Evaluation of Unexplained Elevated Maternal Serum Alpha-Fetoprotein between 16-18 Weeks' Gestation and It's Relation with Pregnancy Outcome

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Abstract:

Background: Maternal serum screening is one of several possible first steps in identifying women whose fetuses are at increased risk for a disorder amenable to antenatal detection. The first test utilized widely was the measurement in maternal serum assay of a fetal–specific protein termed Alpha- fetoprotein (AFP).

Objective: To determine whether pregnant women with unexplained elevated maternal serum alpha-fetoprotein levels are at increased risk for adverse perinatal outcome.

Designs: A prospective follow up (cohort) study.

Setting: The study was carried out in the Department of Obstetrics and Gynecology in Al–Yarmouk teaching Hospital, for the period from June 2002 to November 2003.

Patients & Methods: A total of 348 pregnant women of 16-18 weeks of gestation were chosen to participate in this study. A detailed history was taken concentrating on the presence of risk factors for having congenital abnormal baby or poor pregnancy outcome. All the women were offered maternal serum alpha-fetoprotein testing with simultaneous ultrasonography and follow up of them done with observation of their pregnancy outcome.

Results: Eighteen of women had increased MSAFP level (≥2.0 MOM), five cases discovered to have congenital abnormalities, one case was lost from them and the remaining twelve of these women were regarded as unexplained elevated MSAFP. Follow up was done. Eight of them had adverse perinatal outcome and four cases had normal outcome.330 cases were found to have normal MSAFP levels, 61 cases were excluded from the follow up for variable causes, 89 cases were lost and the remaining 180 cases were completed the follow up. 40 of them had adverse pregnancy outcome and 140 cases had normal outcome.

Conclusions: It is concluded that women with elevated serum level of AFP in the second trimester with no apparent cause might have adverse outcome for their pregnancy like preterm labor, PROM, PIH, IUGR, and spontaneous abortion.

Keywords: £ Fetoprotein, 16-18 Weeks' Gestation, Pregnancy Outcome

Introduction:

Maternal serum screening is one of several possible first steps in identifying women whose fetuses are at increased risk for a disorder amenable to antenatal detection. A positive screen leads to one or more tests and / or procedures directed toward making a definitive diagnosis. The first test utilized widely was the measurement in maternal serum assay of a fetal–specific protein termed alpha-feto protein (AFP) [1].

MSAFP screening became the framework on which other pregnancy screening tests were added. Most subsequent protocols take advantage of the modestly reduced MSAFP [2] and the markedly higher human chorionic gonadotrophin [3] in women whose fetuses have Down's syndrome. Unconjugated esteriol is also decreased in such pregnancies and is incorporated into some screening protocols. [4] Other serum markers and screening modalities shown promise for the detection of fetuses with chromosomal abnormalities and are under evaluation [5].

Traditionally, elevated maternal serum AFP measurement have been used as a screening tool for selection of pregnant women from the general population who are at increased risk for neural tube defects and opened ventral wall defects[6]. The association of elevated MSAFP with other structural open fetal defects such as cystic hygroma or other defects that may affect reabsorption or synthesis of AFP (gastrointestinal atresia, polycystic kidney, teratoma, etc.) and as a predictor of adverse pregnancy outcome has also been observed[7,8,9,10,11].

There are conflicting reports as to whether an elevated MSAFP is a reliable marker for the subsequent development of pre-eclampsia.

Maternal liver disease can be a rare cause of significant MSAFP elevation. Maternal liver function tests and occasionally other diagnostic evaluation may be indicated [5].

The purpose of this study was to determine whether pregnant women with unexplained elevated maternal serum alpha-fetoprotein levels are at increased risk for adverse perinatal outcome.

Patients & Methods:

This prospective follow up (cohort study) had been carried out in AL-Yarmouk teaching hospital for the period from June 2002 to November 2003.

A total of 348 women with a gestational age between 16 – 18 weeks had been chosen to participate in this study. They had been collected from maternity care units and out patient clinics in

the department of obstetrics and gynecology in Al-Yarmouk Teaching Hospital, in addition to referral from private clinics and primary health care centers.

