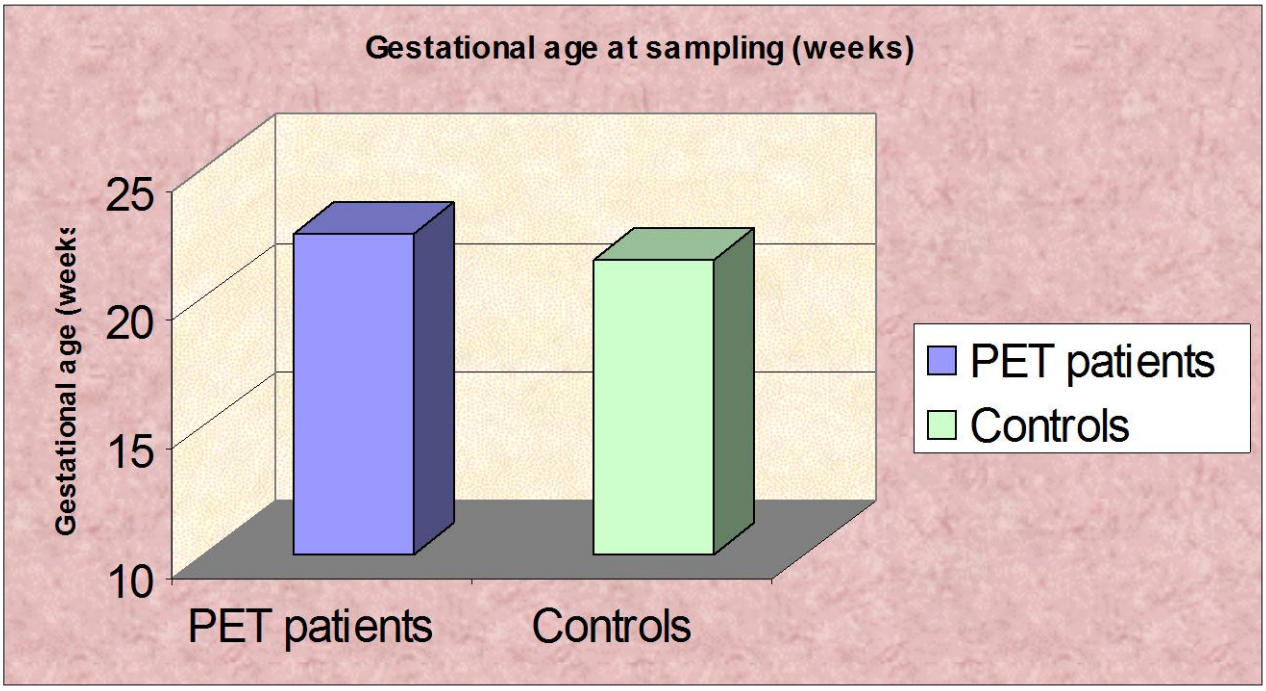

Fig. (2): Shows no significant difference in the gestational age at blood sampling between the PET patients and the controls.

