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ELEVATED SECOND TRIMESTETR SERUM β-hCG LEVEL AS PREDICTOR OF PREECLAMPSIA

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ABSTRACT

Aim: To find any method can predict preeclampsia before 20 weeks gestation to take our precaution to these pregnants as high risk pregnancy. **Study:** This is a cohort study conducted on 96 primigravida with singleton pregnancies at their second trimester period to study the value of elevated serum β-human chorionic gonadotropin in predicting development of preeclampsia. **Objective:** We looked for the differences in serum β-hCG levels between pregnant women with pregnancy induced hypertension and pregnant women with normal pressure. We also studied the predictive value of such assays. **Methods:** We took a blood (serum) from 100 (4 was escape) pregnant lady between 14-20 weeks gestation and test their serum for B-hCG and follow them by signs and physical examinations and investigations for any idea related for preeclampsia till delivery and 2 week post partum period (really we sticky the pregnants by downing all these investigations and examinations freely in especially highly expensive tests and follow up all was freely from any fees. **Result:** Pregnancy induced hypertension was found in 9 in a population of 96 nulliparous. β-hCG levels were significantly higher in women who later developed hypertension (30,000 IU/L – 50,000 IU/C versus 15,650 – 25,620 IU/L). In nulliparous taking 29,250 IU/L as a cut off value of pathology we found that the predictive value of β-hCG was higher [sensitivity (66.7%, specificity (98.9%), positive predictive value (85.7%), negative predictive value (96.7%)], these values can be changed by changing the cut off point for positive test results. Taking a cut off value more than 32,000 IU/L it was possible to predict the occurrence of pregnancy induced hypertension more precisely. **Conclusion:** the mid trimester high serum B-hCG is good predictor for early detection of preeclampsia later on.

KEYWORDS: Serum B-hCG, Preeclapmsia, mid trimestor (2nd trimester).

INTRODUCTION

Definition

Pregnancy can induce hypertension in normotsive women or aggravate already existing hypertension.[1]

Preeclampsia/eclampsia is an unpredictable, multiorgan dosorder unique to human pregnancy.[2]

Preeclampsia is the development of hypertension with protein uria, oedema, or both, after 20th week of gestation. Throughout the world population, preeclampsia-eclampsia is primarily a disease of the indigent primigravida who has received little or no prenatal care. In spite of a vigorous search for a deficiency of a single food substance, vitamin, or trace element, a specific absence or inadequacy of food material has never been demonstrated. It is interesting to note that eclamptic patients in their succeeding pregnancies usually have either normal or mild preeclampsia pregnancies indicating that there are other factors operating besides dietary ones.[3]

Incidence

Pregnancy induced hypertension complicates 5-7 percent of all pregnancies.[4] With a (30%) incidence in multiple gestation, regardless of parity. Perinatal mortality increases progressively with each 5 mm Hg rise in mean arterial pressure.[5]

Table 1.1: Perinatal mortality and hypertension in Singleton pregnancies.[10]

Conditions	Perinatal mortality rate/1000
19.2	Normotensive
15.6	Essential hypertension only
30.7	Essential hypertension & preeclampsia
19.5	Mild preeclampsia
18.1	Moderate preeclampsia
33.7	Severe preeclampsia

The classification of hypertensive disorders recommended by National High Blood Pressure Education Program Working Group report on High

Blood Pressure in Pregnancy (1990) is outlined below. There is uniform agreement that an absolute blood pressure of 140/90 mm Hg is abnormal, because the normal resting arterial pressure is lower in pregnant than in non-pregnant subjects.[6] Classification of hypertensive disorders of pregnancy

1. Preeclampsia/eclampsia (hypertension peculiar to pregnancy).
2. Chronic hypertension (or whatever cause).
3. Chronic hypertension with superimposed preeclampsia.
4. Late or transient hypertension.

Pathophysiology

Pregnancy induced hypertiension which when associated with protein uria is usually called preeclampsia.

Rather than a genuine hypertensive diseases, preeclampsia is mainly a systemic endothelial as causing activation of platelets and diffuse ischaemic disorders whose most obvious clinical manifestations involve the kidney (hence the proteinuria, edema and hyperuricemia), the liver (hence the hemolytic elevated liver enzymes and low platelets, or HELLP syndrome) and the brain (hence eclamptic convulsions).

Hypertension is explained by increased vascular reactivity this is due to endothelial dysfunction with imbalance between prostacyclin and thromboxane A2.

