International Journal of Endocrinology & Metabolism
www.endometabol.com

Mid-Trimester Maternal Serum hCG and Alpha Fetal Protein Levels: Clinical Significance and Prediction of Adverse Pregnancy Outcome

Georgios Androutsopoulos [1,*], Panagiotis Gkogkos [1], Georgios Decavalas [1]

[1] Department of Obstetrics and Gynaecology, University of Patras, Medical School, Rion, Greece

*Corresponding author: Georgios Androutsopoulos, Nikolaou Apostoli 21, Patra, 26332, Greece, Tel.: +30-2613604010, Fax: +30-6974088092, E-mail: androutsopoulosgeorgios@hotmail.com.

ABSTRACT

Context: Maternal serum human Chorionic Gonadotropin (hCG) and Alpha Fetal Protein (AFP) were originally introduced to detect trisomy 21 and neural tube defects. However, in the absence of aneuploidy or neural tube defects, mid-trimester maternal serum hCG and/or maternal serum AFP associated with adverse pregnancy outcomes. Pregnancies with unexplained mid-trimester elevation in maternal serum hCG and/or maternal serum AFP, are at increased risk for pregnancy complications resulting from placental insufficiency.

Evidence Acquisition: Mid-trimester maternal serum hCG > 2.5 MoM associated with an increased risk for pregnancy complications including: late fetal loss, gestational hypertension, preeclampsia, intrauterine growth restriction (IUGR), preterm delivery and intrauterine fetal death(IUFD). Mid-trimester maternal serum AFP levels > 2.5 MoM are thought to reflect a defect in placentation and associated with an increased risk for pregnancy complications including: late fetal loss, gestational hypertension, preeclampsia, IUGR, preterm delivery and IUFD.

Results: Combined mid-trimester elevation in maternal serum hCG and AFP levels suggest a more complex type of placental pathology. They have stronger association with pregnancy complications including: late fetal loss, gestational hypertension, preeclampsia, IUGR, preterm delivery and IUFD.

Conclusions: Mid-trimester maternal serum hCG or AFP levels alone cannot detect all pregnant women with increased risk to develop pregnancy complications. Multiparameter testing of placental function in mid-trimester (maternal serum hCG and AFP screening, uterine artery Doppler and placental morphology) may allow us to identify women with increased risk to develop severe placental insufficiency and pregnancy complications. However, future prospective studies are needed to confirm the prognostic significance of multiparameter testing of placental function in mid-trimester.

Keywords: Maternal Serum Screening Tests; Chorionic Gonadotropin; AFP; Adverse Pregnancy Outcome

▸Article type: Review Article; Received: 03 Apr 2012, Revised: 02 Oct 2012, Accepted: 08 Oct 2012; DOI: 10.5812/ijem. 5014

▸Implication for health policy/practice/research/medical education:
Mid-trimester maternal serum hCG and AFP associated with adverse pregnancy outcomes in the absence of aneuploidy or neural tube defects. Pregnancies with unexplained mid-trimester elevation in maternal serum hCG and/or maternal serum AFP, are at increased risk of pregnancy complications resulting from placental insufficiency [intrauterine growth restriction (IUGR), preeclampsia, intrauterine fetal death (IUFD)].

▸Please cite this paper as:
Androutsopoulos G, Gkogkos P, Decavalas G. Mid-Trimester Maternal Serum hCG and Alpha Fetal Protein Levels: Clinical Significance and Prediction of Adverse Pregnancy Outcome. Int J Endocrinol Metabol. 2013; 11(2):102-6. DOI: 10.5812/ijem.5014

1. Context

Maternal serum human Chorionic Gonadotropin (hCG) and Alpha Fetal Protein (AFP) were originally introduced to detect trisomy 21 and neural tube defects. However, increased ultrasound machine quality and sonographer expertise, has greatly reduced the need for maternal serum hCG (ms-hCG) and maternal serum AFP (ms-AFP) screening in mid-trimester (1, 2).

In the absence of aneuploidy or neural tube defects, mid-trimester ms-hCG and/or ms-AFP associated with adverse pregnancy outcomes (3). Pregnancies with unexplained mid-trimester elevation in ms-hCG and/or ms-AFP, are at increased risk for pregnancy complications resulting from placental insufficiency [intrauterine growth restriction (IUGR), preeclampsia, intrauterine fetal death (IUFD)] (4).

