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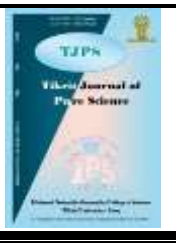

Maternal serum Alpha-fetoprotein Level at 12, 22 and 32weeks' gestation in screening for pre-eclampsia

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DOI: http://dx.doi.org/10.25130/tjps.23.2018.121

ARTICLE INFO.

Article history:

-Received: 10 / 1 / 2018

-Accepted: 9 / 3 / 2018

-Available online: / / 2018

Keywords: preeclampsia, alpha-fetoprotein, screening.

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Abstract

Pre-eclampsia is a major cause of maternal and perinatal morbidity and mortality. Alpha_fetoprotein is measured in pregnant women, as a screening test for pre-eclampsia.This study aimed to examine the maternal serum alpha-fetoprotein at 12, 22 and 32 weeks 'gestation in singleton pregnancies and its performance as a screening test for Pre eclampsia. The study designed as prospective (Cohort) study, it was conducted in Obstetrics department in Salah AL Din Teaching Hospital, from the period of the 15th of December 2016 to the 1st of July 2017. Ninety six pregnant women at first trimester participated in the study, followed up prospectively sixty three of them was attended a second test in second trimester and seventy five of them was attended a third test in a third trimester. The mean age of the studied group was 27.1 ± 5.8 (range: 17-30) years .It had been observed that the mean level of Alphafetoprotein was increased and the mean Multi of Median(MoM) was significantly reduced with advanced gestational age., there are highly significant differences at the different check-points in both mean and MoM, of the maternal alpha-fetoprotein. **T**he mean alpha-fetoprotein level was higher in pre-eclampsia group compared to non-preeclampsia in all of the three testing points. The Comparison of mean alpha-fetoprotein levels of pre-eclampsia vs. non-preeclampsia women revealed that the mean, alpha-fetoprotein level was higher in pre-eclampsia group compared to non-preeclampsia in all of the three testing points**,** alpha-fetoprotein level at the second test was the better than the first and third test.

Introduction

Pre-eclampsia and eclampsia are still among the most important causes of maternal mortality, both in high- and low-income countries. Pre-eclampsia is the 2nd most frequent cause of direct maternal death, [1]. These deaths are avoidable as substandard care complicates 90% of these death. Pre-eclampsia accounts for approximately 25% of all very low birthweight infants, a significant number of preterm births and has perinatal mortality.[2,3].

Accurate prediction of pre-eclampsia remains difficult, there are a number of maternal risk factors which can be easily assessed in early pregnancy, such as nulliparity, obesity, pre-gestational diabetes, maternal and paternal ethnicity, Asthma, coronary heart diseases,[4-9].

Alpha fetoprotein (AFP) is the major serum protein in the embryonic stage and in the early fetal stage. AFP is a glycoprotein that is normally produced (in early pregnancy) by the fetal yolk sac, liver and gastrointestinal tract that is brought to the fetal form of serum albumin. Moreover, a considerable amount of AFP is synthesized by the choroid plexuses and is related to the cerebrospinal fluid, [10]. In fact the human fetus has highest amount of AFP levels than that found in adult. The concentration of AFP increased steadily in both fetal serum and amniotic fluid until 13 weeks, after which levels rapidly decrease. Conversely, AFP is found in steadily increasing quantities in maternal serum after 12 weeks,[11].

The function of AFP in adults is unknown; however, in fetuses it binds estradiol to prevent the transport of this hormone across the placenta. AFP is measured in pregnant women through the analysis of maternal blood or amniotic fluid, as a screening test for a subset of developmental abnormalities, [10,12].

It can also be used as a biomarker to detect a subset of tumors in non-pregnant women, men and children. A level above 500 nanograms /milliliter of AFP in adult can be indicative of hepatocellular carcinoma, germ cell tumors and metastatic cancers of the liver.[13,14].

Patients and Methods

The study was conducted as a longitudinal (prospective study), in gynecological department in Salah Al Din Teaching Hospital, from the period of the 15th of Dec. 2016 to the 1st of July 2017.