The aggressive substances for endothelium are thought to be placentar origin and the cause of their release is explained by placentar ischaemia related to a defect of trophoblastic invasion of spiral arteries, the etiology of this later defect is unknown but involve immunogenic mechanism with genetic predisposition.

In nulliparous the selection of high-risk population is still a subject of research.

The two most promising criteria are abnormal Doppler velocimetry of uterine arteries at around 20th weeks of amenorrhea and abnormally high plasma levels of beta-HCG at 17th week of amenorrhea.[7]

The term pregnancy induced hypertension has not been discarded, because development of hypertension especially in nulliparous cannot be differentiated from transient hypertension except retrospectively.[8]

Most current hypotheses regarding the pathophysiologic mechanisms of pregnancy induced hypertension point to early placental abnormalities.

Consequently, management is directed towards detection of the disorder at an early stage and to effect, or at least ameliorate its progression in an attempt to achieve fetal maturity while preventing maternal complications.[9]

Reduction in platelet count

Occurred early in the development of preeclampsia, being detectable about 7 weeks prior to delivery.[19]

Clotting alterations & platelet activity:

Weiner & Brandt reported significantly lower levels of antithrombin III activity in women with preeclampsia as compared to healthy pregnant women began to decline as much as 13 weeks prior to the development of clinical manifestations.[20]

Tests related to angiotensin II sensitivity:

Sensitivity to infused angiotensin II (A II) is regarded as one of the better predictors of weather pregnancy induced hypertension will develop later in the pregnancy & the A II sensitivity test has been used to select patients who should receive preventive treatment, such as low – dose aspirin.[21]

Platelet A II binding measurements in the subjects who subsequently developed pregnancy induced hypertension was significantly higher than in the subjects who remained normotensive.[22]

Human Chrionic Gonadotropin

Human chrionic gonadotropin (HCG), the "pregnancy hormone" is a glycoprotein with biological activity very similar to luteinizing hormone (LH), both of which act via the plasma membrane LH/HCG receptor. HCG is produced almost exclusively in the placenta. HCG molecular weight about 36.700 with the highest carbohydrate (30%) content of any human hormone.

It's plasma half-life of intact HCG (29 hours), however, is much longer than that of LH (2 hours).

It is synthesized primarily in the syncytiotrophoblast.

HCG is detectable in plasma of pregnant women about 7 ½ to 9 ½ days after the midcycle surge of LH that precedes ovulation.

Thus, it is likely that HCG enters maternal blood at the time of blastocyst implantation.

The levels of HCG in blood increase rapidly thereafter with maximal levels being attained at about 8 to 10 weeks.

There is no predictable rhythmicity in the secretion of HCG during the day.

The concentration of HCG in maternal urine is closely parallel to that in plasma, which is approximately 1000 mIU/mL by 6 weeks after the commencement of last menstrual period, increasing to an average value of about 100.000 mIU/mL between the 60th and 80th days after the last menses. Thereafter the level of HCG in maternal plasma begin to decline, a nadir being reached by about 20 weeks. The levels of HCG in plasma are maintained at this lower level for the remainder of pregnancy.[23]

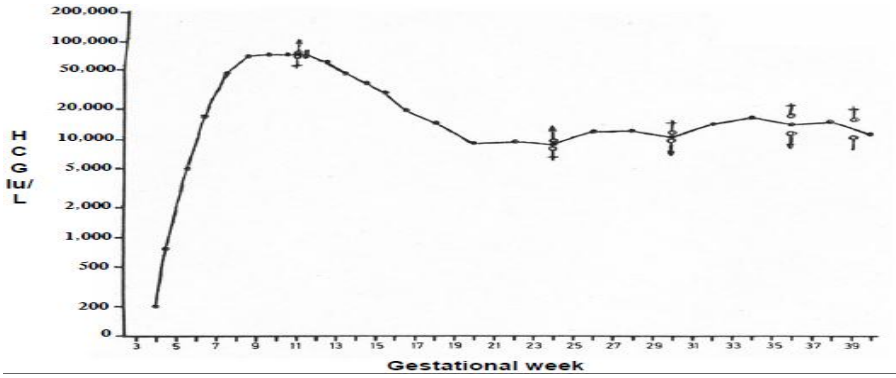

Figure 1.1: The mean plasma concentration of HCG during pregnancy(24). (From A. Klopper, 1980. The placent. In Clinical physiology in Obstetrics (Hytten F., Chairman G. eds.) p. 447. Oxford: Blackwell.