A full history was obtained from each woman concentrating mainly on the presence of risk factors for having congenitally abnormal baby or poor pregnancy outcome, like the presence of personal or family history of abnormal baby, Insulin dependent diabetes mellitus (IDDM), epilepsy, drug intake (e.g. anti epileptic drugs, or prophylactic folic acid intake), social history, quality of food intake, previous abruptio placentae, pregnancy induced hypertension (PIH), intrauterine growth restriction (IUGR), intrauterine death (IUD), preterm labor, and premature rupture of membrane (PROM) …etc.

All women were offered MSAFP testing with simultaneous ultrasonography for confirmation of dating, fetal viability, fetal number and the presence of gross congenital abnormalities.

Women with wrong date, twin pregnancy, missed abortion, those with liver diseases and obvious congenital abnormality had been excluded from follow up.

An elevated maternal serum AFP was defined as ≥ 2.0 multiples of medians adjusted for weight and diabetes mellitus. An abnormal test was repeated in 1 week (unless the patient was past 18 weeks or the multiples of the medians value was very high ≥3.0) and gestational dating ultrasonography was obtained, if previously not performed. If 2 values were ≥ 2.0 multiples of the medians patients were offered genetic counseling, high resolution ultrasonography and amniocentesis for amniotic fluid AFP level and chromosomal analysis.

An elevated maternal serum AFP level was considered unexplained if after erroneous gestational dating (i.e. ≥ 10 day discrepancy between menstrual and ultrasonographic dating was corrected for, no evidence of multiple pregnancy, obvious or gross fetal anomaly, oligohydramnios or fetal death was detected on diagnostic ultrasonographic evaluation…etc.)

Patients with unexplained elevated levels were offered weakly non stress testing with biophysical profile follow up, if non reactive and monthly Doppler ultrasonography starting at 28 –32 weeks' gestation.

Adverse perinatal outcomes were defined as spontaneous loss at < 20 weeks' IUGR, premature rupture of membranes, preterm labor at <37 weeks, pregnancy induced hypertension, abruptio placentae, congenital malformations other than neural tube and ventral wall defects, and stillbirth.

Patients with second trimester oligohydramnios were excluded from unexplained elevated maternal serum AFP group, because this finding is a known cause of elevated levels of MSAFP.

Principle of serum AFP assay:

The AFP assay is a two-step "Sandwich" type assay in which two monoclonal mouse antibodies, directed against two different epitopes of the molecule, are employed.

The unknown samples or standards are first incubated in tubes coated with first monoclonal antibody .The contents of the tubes are then aspirated and the revealed by incubation with the second, I^{125} labeled antibody. The contents of tube are aspirated after this second incubation and unbound labeled anti-body is eliminated by washing. The amount of bound reactivity measured in a gamma counter is proportional to the AFP concentration. The unknown values are determined by interpolation from a standard curve.

Sample collection: After informed consent was obtained from the women, 3ml of venous blood sample were obtained, and collected in dry tubes (without additives). Then blood samples were centrifuged for 15 minutes, sera were collected and stored in a deep freeze until the assay was performed (sera may be kept at (2-8) ˚C, if they are to be assayed within 24hours.

For longer storage they were kept at –20 ˚C (2 months maximum) or preferably at – 80˚C (1 year maximum).

The serum samples were diluted 1: 100 in phosphate buffer prior to assay if the sample has a concentration exceeding that of the highest standard.

The standard curve is established at the same time as the samples are assessed, many steps of laboratory techniques were done to determine the radioactivity of the samples then the results are obtained by extrapolation from a standard curve that established. Adjustment for the diabetes was done by regarding MSAFP ≥ 1.5 MOM as a positive value. According to WHO, each 1 I.U corresponds to 1.21 nanogram AFP.

The results of AFP levels obtained were compared with normal ranges which were determined in maternal sera of pregnant females between 16-18 weeks gestation as follows; for 16 weeks of gestation the concentration range is 21.2-92, median 35.4 IU/ml, for 17 weeks of gestation the concentration is 24.2-105, median 40.3 IU/ml, and for 18 weeks of gestation the concentration is 27.7-120, median 46.2 IU/ml.