Ninety six pregnant women at 1st trimester participated in the study, followed up prospectively sixty three of them was attented asecond test in second trimester and seventy five of them was attended a third test in a third trimester. The follow up regarding visits dates of the pregnant women accomplished by phone call.

These subjects included in the study consist of 96 pregnant women of only **Singletone pregnancy**, attended routine visits to the gynecological outpatient. Clinical examination & ultrasonography done by gynecologist for all patients.

A special questionnaire form was arranged and full information were collected and the clinical details were recorded from each pregnant women which include: name, age, address, ethnicity, parity, gravidity, Last menstrual period, related symptoms, medical history of chronic hypertension, diabetes mellitus and renal disease, family history of PE, previous pregnancy with PE , pregnancy interval, gestational age, maternal height, weight and BMI. The gestational age was calculated from the Last menstrual period, and by ultrasonography.

The informed consent was taken from each patient agreeing to participate in a study

In the first hospital visit, at 11 +0 to 13+6 weeks' gestation, we recorded maternal characteristics and medical history and performed combined screening for aneuploidies. The second visit, at 19 +0 to 24+6weeks' gestation, and the third, at 30 +0 to 34+6weeks, included ultrasound examination of the fetal anatomy and estimation of fetal size.

Exclusion criteria:

Pregnancies that ending in termination, Miscarriage, Fetal death before 24 weeks' gestation, Twin pregnancy, Pregnancies with aneuploidies or major fetal abnormalities.

Methods:

Blood pressure measurement:

That women with hypertension after measuring their blood pressure in addition to the information of questionnaire form, (An optimal blood pressure is one of under 120 over 80 mmHg. The systolic (upper) value is below 120 mmHg and the diastolic (lower) value is below 80 mmHg. A systolic blood pressure of 120 to 139 mmHg or a diastolic value of 80 to 89 mmHg is called high normal. Values of 140 mmHg or more systolic and 90 mmHg or more diastolic are called hypertension. When one of the two values is elevated, this is enough to be in the high normal or the hypertension group. Blood pressure was taken by the indirect method (auscultatory method) by the means of sphygmomanometer, after 10 minute resting.

Anthropometric:

Body height was measured to the nearest one centimeter & body weight was measured by electronic scale, the women measured without shoes, so from these two parameters the body mass index (BMI) was calculated from body weight (kg) divided by height square (m2).

Blood sampling:

About 5 ml blood was drawn from each participant using a tourniquet from median cubital vein in cubital fossa from pregnant women at 12, 22 and 32 weeks' gestation, the blood drawn into a tube without anticoagulation and serum separated by centrifugation (BioTeck) at 4000 rpm for 20 minutes (1-2 ml serum was used. Determine the absorbance of each well at 450 ± 10 nm with a microtiter plate reader.

AFP was measured by using DEMEDITIC AFP ELISA Kit (Germany) is a solid phase ELISA based on the sandwich principle. The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site on an AFP molecule. An aliquot of patient sample containing endogenous AFP is incubated in the coated well with enzyme conjugate, which is an anti=AFP antibody conjugated with horseradish peroxidase.

Statistical analysis:

Data were entered and analyzed using the statistical package for social sciences (SPSS) version 24. Descriptive statistics presented as mean, median, multiple of median standard error of mean, 95% confidence interval for mean, frequencies and proportions. Analysis of variances (ANOVA) test was used to compare 3 means or more of a continuous variable. Chi square test was used to compare frequencies of categorical variables.

Level of significance (P. value) set at $P \leq 0.05$ to be considered as significant difference or correlation.

Results

A total of 96 women were enrolled in this study and followed up prospectively, the mean age of the studied group at the beginning of the study was 27.1 ± 5.8 (range: 17-30) years. Eighteen 18.8% were nulliparous, 24 women (25%) had history of one or more abortions, 30.4% had an inter pregnancy interval IPI of ≤ 1 year and majority (84.4%) had spontaneous conception.