1.1. Maternal Serum hCG

hCG is a member of the glycoprotein hormone family [hCG, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Thyroid Stimulating Hormone (TSH)] (5). All of them are dimers consisting of a common α-subunit and distinct β-subunits that are associated noncovalently (5-7). The distinct β-subunits confer biological activity and display various degrees of homology (5). In adults hCG expression is often associated with pregnancy (6). However, hCG can be found in other conditions such as gestational trophoblastic disease and non-germinomatous germ cell tumors (6, 8). During pregnancy hCG is produced almost exclusively by the syncytiotrophoblast of the placenta (6). However, it is synthesized by the fetal kidney and fetal liver (9). Most of the hCG in circulation is metabolized by the liver (10). Also, 20% of the circulating hCG is excreted by the kidneys (10). However, hCG appears early during pregnancy (6). Its concentration increases gradually by reaching a peak at 8 to 10 weeks of gestation (3, 4,6). After that peak, it progressively declines to reach a plateau at 18 to 20 weeks of gestation (3, 4,6). During early pregnancy, the main function of hCG is the maintenance of the corpus luteum (3, 4). It takes over from LH in promoting progesterone production by ovarian corpus luteal cells, preventing menstrual bleeding (7). It promotes progesterone production only for 3-4 weeks following pregnancy implantation (7). Also hCG receptor gene expressed by uterine spiral arteries and hCG acts on them promoting angiogenesis of uterine vasculature and uterine growth in line with fetal growth (7). During pregnancy hCG also promotes: cytotrophoblast differentiation, immunosuppression and blockage of phagocytosis of invading trophoblast cells (7, 11,12).

1.2. Maternal Serum AFP

AFP is a glycoprotein and it is a member of the albuminoid gene family [AFP, albumin (ALB), alpha albumin (αALB), vitamin D binding protein (DBP)] (13, 14). In adults AFP expression is often associated with hepatocellular cancer, non-germinomatous germ cell tumors and gastrointestinal cancers (13). However, AFP can be found in non-neoplastic conditions such as hepatitis, cirrhosis and pregnancy (13, 14). During pregnancy it is synthesized by the fetus (13, 14). AFP is normally produced in early pregnancy primarily by the fetal liver and yolk sac (15, 16). It is also produced to a lesser extent by the fetal gastrointestinal tract (15, 16). As the yolk sac involutes at the 9th week of gestation, the fetal liver is the principal source of AFP during development (15, 16). AFP synthesis by the proliferating fetal liver actually increases through the 20th week of gestation, after which it remains fairly constant until 32nd week (15-17).AFP is excreted into fetal urine and transported to maternal serum through the placenta or by diffusion across fetal membranes (14, 18). The transplacental passage of AFP involves additional and more complicated mechanisms (14). It is asymmetrical and unidirectional, displaying a faster transfer rate of AFP from the fetal to maternal circulation (14). Small amounts of AFP is transported and can be measured in the maternal serum (13). Despite the decrease in fetal serum AFP (fs-AFP) during mid-trimester of gestation, ms-AFP continues to rise until the 32nd week of gestation (15-17). After the 32nd week of gestation, ms-AFP begins to decline until parturition (17). AFP is involved with ontogenic and oncogenic growth (13, 14). It can bind and transport a multitude of ligands (bilirubin, fatty acids, retinoids, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxin and various drugs) (14). Also, it is capable of regulating growth in reproductive, hematopoietic, placental, hepatic, inflammatory and lymphatic cells (14). However, AFP regulating function during pregnancy remains controversial and relatively unknown (19). AFP involvement in the regulation of placental growth, is also unknown.

2. Evidence Acquisition

2.1. Mid-Trimester Elevation of Maternal Serum hCG

Elevated mid-trimester ms-hCG levels have been associated with congenital abnormalities, placental dysfunction and adverse pregnancy outcome (3). Although there are various cut-off points, the most common cut-off values are between 2 and 2.5 MoM (20). However there are no significant differences among them (21, 22). In the absence of fetal chromosomal or structural anomalies, mid-trimester ms-hCG > 2.5 MoM associated with an increased risk for pregnancy complications including: late fetal loss [OR 2.2 (95% CI: 1.3-3.0)], gestational hypertension [OR 1.4 (95% CI: 1.1-1.8)], preeclampsia [OR 1.19 (95% CI:

0.88–1.61)], IUGR [OR 1.3 (95% CI: 0.9-1.7)], preterm delivery [OR 1.7 (95% CI: 1.4-2.1)] and IUFD [OR 2.7 (95% CI: 1.8-4.0)] (3, 4, 23-27). The risk of adverse pregnancy outcome increases as the mid-trimester ms-hCG levels become more elevated (3, 23,27). Extremely high mid-trimester ms-hCG levels (≥ 10 MoM) imply a poor pregnancy outcome (27). Mechanisms for elevated mid-trimester ms-hCG levels with a structurally normal fetus include: placental ischemia and inadequate trophoblastic remodeling (26, 28-30). Trophoblastic cells cultured in vitro showed that hypoxia increases hCG production (31). Placental pathology studies suggest that unexplained mid-trimester elevation of ms-hCG levels associated with villitis, placental vascular lesions (infarction, ischemic changes, intervillus thrombosis), velamentous cord insertion and placental mosaicism (26, 28-30, 32, 33). It is unknown whether elevated ms-hCG levels, are the cause or the result of villitis and placental vascular lesions.