After taking the results of MSAFP those who have increased MSAFP underwent a diagnostic transabdominal ultrasonography, if they didn't have previously to detect any congenital abnormality. But those who had MSAFP ≥3.0 multiples of medians offered for amniocentesis. The normal scan+ increased MSAFP regarded as a group of unexplained elevated MSAFP and they had been followed up till the 3rd trimester and delivery to observe the outcome. Our follow up was done during the antenatal visits (every 4 weeks till the 28th week, 2 weeks till the 36th weeks then weekly

till delivery) by blood pressure measurement non stress test after 28 weeks with biophysical profile if NST is non reactive. During the follow up we missed 90 cases and we excluded 66 cases from both elevated and normal level MSAFP groups, so 192 cases remained till the end of the follow-up the data were expressed as numbers, percentages and whenever possible as mean of number of the observations. The results were analyzed by using Chi–Square test. Probability (P– Value <0.05 was considered statistically significant).

Results:

Among the 348 women who were underwent MSAFP testing between June 2002 and November 2003, eighteen of them (5.17%) were found to have elevated MSAFP level (≥ 2.0 MOM), one case was lost. All the possible causes of elevated MSAEP listed had been taken in consideration for exclusion, for example congenital abnormalities, five patients with gross congenital abnormalities diagnosed by ultrasound had been excluded. Twelve patients remained as possible causes of unexplained high AFP up to our maximum efforts and facilities we didn't find possible causes of elevation at the time of study and they were subjected to follow up till the end of their pregnancies.

330 women had normal MSAFP levels, of them 61 women (18.5%) were excluded from the study for wrong date, missed abortion hydrocephaly and hydatidiform mole. Therefore, 269 women remained, 89 of them (26.96%) were lost while 180 women (54.5%) were followed up till the end of their pregnancies, as shown in figure 1.