The distribution of AFP of the studied groups at the different check points is shown in figure 1, the mean AFP and 95% confidence interval (95% CI) for the

mean of the 96 women at the first test was 41.83 (30.06 – 53.61), that of the 63 women at the second test was 156.14 (135.07 - 177.88) for the 75 women at the third test it was 208.82 (194.36 - 223.74) , the corresponding values for the multiple of median (MoM) was 1.74 (1.26 – 2.22) at first test, 1.55 (1.33 – 1.77) at the second and 1.01 (0.94 – 1.05) at the third test is shown in figure 2. It had been observed that the mean AFP level was significantly increased and the mean MoM was significantly reduced with advanced gestational age, (P< 0.001) The multiple comparison using the analysis of variances (ANOVA) test revealed a highly significant differences at the different check-points in both mean AFP and MoM .

The comparison of mean AFP levels of pre-eclampsia vs non-preeclampsia women revealed that the mean AFP level was significantly higher in pre-eclampsia group compared to non-preeclampsia in all of the three testing points, (P<0.001), furthermore, the differences was also significant when compare the mean AFP level within each group, i.e. the same trend when check for AFP level in the whole group however, the change in mean AFP was significantly larger in PE than non-PE group, (P=0.011), (Figure4). On the other hand comparison of MoM of PE vs. non PE revealed inverse trend in both groups where the mean MoM of AFP in PE group at first test was 4.985, 2.659 at second test and 1.214 at the third test and the difference were statistically significant on the subsequent testing in PE group while in non-PE group the differences in mean MoM were not significant when compare Second vs. Third tests, (Figure 5).

The cutoff points used for calculation of sensitivity and other validity parameters by ROC curve analysis were depending on the median value at each test, according to these cutoff points then the sensitivity specificity and accuracy of AFP in prediction of PE were calculated from the ROC curves of each testing point, it is worth mentioning that in addition to these validity parameters, area under the curve (AUC) is an important indicator for any diagnostic test, a test with an AUC of 0.5 is failed test. A test with larger AUC close to one is the better and good predictor. According to these findings, AFP level at the second test was the better than the first and third testing of AFP, and the first test was better than the third one.

Table 1. Age, residence and BMI of the studied group (N = 96)

Variable		No.	%
Age (year)	≤ 20	11	11.5
	21 - 30	55	57.3
	> 30	30	31.3
	mean ± SD	27.1 ± 5.8	-
	Range	17 - 30	-
House	Urban	82	85.4
	Rural	14	14.6
BMI	Normal	33	34.4
	Overweight	42	43.8
	Obese	21	21.9
	mean ± SD	27.3 ± 4.5	-
	range	21.2 – 47.8	-

Table 2. Obstetric history of the studied group (N = 96)

Obstetric history		No.	%
Gravidity	Gravida 1-2	36	37.5
	Gravida 3-4	37	38.5
	Gravida 5 or more	23	24.0
Parity	Nulliparous	18	18.8
	One	24	25.0
	Two	19	19.8
	Three	20	20.8
	Four or more	15	15.6
Abortion	One	20	20.8
	Two or more	4	4.2
	None	72	75.0
	Total	24	100.0
Interpregnancy interval (year) (Parous only)	≤ 1	24	30.4
	2 – 3	40	50.6
	≥ 4	15	19.0
	Total	79	100.0
Mode of conception	Spontaneous	81	84.4
	Ovulation induction	15	15.6

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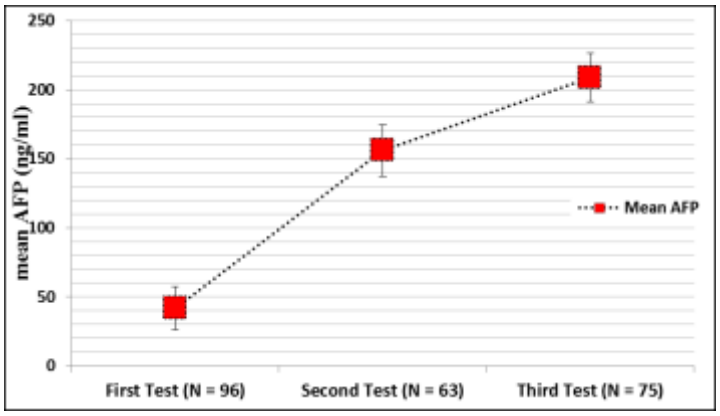
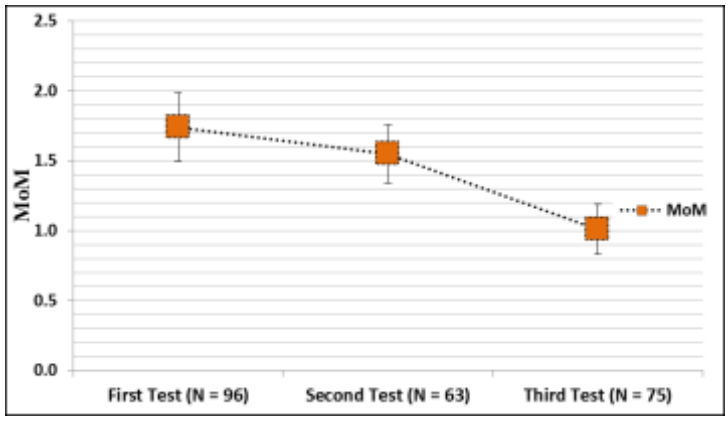