Table 1.2: Methods for β–HCG measurements.[25]

Methods	Type of analysis	Principle	Usage	Comments
Urine 1. "Slide tests"	a. Agglutination b. Agglutination inhibition	Colored latex or other visible particles coated with antibodies to β-HCG Negative urines remain homogeneous: presence of HCG results in visible agglutination of particles ("clumpiness") Colored latex or other visible particles (red blood cells) coated with HCG; antibodies to HCG and urine are mixed with particles Negative urine results in visible agglutination; presence of HCG in urine inhibits agglutination (or protein flocculation)	Infrequently used as stat. urinary pregnancy test Most frequently used as stat. urinary pregnancy test	Least sensitive of all HCG methods Most rapid (2 to 3 min)
2. "Tube tests"	Same as method 1	Same as method 1 reaction occurs in min.	Also frequently used for stat urine pregnancy tests	More sensitive than slide some approach upper limit sensitivity of RIA methods 45 to 120 min/assay
Serum and urine 3. Radioimmunoassay (RIA)	Competitive inhibition	Radiolabeled (radioactive iodine, 1251) HCG competes with sample analyte for binding to anti-HCG. Increased HCG in sample, decreased bound radioactivity	Infrequently used as stat. procedure Serum or urine	Most sensitive HCG assay available 40 to 60 min/assay
4. Enzyme- linked immuno-sorbent assay (ELISA)	Sandwich assay	a. Enzyme-labeled HCG reacts with sample HCG and binds to solid-phase anti-HCG Amount of bound enzyme activity directly proportional to amount of HCG in sample b. Solid-phase, double-antibody sandwich ELISA in which HCG binds to antibody Enzyme-labeled antibody added, and residual activity directly related to HCG concentration	Recently introduced serum assay Recently introduced for urine and serum testing	Reported sensitivity of 5 to 10 U/mL Assay time 1 to 3 hours Reported sensitivity 25-50 mU/mL 60-90 min assay Qualitative or quantitative assay

There were 100 women, primigravida with singleton pregnancies, who had a maternal serum β-HCG during the second trimester (~ 16-20 week) between August, 20, 2000 and May, 16, 2001 at the antenatal care center at Saddam Maternal Teaching Hospital in Al-Habibia.

Four cases from the analyzed sample were excluded because of (multiple pregnancy, maternal diabetes & ending in abortion).

Of the remaining 96 pregnancies had adequate follow up data and constituted our study population.

All β-HCG samples were assayed in the same laboratory. Blood pressure measured at two periods of gestation (first at 16-20 weeks) (second at 30 week) in all pregnant women included in this study.

After informed consent was obtained from women, venous blood samples were obtained.

Blood samples were centrifuged for 15 minutes, sera were collected & stored in a deep freeze until the assay was performed.

As recommended by the consensus report on high blood pressure in pregnancy.

Pregnancy induced hypertension corresponds to supine systolic blood pressure > or = 140 mmHg or diastolic blood pressure > or = 90 mmHg (measured twice at an interval of 10 minutes at rest), preeclampsia corresponds to hypertension accompanied by proteinuria > or = 0.3 g/L (> or = 30 mg/dL or > or = 1+ albumin in urine on dipstick sample).

Non of these patients had previous history of hypertension, diabetes or renal disease.

These women were followed during their subsequent pregnancies and categorized into those who remained normotensive and those who developed preeclampsia on both clinical and biochemical grounds.

Statistical Analysis

Data were translated into codes using a specially designed coding sheet, and then converted into a computerized data structure. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 7.5 computer software (Statistical Package for Social Sciences).

HCG measurements for pregnant women did not fit a normal distribution as shown by a statistically significant departure from a normal distribution hypothesis (P (Kolmogorov-Semirnov test) < 0.001), therefore this variable was converted into multiples of median (each value was divided by 19,520, which is the value of median in the present study sample). Different cutoff values for HCG level were selected to test the validity parameters of HCG test in the prediction of PET development. Chi-square test (or were appropriate Fisher's exact test) was used to assess the statistical significance of association between HCG level (at different cutoff points) and development of PET. Relative risk (RR) was calculated to assess the magnitude of risk for developing PET for subjects with HCG level above a certain cutoff point compared to those with HCG level below this cutoff point. This kind of risk assessment is valid for a cohort design in which the risk factor status is known at the start of study and the study subjects are followed for a certain period of time to record the development of the outcome of interest. while OR is an estimate of RR calculated in case-control studies. The 95% confidence interval gives an idea about the expected range of the calculated RR in the target population, it is affected by sample size and is useful in assessing credibility of the calculated estimate from the present random sample.