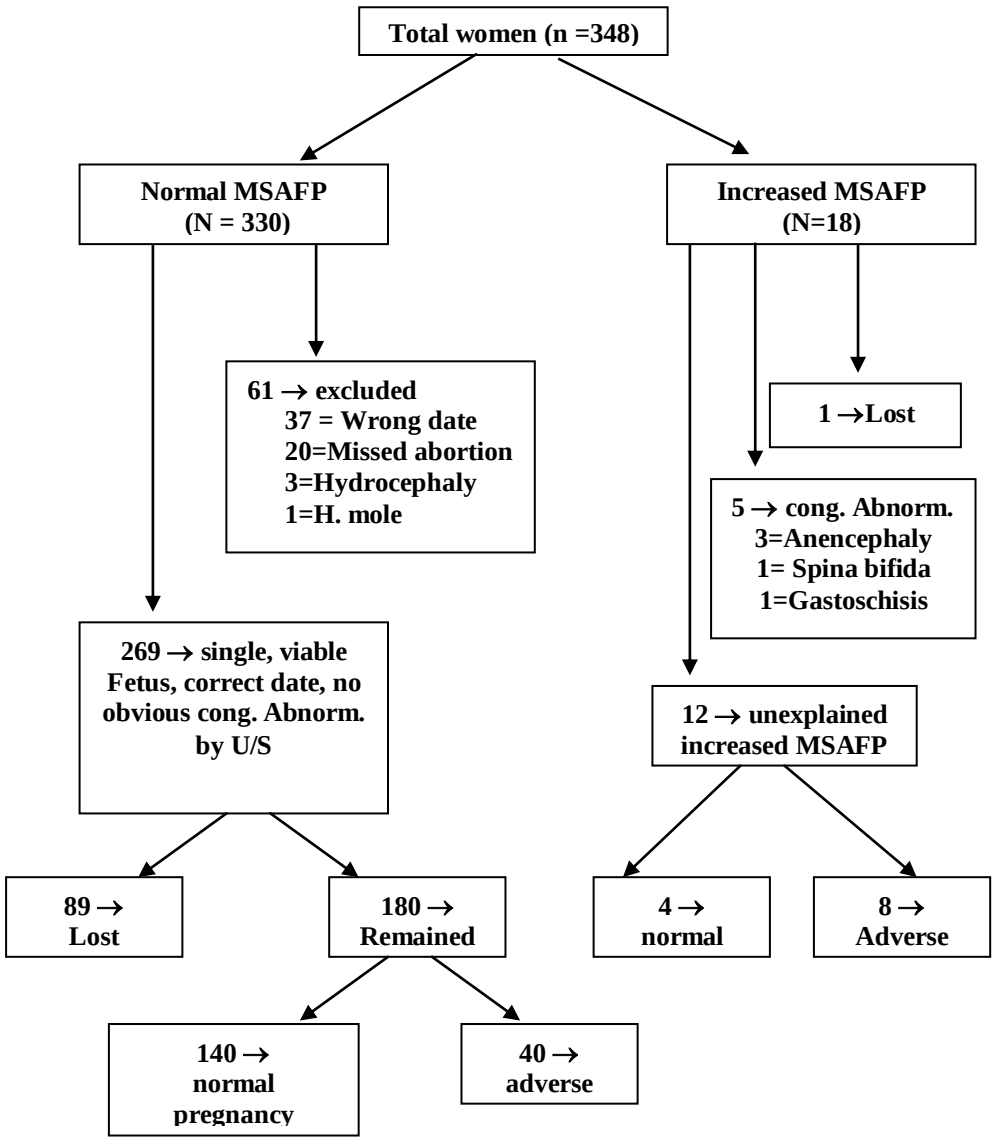

Figure 1: Total number of women participated in the study and their follow-up

Table 1 shows the characteristics of the studied women. The age of the women in the study ranged from 16-42 years. The mean age was 29.4 years in the study group (elevated MSAFP) and 28.3 years was in the control group (Normal MSAFP). The parity range of the studied women was (0 – 11): 93 women (26.7%) were primigravida, 187 women (53.7%) were multipara while 68 women (19.5%) were grand multipara (≥5).

About the gestational age during which the women were studied; 52 women (14.9%) were 16 weeks pregnant, 88 women (25.3%) were 17 weeks pregnant & while 208 women (59.8%) were 18 weeks pregnant.

About the medical history; 6 women (1.7%) had history of IDDM and 4 women (1.1%) were epileptics.

For drug history; 135 women (38.7%) had positive history of drug intake, 4 women (1.1%) take anti-epileptic drugs, 90 women (25.8%) were taking folic acid, and 41 women (11.7%) were taking miscellaneous drugs.

Table 1: The characteristics of the studied women

Criteria	**Total sample (n=348)**	
	No	**%**
Age (years)<18	32	9.2
18-34	259	74.4
≥35	57	16.4
Parity Primi	93	26.7
1 – 4	187	53.7
5 & more	68	19.5
Gestational age (weeks)16	52	14.9
17	88	25.3
18	208	59.8
Medical history IDDM	6	1.7
Epilepsy	4	1.1
Drug history Yes	135	38.7
No	213	61.2
Family history of abnormality Baby Yes	22	6.3
No	326	93.6
Personal history of abn. Baby Yes	9	2.6
No	339	97.4
Past obstetrical history Previous IUGR infant	10	2.8
History of prematurity	32	9
Prior stillbirth	9	2.6
PIH	15	4.3
Previous APH	8	2.3
Current pregnancy Bleeding	24	6.8

Regarding family history; 22 (6.3%) women gave positive family history of previous delivery of congenitally abnormal babies (in form of NTD, hydrocephaly, Down's syndrome, etc).

Personal history; 9 women (2.6%) had positive personal history of previous delivery of congenitally abnormal babies.

Regarding the past obstetric history; 98 women (28%) had some complications in their previous obstetric history, 10 women (2.8%) had previous IUGR, 32 women (9%) had previous premature delivery, 9 women (2.6%) had previous stillbirth, 15 women (4.3%) had previous PIH, 8 women (2.3%) had previous placental abruption. Lastly about the current pregnancy; 24 women (8.6%) had history of vaginal bleeding.