Figures 1. Comparison of mean AFP level on the three tests



Figures 2. Comparison of mean multiple of median of AFP on the three tests

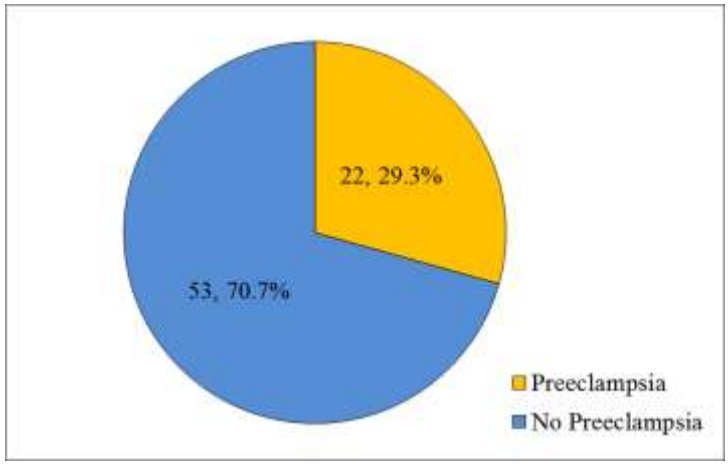


Figure 3. Distribution of PE among 75 women at the third trimester

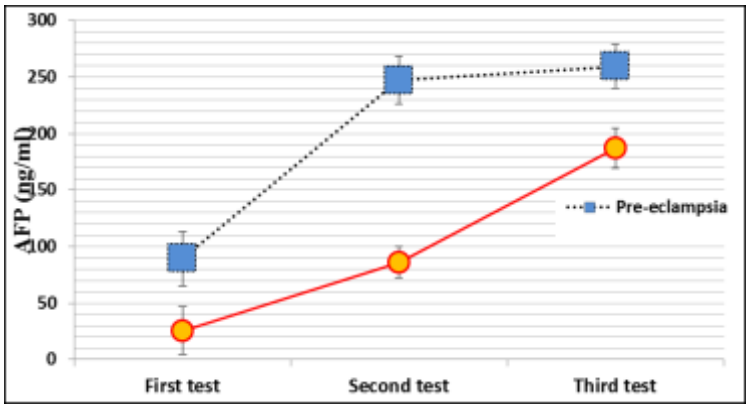


Figure4 . Trends of AFP P in PE and non-PE group

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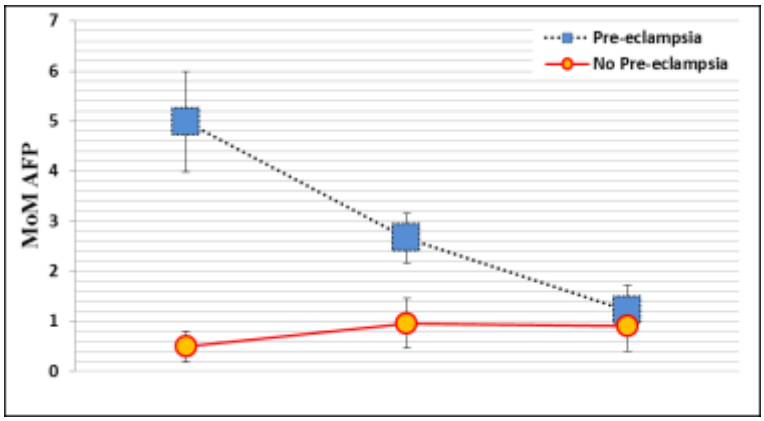