Mann-Whitney U test was used to assess the statistical significance of difference in median between 2 groups. This test is the non-parametric counterpart of t-test and is used when the assumption of normal distribution of the tested variable is not valid as is the case for HCG level. Independent samples t-test was used to assess the statistical significance of difference in age, weight and blood urea between 2 study groups. P value less than the 0.05 level of significance was considered statistically significant. Different cutoff values of β-HCG level were used to construct a ROC (Receiver Operator Characteristics) curve to assess the screening potential of this test for preeclampsia.

Test performance Characteristics

The performance characteristics of a test or criteria, sometimes called test operating characteristics include, among others: Sensitivity, specificity, positive predictive value and negative predictive value.

Sensitivity, is the conditional probability that a diseased person has a positive result. Its value can be changed by changing the cutoff point for positive test results. *Specificity* is the conditional probability that a disease-free person has a negative test result (Sorlie DE, 1995).

Positive Predictive Value (PPV), is the conditional probability that a person with a positive test result is truly diseased. Its value depends on the cutoff point for positive test result and the prevalence of the disease in the screened population. *Negative predictive value (NPV)* is the conditional probability that a person with a negative test result is truly free of the disease (Sorlie DE, 1995).

Proportion of false negative, is the conditional probability that a diseased person has a negative test result. It is the complement for the probability of true positive (sensitivity). *Proportion of false positive*, is the conditional probability that a diseased free person has a

positive test result. It is the complement for the probability of true negative (specificity).

Accuracy (percent agreement), represent the proportion of subjects in which the test agreed with their status (true positive and negative) out of all tested subjects.

Formulas used in calculation

$$Sensitivity = \frac{Number\ of\ true\ positives}{Number\ of\ diseased\ people}$$

$$Specificity = \frac{Number\ of\ true\ negatives}{Number\ of\ non\text{-}diseased\ people}$$

$$PPV = \frac{Number\ of\ true\ positives}{Number\ of\ positive\ test\ results}$$

$$NPV = \frac{Number\ of\ true\ negatives}{Number\ of\ negative\ test\ results}$$

$$Accuracy = \frac{Number\ of\ (true\ positives + true\ negatives)}{Total\ number}$$

$$False\ -ve = 1\text{-}Sensitivity$$

$$False\ +ve = 1\text{-}Specificity$$

The results presented in this chapter were based on the analysis of HCG tests on 96 normal singleton primgravida females in the second trimester. These patients were followed up screening for the development of PET.

Type of distribution for HCG

The box plot in (Figure 1.1) shows that the majority (six out of seven) points that were extreme HCG values (extremely high compared to average values of HCG in the present sample) belonged to patients who developed preeclampsia later on. As a definition extreme values are those data points that are more than three box widths away from the margin of the box (which represent the HCG values of the subjects in central (50%) of HCG distribution). One can notice that (Figures 3.2 and 3.3) that represent the distribution of study subjects by weight and blood urea have no extreme high values.

When subjects were divided according to development of PET later on into PET positive and negative groups, the box plot that represented PET positive group showed no extreme values, (Figures 3.4 and 3.5). Which point out that this group is more or less homogenous regarding HCG values.

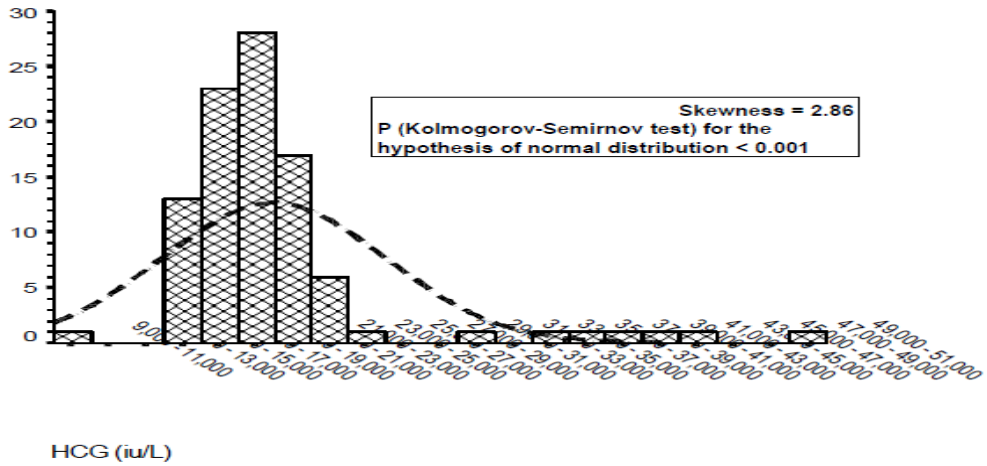

Figure 3.6: Histogram showing the frequency distribution of HCG concentration (with a proposed normal curve) in the study sample (n=96).