Table 2 shows the women with elevated MSAFP and their description. Five women (27.8%) found to have congenital abnormalities by ultrasonography, so they were excluded from the follow up, one case (5.5%) was lost after the appearance of the result of MSAFP, so she was lost from further assessment (her MSAFP level was $\geq$2.0MOM). 12 cases (66.6%) of them were regarded as unexplained elevated MSAFP, because no identifiable cause had been detected in them.

Table 3 shows the types of fetal congenital abnormalities who were diagnosed by ultrasound in the study group (elevated MSAFP), these were 5 cases (27%); 3 women of them (16%) had fetuses with anencephaly, one case (5.5%) had fetus with spina bifida and one case (5.5) had fetus with gastroschisis.

Table 2: The description of women with elevated MSAFP by U/S findings

Women with elevated MSAFP	**Elevated MSAFP (n=18)**	
	No	**%**
Abnormality	5	27.8
Unexplained	12	66.7
Missing cases	1	5.5
	18	100%

Table 3: Types of fetal abnormalities diagnosed by U/S in the study group (N=18).

Type of abnormality	**Number**	**%**
Anencephaly	3	16
Spina bifida	1	5.5
Gastroschisis	1	5.5
Total	5	27

Table 4 shows the causes of exclusion by ultrasonography in those women with normal MSAFP. 61 women (18.4%) were excluded because 37 cases (11.2%) were having wrong date, 20 cases (6%) had missed abortion, 3 cases (0. 9 %) had hydrocephaly, one case (0. 3%) was discovered to have hydatidiform mole.

Table 5 shows the comparison of pregnancy outcome between study and control groups i.e. those with unexplained elevated MSAFP who were 12 women and those with normal MSAFP who were 180 women.

For preterm delivery one of 12 women (8.3%) in the study group Vs 10 women (5.6%). There is no significant difference between the two groups (P>0.05).

For PROM; in the study group there was no case of PROM while in the control group there were 6 cases (3.3%), the difference did not reach statistical significant value (P> 0.05).

Regarding stillbirth also there is no cases of stillbirth in the study group Vs 2 cases (1.1%) in the control group. Also there is no significant difference between the two groups.

For abruptio placentae; in the study group there was one case (8.3%) VS 4 cases (2.2%) in the controls (P<0.036), which is statistically regarded as significant.

A comparable figure was obtained regarding IUGR in both groups where significant differences (P<0.036).

Spontaneous abortion in the study group we found 2 cases (16.7%) Vs 3 cases (1.7%) in the controls, there is a highly significant difference (P<0.0001).

Regarding PIH, in the study group were 2 cases 16.7% Vs 9 cases (5%) in the controls (P<0.01).

Lastly, congenital abnormalities in study group was one case (8.3%) (Spina bifida) Vs 2 cases (1.1%) (One cleft lip and palate and other polydectaly) in the controls which is significantly different (P<0.004).

The overall adverse pregnancy outcome in the study group was 8 cases over 12 (66.6%) Vs 40 cases over 180 (22.2%) in the control group. This overall difference is significant when compared with the normal pregnancy outcome.

Table 6 shows the comparison of women with elevated MSAFP with women with normal MSAFP according to the age.

In the study group 16.6%, 55.5% and 27.7% were located in the age groups (<18) years, (18–34) years and ($\geq$35) years respectively. The comparable figures for the control group were 8.8%, 75.45% and 15.75% respectively (P>0.05) (age matched).