Figure5 . Trends of MoM of AFP in PE and non-PE group

Table3. Correlation between PE and maternal variables

Variable		Preeclampsia				P.value
		Pre-eclampsia (n = 22)		No Pre-eclampsia (n = 53)		
		Count	Row N	Count	Row N	
Age (year)	**≤ 20**	0	0.00	8	100.00	**0.030**
	21 - 30	12	27.30	32	72.70	
	> 30	**10**	**43.50**	13	56.50	
Residence	**Urban**	18	28.10	46	71.90	0.58
	Rural	4	36.40	7	63.60	
BMI	**Normal**	2	8.70	21	91.30	**0.002**
	Overweight	9	27.30	24	72.70	
	Obese	11	57.90	8	42.10	
Gravidity	**Gravida 1-2**	4	13.80	25	86.20	0.061
	Gravida 3-4	11	40.70	16	59.30	
	Gravida 5 or more	7	36.80	12	63.20	
Parity	**Nulliparous**	**1**	**6.70**	14	93.30	**0.011**
	One	4	21.10	15	78.90	
	Two	4	30.80	9	69.20	
	Three	10	62.50	6	37.50	
	Four or more	3	25.00	9	75.00	
Interpregnancy interval (year)	**≤ 1**	7	36.80	12	63.20	0.19
	3-Feb	11	36.70	19	63.30	
	≥ 4	3	25.00	9	75.00	
	Nulliparous	1	7.10	13	92.90	
Abortion	**One**	4	26.70	11	73.30	0.37
	Two or more	2	66.70	1	33.30	
	None	16	28.10	41	71.90	
Mode of conception	**Spontaneous**	17	27.40	45	72.60	0.43
	Ovulation induction	5	38.50	8	61.50	
PE in previous pregnancy	**Yes**	**18**	**85.70**	3	14.30	**< 0.001**
	No	4	7.40	50	92.60	
Family history of PE	**Yes**	**10**	**76.90**	3	23.10	**< 0.001**
	No	12	19.40	50	80.60	

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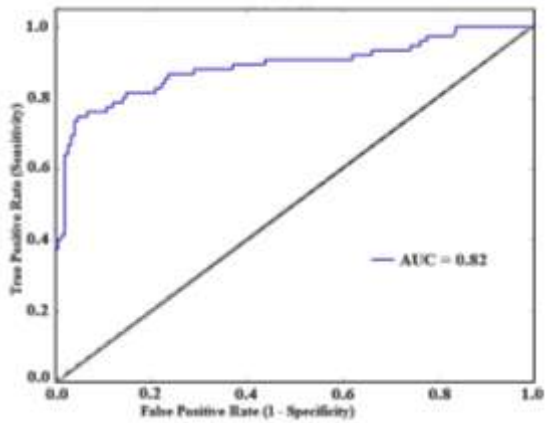