Fig. (3): Gestational age at delivery (weeks) in patients and controls

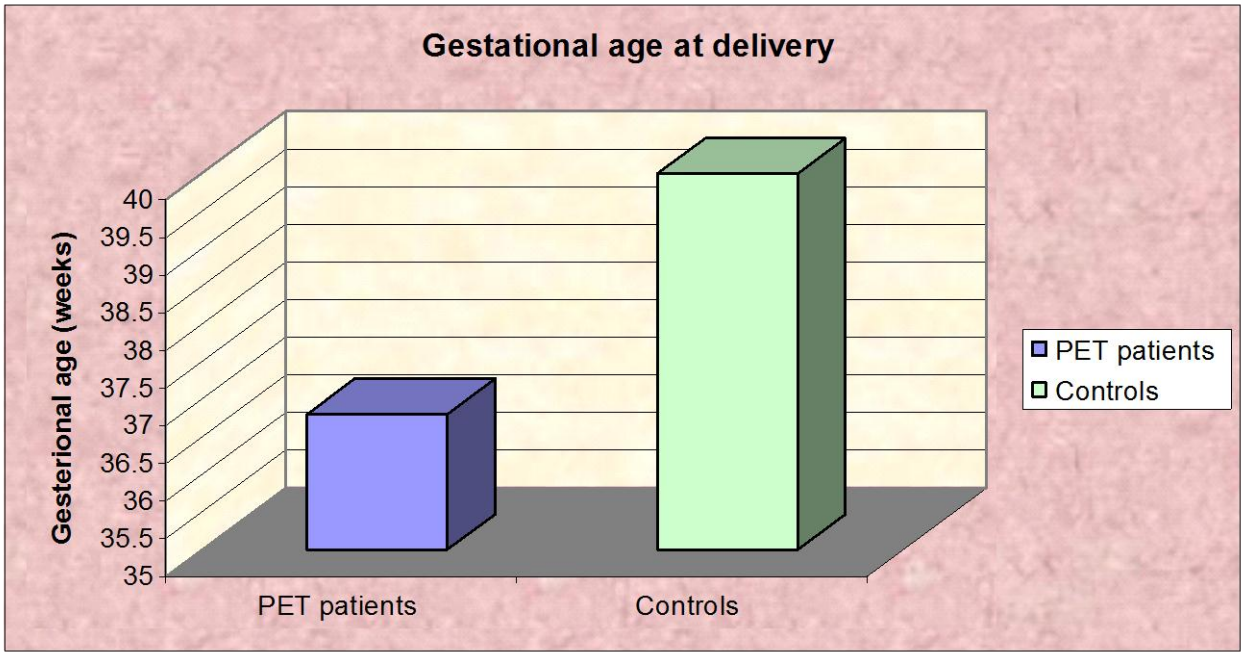

Fig. (3): Shows significant decrease in the gestational age at delivery in the PET patients compared to the controls.