As shown in (Figure 3.6), the HCG value for subjects in the present sample do not provide enough evidence to support the normal distribution hypothesis, because of the long positive tail (positive skewness). The present data significantly depart from what is expected based on a normal distribution hypothesis. Therefore in the remaining part of the result chapter HCG will be converted into multiples of median and dealt with as a distribution free variable.

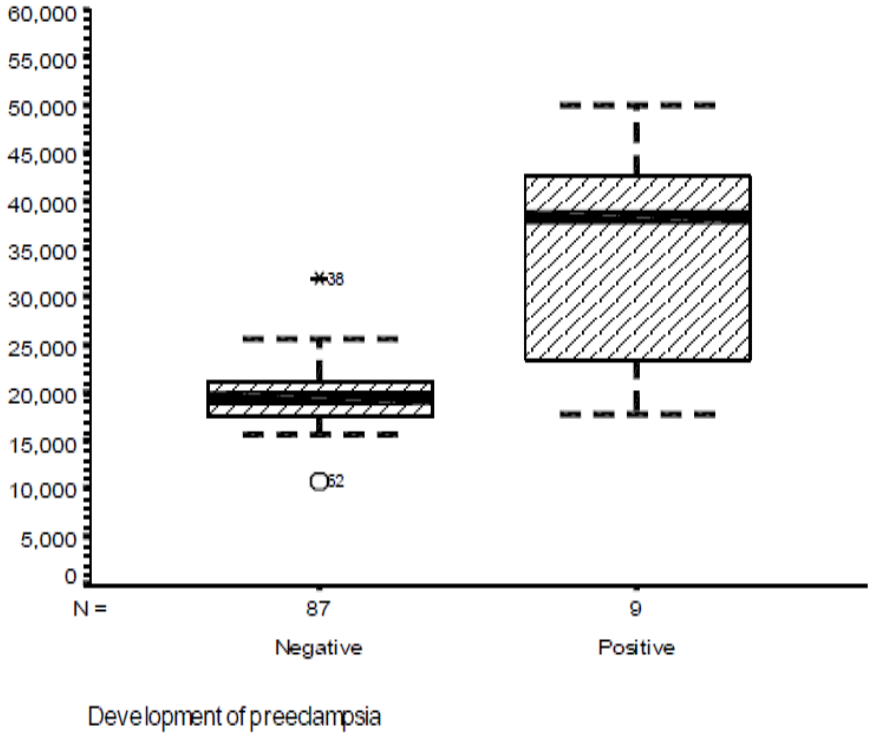

Figure 3.4: Box plot showing the distribution of two study groups (PET positive and negative) by HCG concentration (iu/L).