Figure 6. ROC curve for the validity of AFP at first test in prediction of P

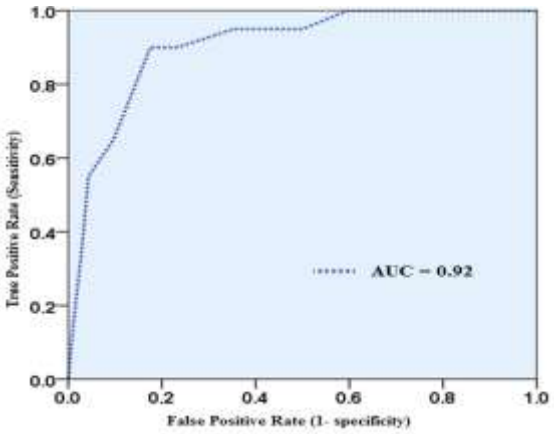

Figure 7. ROC curve for the validity of AFP at second test in prediction of PE

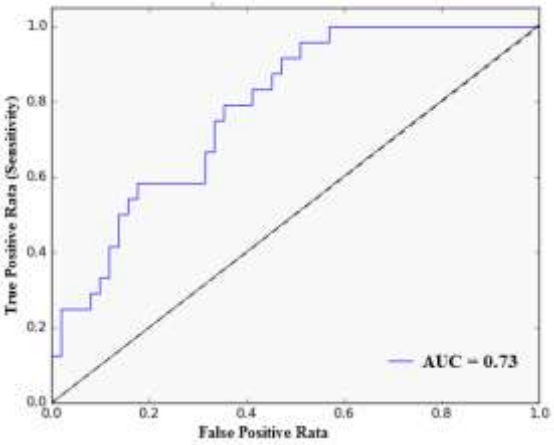

Figure 8. ROC curve for the validity of AFP at third test in prediction of PE

Discussion

Maternal serum alpha-fetoprotein (AFP) levels during pregnancy is altered, abnormal change in AFP could lead to adverse pregnancy outcome, including pre-eclampsia (PE) and fetal death. The current study tried to assess the role of AFP in screening for PE in 1st, 2nd and 3rd trimesters as a biochemical screening test.[19]. The present study has established a reference distribution for maternal serum AFP at 11-13 weeks, 19-24 and 32 weeks in our cites. In the current pregnancy PE was reported in 22 out of 75 women giving an incidence of 29.3% and this was higher than that reported in previous studies; in a study conducted in India 20% of women developed PE, [20], while another study from Iran in 2010 a lower rate of 5.1% was reported, [21]. However, the difference in the incidence of PE might attribute to population variation.

The data of this study demonstrated that in women who developed PE, there is increased levels of maternal serum AFP progressively during the first, second and third trimesters. The MoM values changed adversely as greater with earlier, compared to later.

The current study found that the mean AFP of the studied group was significantly increased with advancing gestational age, with a mean at first, second and third trimester of 41.83, 156.14, and 208.82 ng/IU, respectively.

Previous studies and literatures documented that AFP level increased with the gestational age and progression of pregnancy. Kuo *et al* (2003) investigated the association between elevation of AFP and pregnancy outcomes on 168 singleton pregnancies. They suggested that screening for pregnancies with elevated AFP and pregnancy outcomes included preterm labor, preeclampsia, intrauterine fetal death would help to identify the low-risk cases and facilitate cost-effective management [22].

Previous studies showed that increases risk of pregnancy-induced hypertension, preterm labor, oligohydramnias and abruptio placenta are associated with elevated AFP levels.[23,24].

Walters *et al*. reported that 13% of women with elevated AFP developed pre-eclampsia compared to 1% of the women with normal AFP, [25]. Williams *et al*. compared 201 women with unexplained elevated AFP (greater than and including 2.0 MoM) with 211 women with normal AFP. A significant association was found between elevated AFP and pre-eclampsia, [26]. The current study found that pre-eclamptic women had significantly higher AFP levels across the three trimesters than non-pre eclamptic women ($P<0.001$), on the other hand, the comparison of AFP levels across the three trimester within each group revealed that AFP levels increased significantly with progression of pregnancy and advanced gestational age. Using a threshold value of two multiples of the median (MOM), elevated AFP in the mid-trimester has been shown to be associated with a 2.3 to 3.8 fold increased risk of developing pre-eclampsia, [27]. According to these findings and further analysis using ROC curve, the current study found that AFP is a good predictor for both the incidence and severity of PE, The best validity was reported in the second rather than first or third trimesters. These results are in good agreement with the results of **Bredaki** (2016), who found that the screening for preeclampsia is superior with screening at 19 -24 than at 11-13 weeks[27].

Other findings reported in the present study included the association between maternal variables and PE where it had been significantly found that PE was more frequent in older age (>30 years), this might explained by the fact that older women had more prior terminations, were more likely to have a body mass index (BMI) >25, had more in vitro fertilization (IVF) and other fertility treatments and a higher incidence of maternal diabetes and chronic hypertension (P<0.001). A registry-based study on pregnant women in Finland (2004) by Jacobsson B. *et al* (2004), showed that women of advanced maternal age exhibited more preeclampsia (9.4%) than younger women (6.4%).[28]. The incidence of obesity is increasing at an alarming rate. There is compelling evidence that obesity increases the risk of preeclampsia about 3-fold, and in developed countries is the leading attributable risk for the disorder,[29]. The present study found that obesity increases the risk of preeclampsia (21.9%) , these findings agreed that reported in an USA study was conducted by Bodnar LM *et al* in 2005 who found that obesity is the leading attributable risk for preeclampsia in 30% of cases,[29].