2.2. Mid-Trimester Reduction of Maternal Serum hCG

Isolated mid-trimester ms-hCG levels < 0.5 MoM have no association with adverse pregnancy outcome (3, 34).

2.3. Mid-Trimester Elevation of Maternal Serum AFP

Elevated mid-trimester ms-AFP levels have been strongly associated with congenital abnormalities, placental dysfunction and adverse pregnancy outcome (3, 17). Although there are various cut-off points, the most common cut-off values are between 2 and 2.5 MoM (20). However there are no significant differences among them (21, 35). In the absence of fetal chromosomal or structural anomalies, mid-trimester ms-AFP levels > 2.5 MoM are thought to reflect a defect in placentation [placental abruption, placenta previa, abnormal placental adherence (placenta accreta, increta and percreta)] (3, 36, 37). They also associated with an increased risk for pregnancy complications including: late fetal loss [OR 10.1 (95% CI: 7.5-13.5)], gestational hypertension [OR 1.6 (95% CI: 1.3-2.1)], preeclampsia [OR 0.83 (95% CI: 0.44–1.56)], IUGR [OR 2.3 (95% CI: 1.8-2.9)], preterm delivery [OR 1.8 (95% CI: 1.5-2.3)] and IUFD [OR 5.3 (95% CI: 3.8-7.3)] (3, 4, 17, 23-25, 38, 39). Mechanisms for elevated mid-trimester ms-AFP levels with a structurally normal fetus include: disruption of the fetal-maternal placental barrier, placental vascular damage from early abruption, fetal-maternal bleeding and fetal-placental ischemia (3, 36, 40-42). Placental pathology studies suggest that unexplained mid-trimester elevation of ms-AFP levels associated with chorionic villitis and placental vascular lesions (43). It is unknown whether elevated ms-AFP levels, are the cause or the result of these lesions. Probably the factor that leads to adverse pregnancy outcome also increases ms-AFP (3, 38).

2.4. Mid-Trimester Reduction of Maternal Serum AFP

Mid-trimester ms-AFP levels < 0.25 MoM have been associated with late fetal loss [OR 15.1 (95% CI: 9.3-24.8)], preterm delivery [OR 2.2 (95% CI: 1.3-3.8)], stillbirth [OR 4.0 (95% CI: 1.0-16.0)] and macrosomia (3, 23, 38, 44, 45).

2.5. Mid-Trimester Combined Elevation of Maternal Serum hCG and AFP

Although isolated abnormal mid-trimester ms-hCG or ms-AFP levels associated with an increased risk for with congenital abnormalities and adverse pregnancy outcome, the risk increases when they are combined (3, 4, 24, 25, 46). The risk for adverse pregnancy outcome also increases as mid-trimester ms-hCG and/or ms-AFP levels become more extreme. In the absence of fetal chromosomal or structural anomalies, combined mid-trimester elevation in ms-hCG and ms-AFP levels suggest a more complex type of placental pathology which have stronger association with pregnancy complications including: late fetal loss [OR 7.05 (95% CI: 1.18-29.88)], gestational hypertension [OR 0.78 (95% CI: 0.13-3.24)], preeclampsia [OR 6.67 (95% CI: 3.84-11.58)], IUGR [OR 3.54 (95% CI: 1.26-9.14)], preterm delivery [OR 3.20 (95% CI: 1.49-6.61)] and IUFD [OR 6.87 (95% CI: 1.71-22.93)] (3, 4, 23-25, 39, 46, 47). Placental pathology studies suggest that unexplained combined mid-trimester elevation in ms-hCG and ms-AFP levels associated with villitis and placental vascular lesions (48).