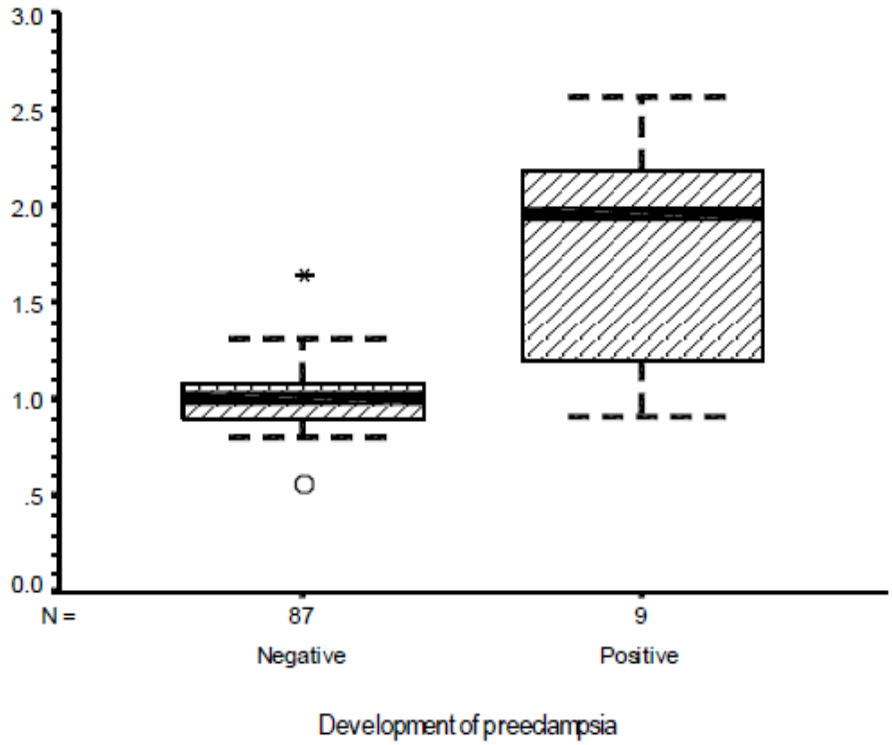

Figure 3.5: Box plot showing the distribution of two study groups (PET positive and negative) by HCG level measured in multiple of median (MOM).

As shown in (Table 3.1), the crude incidence rate of PET in normal singleton primigravida females was (9.4%). The incidence rate is almost doubled (16.7%) in females with a second trimester reading of HCG above 1 MOM (multiple o median) or 19,520 iu/L, (Figure 3.7). The incidence further increased to 85.7% among females with a second trimester HCG level above 1.5 MOM (29,250 iu/L), (Figure 3.8). All the 6 female patients (100% incidence rate) with an HCG value above 32,000 iu/L (1.65 MOM) develpoped PET lateron, (Figure 3.8). HCG level at different cutoff points significantly affected the incidence rate of PET, (Table 3.1).

The risk of developing PET in females with an HCG level above a certain cutoff value was compared to those with HCG level below this point. The risk of development of PET was highest (RR=30 with a 95% CI of 9.9-91.3) in female subjects with a second trimester HCG level above 1.65 MOM (32,000 iu/L). The lowest risk was observed in females with HCG level above 1 MOM (RR=8 with a 95% CI of 1.1-61.5).

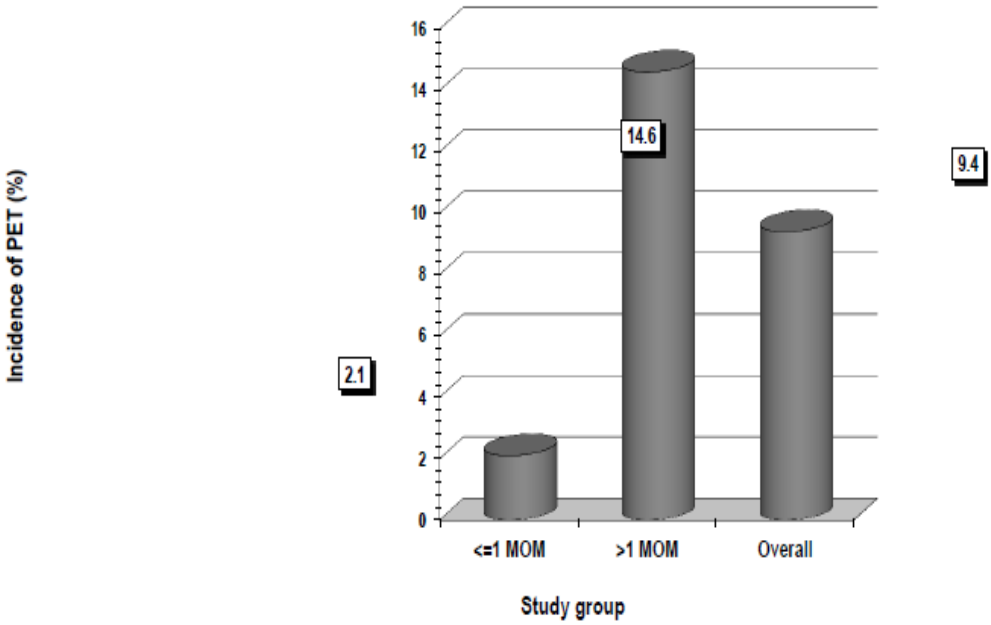

Figure 3.7: Bar chart showing the incidence rate of PET in 3 study groups.