History of PE in previous pregnancy considered as an important risk to develop PE in next pregnancy and those with family history of PE, [30]. In the present study, pregnant women who had PE in their previous pregnancy (27.1%) and those with positive family history of PE (18.8%) were more likely to develop PE in their current pregnancy. Similar findings were also documented by Ros H S *et al* in 1998, where authors in this case two studies documented that women who developed PE in previous pregnancies were about 2-3 folds more likely to have PE in the next pregnancy, 11.5% of cases had a past history of hypertension and 9% of PE cases had a family history of PE,[31].

Conclusion

The Comparison of mean AFP levels of pre-eclampsia vs. non-preeclampsia women revealed that the mean AFP level was significantly higher in pre-eclampsia group compared to non-preeclampsia in all of the three testing points AFP level at the second test was the better than the first and third testing of AFP, and the first test was better than the third one.

Recommendations

To further investigate our hypothesis whether the association between Alphafetoprotein and preeclampsia is dependent, we suggest;

1. Different biochemical markers that might demonstrate differences at different stages of pregnancy.
2. Larger sample size .
3. Iraqi references regarding AFP screening in normal women.
4. Follow up of obstetrical outcome till labour.

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Tikrit Journal of Pure Science 23 (8) 2018 *ISSN: 1813 – 1662 (Print)*
E-ISSN: 2415 – 1726 (On Line)

مستوى (الفا فيتوبروتين) في مصل الام للأسابيع 12، 22 و 32 من الحمل للتحري عن مرض ما قبل الارجاج الحاد

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الملخص

يعد مرض ما قبل الارجاج الحاد هو سبب رئيسي لاعتلال ووفيات الأمهات يصيب 2–3% من جميع حالات الحمل. يتم تقدير تركيز هورمون الفا فيتو بروتين في النساء الحوامل من خلال دم الام او السائل الامنيوسي ويستخدم للتحري عن مرض ما قبل الارجاج الحاد والتشوهات الخلقية الولادية. جاءت هذه الدراسة لاختبار توزيع نسبة (الفا فيتو بروتين) في مصل الام للاسابيع 12، 22 و 32 من الحمل المفرد اللواتي تعانين من مرض ما قبل الارجاج الحاد واستخدام هذه العلامة البايلوجية للتحري عن مرض ما قبل الارجاج الحاد.تم اجراء الدراسة في قسم النسائية والتوليد في مستشفى صلاح الدين التعليمي للفترة من 15 كانون الاول 2016 ولغاية 1 تموز 2017. كان عدد النساء الحوامل 96 امراة في الثلث الاول من الحمل في الفحص الاول: وتمت متابعتها بشكل محتمل و63 في الفحص الثاني في الثلث الثاني و 75 الفحص الثالث في الثلث الاخير من الحمل اللواتي شاركن في الدراسة. بينت الدراسة ان متوسط اعمار المجموعة المدروسة في بداية الدراسة كان بحدود 27,1 ± 5,8 (المدى: 17–30 سنة)، تم ملاحظه متوسط مستوى (AFP) وكان مرتفعاً بصورة كبيرة في مجموعة مرضى ما قبل الارجاج الحاد مقارنة بالنساء الخاليات من المرض على مدى الفحوصات الثلاثة. ومن خلال المقارنة بين مستوى (AFP) لمرضى ما قبل الارجاج الحاد والنساء الخاليات من المرض تبين ان مستوى (AFP) كان عاليا في مجموعة مرضى ما قبل الارجاج الحاد مقارنة بالنساء الخاليات من المرض على مدى الفحوصات الثلاثة، كان مستوى (AFP) في الثلث الثاني افضل من الثلثين الاول والثالث، والفحص الاول افضل من الثالث.