Fig. (4): Plasma Total ds DNA concentrations (µg/ml) in patients and controls

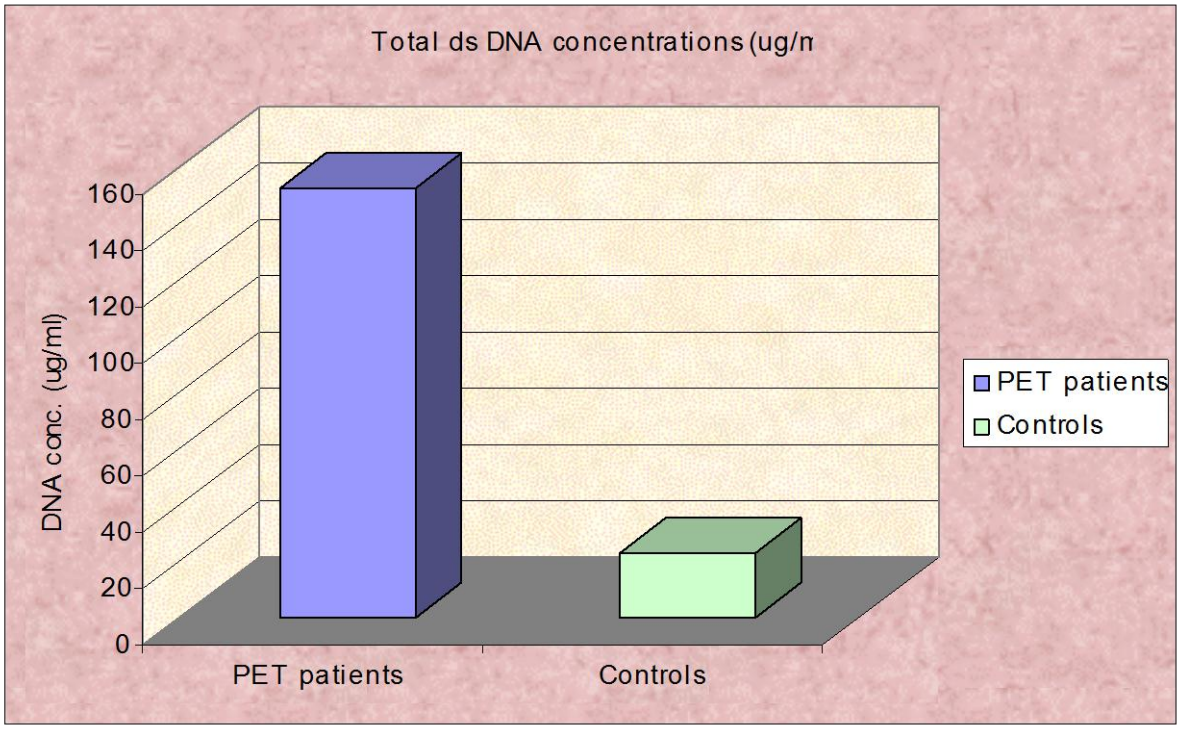

Fig. (4): Shows the highly significant increase in the total ds DNA concentrations (µg/ml) in PET patients compared to the controls.

Fig. (5): Plasma total dsDNA concentrations in early and late onset of PE compared to controls

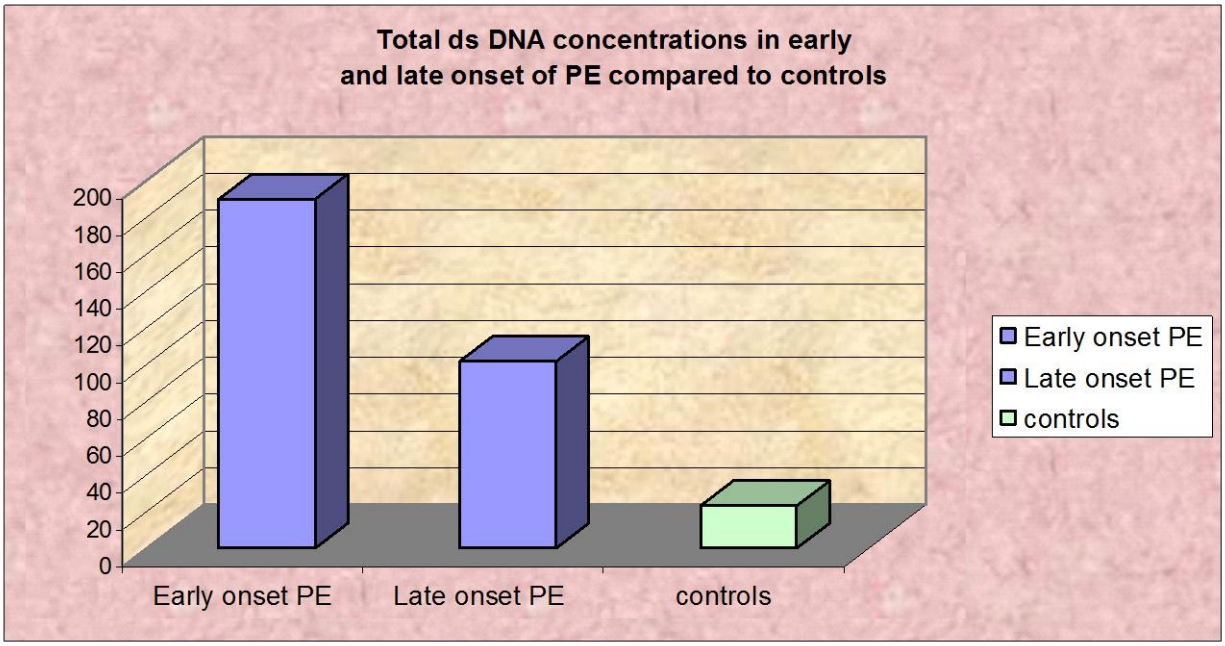

Fig (5): Shows significant elevation in the total ds DNA concentrations in patients with early onset of PE than those of late onset one and both compared to the controls.