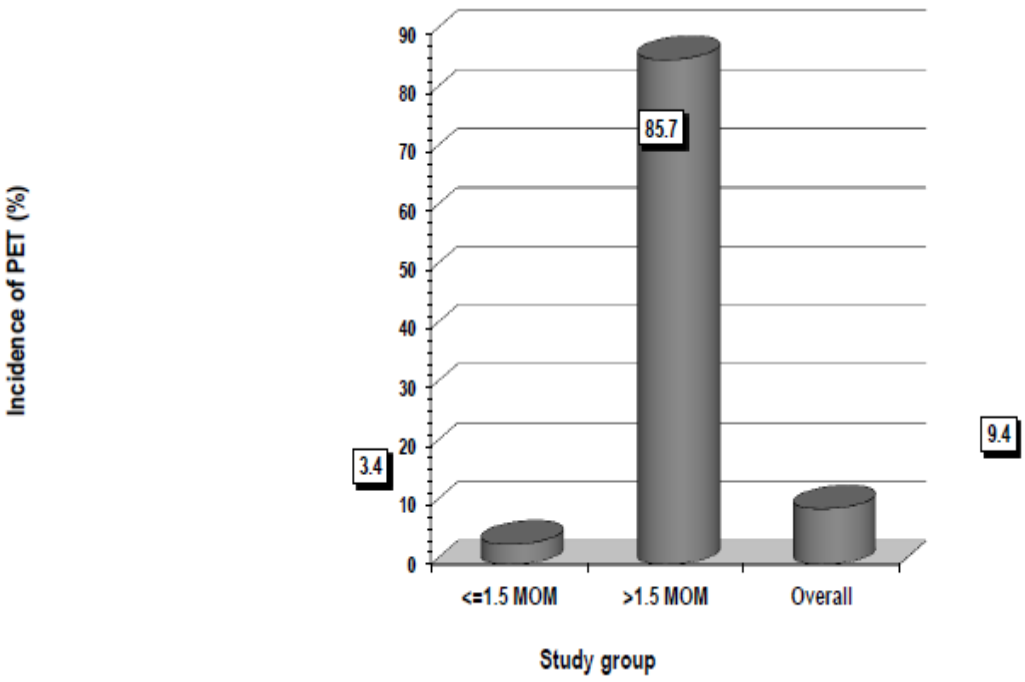

Figure 3.8: Bar chart showing the incidence rate of PET in 3 study groups.

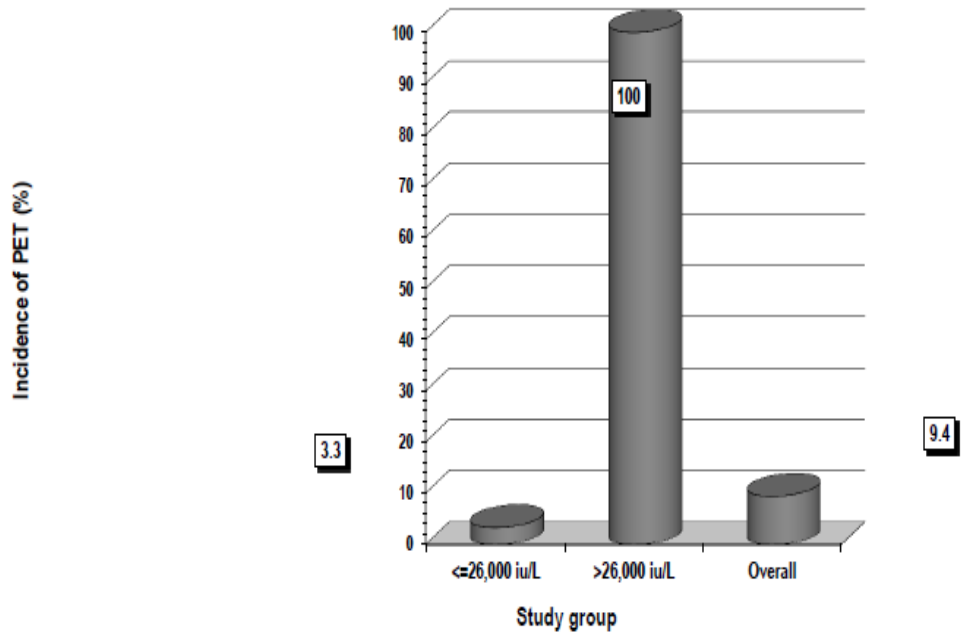

Figure 3.9: Bar chart showing the incidence rate of PET in 3 study groups.

As shown in (Table 3.2), the median HCG level (measured in the second trimester of pregnancy) in PET positive group (38,230 iu/L or 2 MOM) was significantly higher than that of PET negative group (19,480 iu/L or 1 MOM).

The mean age of PET positive group (26 years) was not significantly different from that of PET negative group (26 years). The mean blood urea nitrogen in PET positive group (22.7 mg/dl) was not significantly different from that of PET negative group (21.6 mg/dl). The mean body weight in PET positive group (69.1 Kg) was not significantly different from that of PET negative group (69.5 Kg), (Table 3.2).