Table 4: Description of women with normal MSAFP (control group) by U/S findings who had been excluded (N=330)

women with normal MSAFP excluded by U/S findings	**Number**	**%**
1. Wrong date	37	11.2
2. Missed abortion	20	6
3. Hydrocephaly	3	0.9
4. H – mole	1	0.3
Total	61	18.4

Table 5: Comparison of pregnancy outcomes between study and control groups

Pregnancy outcome	Study group (N=12)		Control group (N=180)		P
	No	%	No	%	
Preterm labor	1	8.3	10	5.6	>0.05
PROM	-	0	6	3.3	-
io placenta	1	8.3	4	2.2	<0.036*
IUGR	1	8.3	4	2.2	<0.036*
Spontaneous abortion	2	16.7	3	1.7	<0.0001*
Stillbirth	-	0	2	1.1	-
PIH	2	16.7	9	5.0	<0.01*
Cong. Abnormality	1	8.3	2	1.1	<0.004*
Total no. of adverse outcome	8	66.6	40	22.2	0.0001*
Normal	4	33.4	140	77.8	-
Total	12	100%	180	100%	-

*Significant differences when compared to normal pregnancy outcome

Table 6: Comparison of women with elevated MSAFP with women with normal MSAFP according to the age

Age (years)	Study group (N=18)		Control (N=330)		P .value
	No	%	No	%	
<18	3	16.6	29	8.8	0.168
18-34	10	55.5	249	75.45	
≥35	5	27.7	52	15.75	
Total	18	100%	330	100%	

Discussion:

Elevated MSAFP concentration have been found to be associated with a variety of fetal abnormalities such as neural tube defects, ventral wall defects, fetal death, twin gestation, placental abnormalities, oligohydramnios and advanced gestation [12].

Initial evaluation of an elevated MSAFP concentration involves ultrasound for confirmation of dating, fetal number and fetal viability [12].

Once these are determined, options have included detailed ultrasound and/or amniocentesis to seek evidence of a structural abnormality. Determination of amniotic fluid AFP concentration in conjunction with acetyl cholinesterase assay will detect some structural abnormalities associated with elevated MSAFP concentrations [13]

Many screening programs encourage amniocentesis for all pregnancies in which ultrasound findings do not explain the elevation [14]. Because second trimester amniocentesis had

been associated with occasional fetal trauma [15,16] and involves a 0.3 increase to 1.3% in the rate of fetal loss [14] the patients decision to proceed requires optimal informed consent.

MSAFP screening has a known detection and false positive rate, but the corresponding rates for routine diagnostic ultrasonography are not known. Although ultrasonography can detect a wider range of abnormalities than AFP screening, most are rare and many have an uncertain natural history. There is no scientific case for abandoning AFP screening for routine ultrasound based screening or diagnosis. If the routine use of diagnostic ultrasonography were shown to achieve accuracy comparable with its selective use for high risk women at an acceptable cost, the position could be reviewed. The best approach at present is to carry out AFP screening in conjunction with an ultrasound examination to determine the BPD at 16–18 weeks' pregnancy [17].

The explanation for the association between elevated MSAFP level and adverse pregnancy outcome remains elusive, although placental dysfunction, including partial abruptio placentae, pathologic findings, feto–maternal bleeding, and abnormal implantation are often implicated. Additionally, the optimal perinatal management strategy for patients with unexplained elevated levels is not known[18]. Our population of pregnant women with unexplained elevated MSAFP values was advised to have weekly non stress tests and Doppler velocimetry studies beginning between 28 and 32 weeks. In spite of this recommendation for increased surveillance, an extremely high adverse perinatal outcome rate was identified [19].

There were significant differences regarding overall adverse perinatal outcome rate among patient with MSAFP value $\geq$2.0 MOM, 66.6% (8/12) of patients with unexplained elevated MSAFP compared with 22.2% (40/180) of the control group. This difference is statistically significant, (P. value <0.05).

Similar results were obtained in some studies [19,20,21], comparable figures were 57.9% (33/57) of the patients with unexplained elevated MSAFP compared with 22.7% (163/719) of controls. Also Robinson *et al.*[20], and Nelson *et al.* [21] found 72.9% adverse perinatal outcome among patients with MSAFP $\geq$2.5 MOM. However, our results have disagreement with observation of Philips *et al.*[22]. In Memphis study they found 39.9% adverse perinatal outcome and concluded that unexplained elevated MSAFP levels and adverse perinatal outcome may not apply to all obstetric population but suggested further studies.