Fig. (6): Correlation coefficient between plasma total DNA concentrations and gestational age at onset of PE

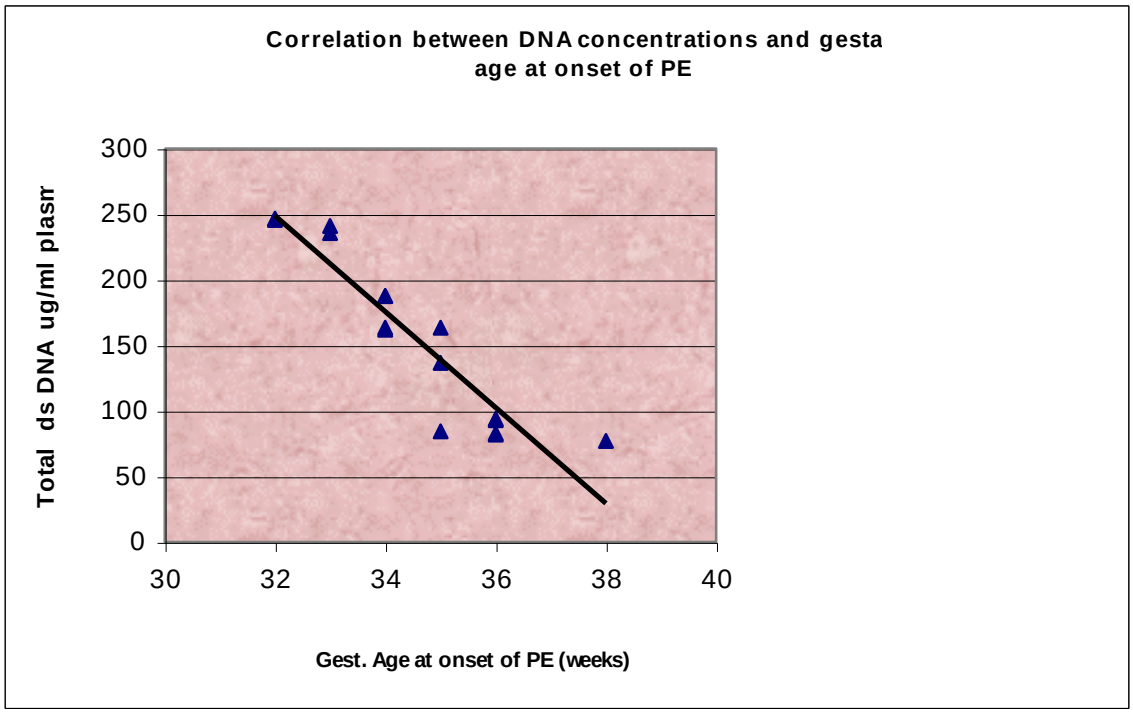

Fig. (6): Shows significant negative correlation between the plasma total ds DNA concentrations (µg/ml) and gestational ages at onset of PE (weeks) in PET patients.

Fig. (7): Correlation coefficient between plasma total DNA concentrations and gestational ages at delivery (weeks) in PET patients