Four HCG cutoff points were used to study the validity parameters of HCG test in the prediction of PET development. Three cut off points, namely 1, 1.5 and 2 MOM were used for the sake of comparison with other previous studies. The 32,000 iu/L (1.65 MOM) point was used because it provided the unique opportunity of having a test that is (100%) diagnostic.

Considering HCG value above 1 MOM (19,250 iu/L) as a positive test the following validity parameters will result. When used as a screening tool it can predict 88.9% of possible PET cases, however a high proportion of these positive results will be false positive results (46%) and positive predictive value of HCG will be (16.7%), i.e. given a positive result one can be only 16.7% confident that this specific female will develop PET in the coming months of pregnancy. Given a negative result of HCG test on the other hand one exclude the possible development of PET with (97.9%) confidence.

Considering HCG value above 1.5 MOM (29,250 iu/L) as a positive test the following validity parameters will result. When used as a screening tool it can predict 66.7% of possible PET cases, almost all of these cases will be true positive cases, i.e. given a positive result one can be 85.7% confident that this specific female will develop PET in the coming months of pregnancy. Given a negative result of HCG test on the other hand one can exclude the possible development of PET with (96.6%) confidence. Its worth to notice here that although the probability of false negative test is high (33.3%) the NPV is very high, this is because of low incidence of PET in the present sample. This need not to be the case in females with a high chance of developing PET because of other reasons.

Considering the HCG test as positive when it is above 32,000 iu/L (1.65 MOM) a test with 100% specificity and 100% PPV (diagnostic). The sensitivity of the test however will be low (66.7%). The PPV although 100% in the present sample depends on the clinical index of suspicion based on history and other clinical parameters. Since a high clinical suspicion can increase the PPV and decrease the NPV, while a low index of suspicion (like in the present study sample) can decrease the PPV and increase the NPV. The ORC curve shows the reciprocal changes in sensitivity of detecting development of PET versus false positive (1-specificity) at various cutoff values of HCG measured in the second trimester, figure 3.10.

A link between elevated material HCG and pregnancy induced hypertension has been long suspected. As early as 1950 the placental hormone HCG was reported to be elevated in toxemia affected pregnancies.[28] In our study there was significant correlation between elevated second trimester HCG levels and pregnancy-induced

hypertension (P<0.001), similar findings were observed by Gonen et al.(26) Our results show an incidence of preeclampsia in our study group (primigravida) was (9.4%), it appear to be higher than that observed by Luckas et al.[30] which was only (4.4%). More recently, the predictive value of maternal HCG for pregnancy-associated vascular disease was investigated by Vaillant et al.[29] We found that our results about the validity parameters of β-HCG test at a cutoff point of 1.5 MOM (29,250 iu/L) showed a sensitivity of (66.7%), specificity (98.9%), positive predictive value (85.7%) and negative predictive value (96.6%), a false negative rate of (33.3%) and a false positive rate of (1.1%), which agree with that observed by Vaillant et al.[29] [sensitivity (67%), specificity (91.6%), positive predictive value (86%) and negative predictive value (97.4%)].

Different cutoff values of β-HCG level were used to construct a ROC (Receiver Operator Characteristics) curve to assess the screening potential of this test for preeclampsia. In the present study the ROC curve shows that for β-HCG level > 1.5 MOM (29,250 iu/L) the sensitivity was 66.7% and specificity was 98.9% compared to that obtained by Luckas et. al.[30] which reported a sensitivity of 79% and a specificity of 54%.

CONCLUSIONS

Our data confirm previous reports that demonstrated an association between elevated second trimester maternal serum β-HCG and the subsequent development of pregnancy induced hypertension and preeclampsia.[26,27] It is clear that early in gestation the stage is set for pregnancy induced hypertension. Investigators have been shown that women destined to have the disorder may exhibit multiple physiologic changes by the second trimester. All these indicate that pregnancy induced hypertension is not a disease of late pregnancy or of hypertensive origin but is instead a progressive systemic disease. Maternal serum β-HCG measured in the second trimester has some predictive value for preeclampsia in primigravida women. The study demonstrated an increased risk of preeclampsia in women with increased second trimester HCG levels and suggested that a second trimester screening program might be useful to anticipate those at risk for pregnancy induced hypertension.

RECOMMENDATIONS

Because of the relatively high prevalence rate of preeclampsia in our population (9.4%) among primigravida, the second trimester serum β-HCG measurement would be a potentially cost-effective tool for identifying women at risk for the development of preeclampsia, such a screening program would facilitate closer surveillance and possible intervention with low dose aspirin or calcium supplementation.

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