In our study there were significant differences in regard to abruptio placentae between the study group and control group (8.3% [one of 12] vs. 2.2% [Four of 180], P<0.036), this agrees with some studies like Chandra *et al.*[23] study, and William *et al.*[19] study in which the comparable figure between study and control group was (7% [four of 57] vs. 1.9% [14/719], P. < 0.025), while Phillips *et al.*[22] disagrees with our study where they found (2.8%[2/72] vs. 2.8% [2/72]).

Regarding IUGR; there is significant differences between study and control groups (8.3% [1/12] vs. 2.2% [4/180], P<0.036). Several studied [19,22,23] have reported similar results, William *et al.*[19] where they found (10.5% [six of 57] vs. 4% [29/719], P<0.025), Philips *et al.*[22] found (8.3% [6/72] vs. 1.4% [1/72], P<0.025). Chandra *et al.*[23] reported similar results to our study.

Also in this study documented significant difference in regard to spontaneous abortion between study and control groups (16.7% [2/12] vs. 1.7% [3/180], P<0.0001). However, this result disagrees with other trials, like William *et al.*[19] were found (5.2% [3/57] vs. 1.7% (12/719), P>0.05), and in Phillips *et al.*[22] were found (5.6% [4/72] vs. zero) which is not significant.

For PIH there is significant difference between study and control group (16.7 [2/12] vs. 5% [9/180], P<0.01). This agrees with some of studies like William *et al.*[19] Where they found (12.3% [7/57] vs. 4.3% [31/719], P<0.01), which is significant. Phillips *et al.*[22] disagree with our study where comparable figure (12.5% [9/72] vs. 13.4% [10/72].

As national perinatal mortality rates progressively fall, congenital malformations and chromosomal abnormalities contributes an ever-increasing share to both residual death and long-term disabilities of children[24]. The present study revealed statistically significant difference in regard to congenital malformations between study and control groups (8.3% [1/12] vs. 1.1% [2/18], P<0.004). William *et al.* [19] were found dissimilar results (1.8% [1/57] vs. 1.7% [12/719]) which is not significant, also Phillips *et al.* [22] disagree with our study where comparable figure (2.8% [2/72] vs. 1.4% [1/72]) which is significant.

On other hand, our study revealed insignificant difference between the study and controls groups regarding preterm labor (8.3% [1/12] vs. 5.6% [10/180], P>0.05). This agrees

with William *et al.* [19] were found (7% [4/57] vs. 5.1% [37/719]) which is insignificant.

Regarding PROM, our study revealed insignificant difference between the study and control groups we found (zero vs. 3.3% [6/180]). William *et al.* [19] revealed similar results (7% [4/57] vs. 3.1% [22/719] and also Phillips *et al.* [22] were found (8.3% [6/72] vs. 5.6% [4/72] agree with our study.

For stillbirth there is insignificant differences in our study (zero vs. 1.1% [2/180]). William *et al.* [19] not agree with our study were found (7% [4/57] vs. 0.8% [6/719], P< 0.001) which is significant. Phillips *et al.* [22] study revealed no stillbirths in both study and control group which is agree with our study.

During the follow up of the study group one women with elevated MSAFP was found to have non reactive NST at 35 weeks, then she was subjected to the biophysical profile and Doppler study which gave results going with IUGR and the patient was arranged for termination of pregnancy at 35 weeks, the outcome was satisfactory. She delivered a live male baby. The baby was fully assessed by us sharing with pediatrician for the evidence of signs of IUGR. William *et al.* study reported early delivery because of non reassuring fetal testing was performed on several occasions during this study [19].

In conclusion, maternal serum alpha-fetoprotein is a true screening test. An abnormal level, whether high or low, represents a high risk, rather than a diagnosis of a problem. It indicates that further investigations should be considered and the primary impact is to facilitate improved management of ongoing pregnancy. The process of counseling and investigation following the detection of abnormal results includes detailed explanation and may also include; amniocentesis for determination of amniotic fluid AFP (in case of high levels), genetic testing (in case of low levels), measurements of fetal growth and normal fetal functions, serial U/S monitoring of placental complications for which specific management at the time of delivery may be recommended with increasing antenatal surveillance of those patients with unexplained elevated MSAFP regardless the pregnancy risk status.

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