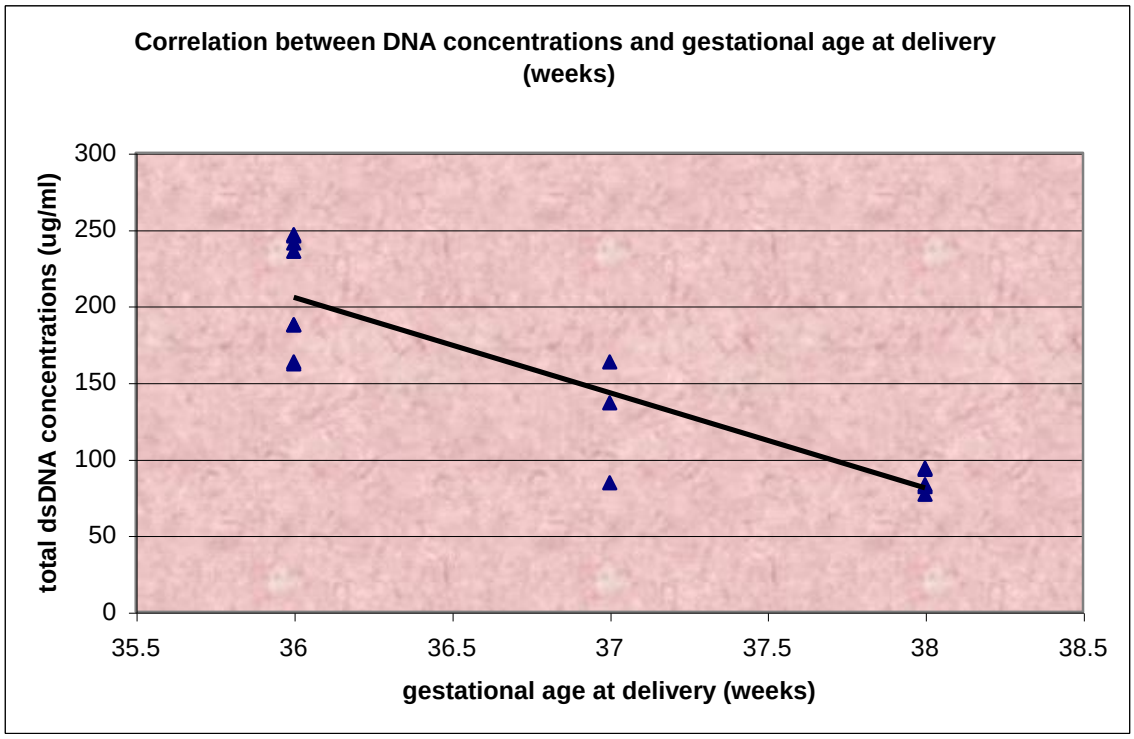

Fig. (7): Shows significant negative correlation between the plasma total ds DNA concentration (µg/ml) and gestational age at delivery (weeks) in PET patients.

Fig. (8): Correlation coefficient *between plasma total DNA concentrations (µg/ml) gestational age at sampling (weeks) in controls*

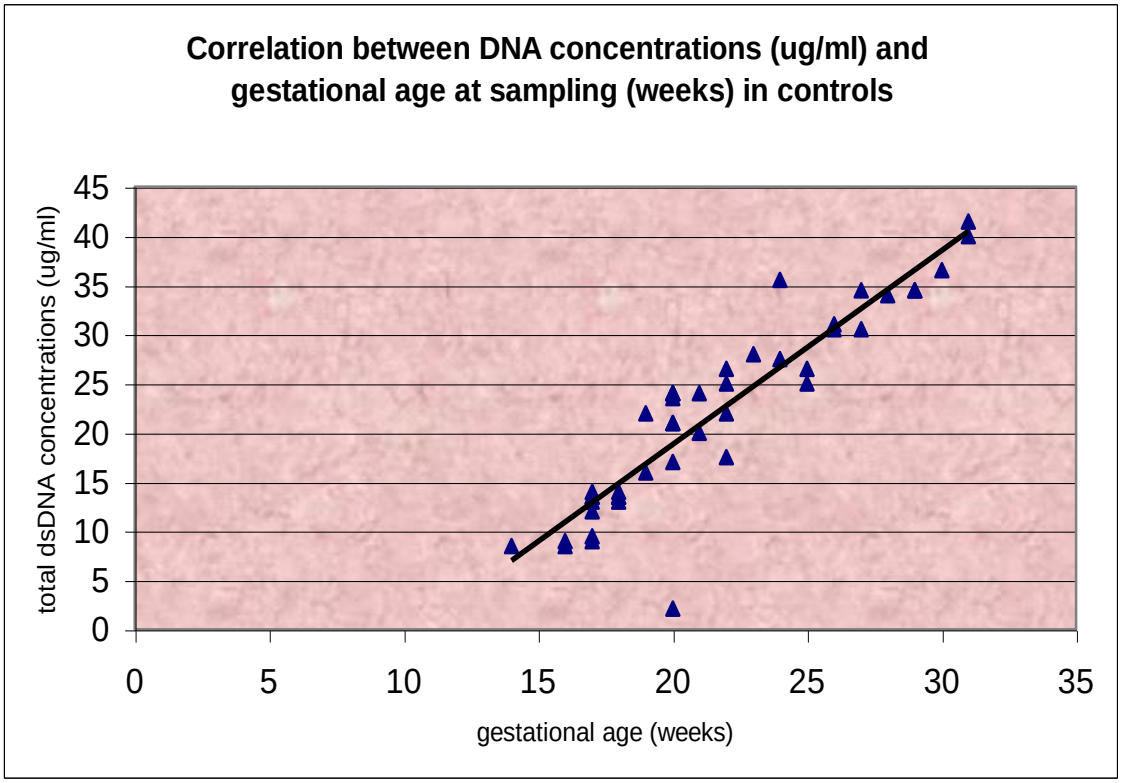

Fig. (8): Shows significant positive correlation between plasma total ds DNA concentrations (µg/ml) and gestational age at sampling (weeks) in controls .

Fig. (9): Correlation coefficient *between plasma total DNA concentrations (µg/ml) gestational age at delivery (weeks) in controls*

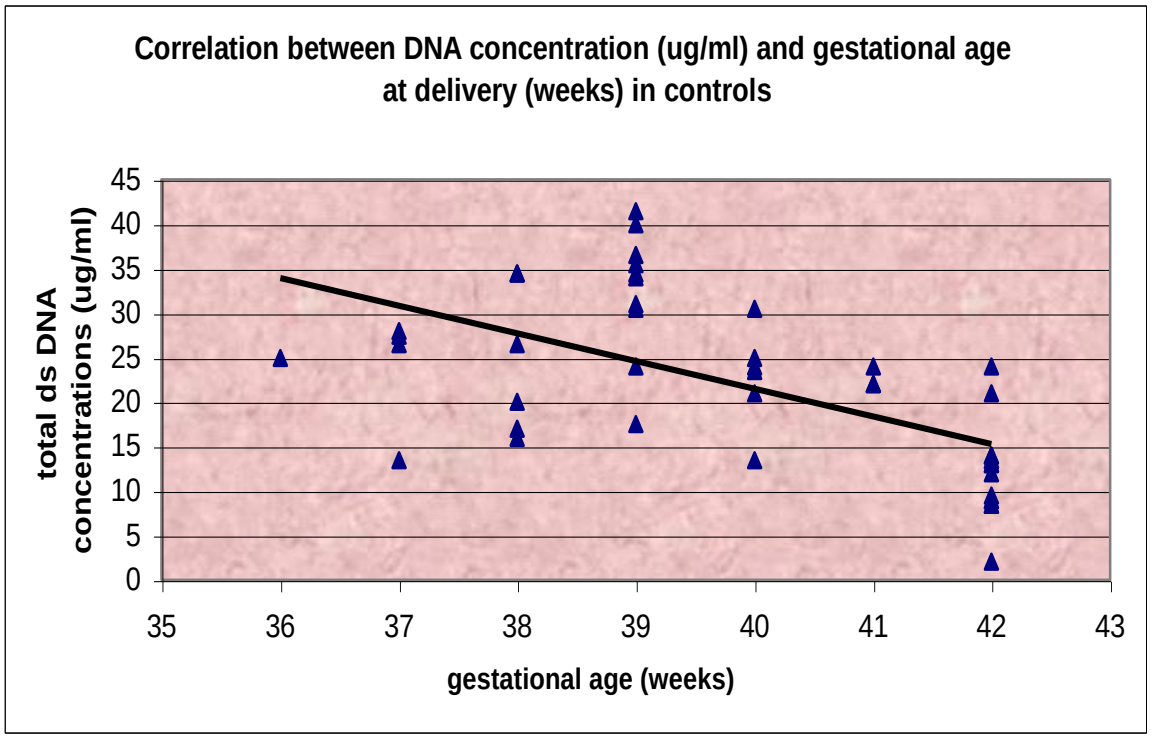

Fig. (9): Shows significant negative correlation between the plasma total ds DNA concentration (µg/ml) and gestational age at delivery (weeks) in controls.