3. Results

Mid-trimester ms-hCG and/or ms-AFP levels alone cannot be detected in all pregnant women with increased risk to develop pregnancy complications (21, 35,49). Although many of the associations between mid-trimester ms-hCG and/or ms-AFP levels and adverse pregnancy outcomes are statistically significant, the sensitivity and positive predictive value are too low for them to be clinically useful as screening tests (3). All these women with unexplained elevation of mid-trimester ms-hCG and/or ms-AFP levels should receive, increased fetal surveillance (3). There are no proven protocols to manage these women with increased risk to develop pregnancy complications(3, 23,25). The optimal type and frequency of testing, remains undetermined (3, 4, 23,25). Ultrasonography is the most cost-effective approach and should be used as the initial diagnostic examination (3, 4). Blood flow in the uterine arteries during mid-trimester can be evaluated noninvasively using Doppler ultrasonography (3, 50). Uterine artery Doppler measurements show that impedance to blood flow in the uterine arteries progressively decreases with gestational age in normal pregnancies (50, 51). Increased impedance and reduced blood flow in the uterine arteries associated with subsequent development of pregnancy complications characterized by spiral

artery vasculopathy (preeclampsia, IUGR, IUFD) (3, 51,52). Also, reduced uteroplacental blood flow is more prevalent in women with elevated mid-trimester ms-hCG and/or ms-AFP levels and may be a useful marker for the subset of women with increased risk to develop pregnancy complications (late fetal loss, gestational hypertension, preeclampsia, IUGR, preterm delivery and IUFD) (3, 23, 48, 53, 54). Likewise, abnormalities of placental shape or texture in women with elevated mid-trimester ms-AFP levels have been associated with poor pregnancy outcome (48). However, uterine artery Doppler screening alone is superior to mid-trimester ms-hCG and ms-AFP screening to identify a significant placental pathology leading to pregnancy complications (55).

4. Conclusions

Multiparameter testing of placental function in mid-trimester (ms-hCG and ms-AFP screening, uterine artery Doppler and placental morphology) may allow us to identify women with increased risk to develop severe placental insufficiency and pregnancy complications (4, 21, 35, 48, 49, 56). However, future prospective studies are needed to confirm the prognostic significance of multiparameter testing of placental function in mid-trimester (21, 49,57).

Acknowledgements

None declared.

Authors' Contribution

Georgios Androutsopoulos reviewed the literature and wrote the manuscript. Panagiotis Gkogkos helped in literature review. Georgios Decavalas edited the manuscript. All the authors read and approved the final manuscript.

Financial Disclosure

None declared.

Funding/Support

None declared.

References

1. Morrow RJ, McNay MB, Whittle MJ. Ultrasound detection of neural tube defects in patients with elevated maternal serum alpha-fetoprotein. *Obstet Gynecol*.1991;**78**(6):1055-7
2. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet*.1998;**352**(9125):343-6
3. Dugoff L. First- and second-trimester maternal serum markers for aneuploidy and adverse obstetric outcomes. *Obstet Gynecol*.2010;**115**(5):1052-61
4. Yaron Y, Cherry M, Kramer RL, O'Brien JE, Hallak M, Johnson MP, et al. Second-trimester maternal serum marker screening: maternal serum alpha-fetoprotein, beta-human chorionic gonadotropin, estriol, and their various combinations as predictors of pregnancy outcome. *Am J Obstet Gynecol*.1999;**181**(4):968-74
5. Pierce JG, Parsons TF. Glycoprotein hormones: structure and function. *Annu Rev Biochem*.1981;**50**:465-95
6. Alfthan H, Schroder J, Fraser R, Koskimies A, Halila H, Stenman UH. Choriogonadotropin and its beta subunit separated by hydrophobic-interaction chromatography and quantified in serum during pregnancy by time-resolved immunofluorometric assays. *Clin Chem*.1988;**34**(9):1758-62
7. Cole LA. Biological functions of hCG and hCG-related molecules. *Reprod Biol Endocrinol*.2010;**8**:102
8. Nakamura H, Makino K, Kochi M, Ushio Y, Kuratsu J. Evaluation of neoadjuvant therapy in patients with nongerminomatous malignant germ cell tumors. *J Neurosurg Pediatr*.2011;**7**(4):431-8
9. McGregor WG, Kuhn RW, Jaffe RB. Biologically active chorionic gonadotropin: synthesis by the human fetus. *Science*.1983;**220**(4594):306-8
10. Nisula BC, Blithe DL, Akar A, Lefort G, Wehmann RE. Metabolic fate of human choriogonadotropin. *J Steroid Biochem*.1989;**33**(4B):733-7
11. Berndt S, Blacher S, Perrier d'Hauterive S, Thiry M, Tsampalas M, Cruz A, et al. Chorionic gonadotropin stimulation of angiogenesis and pericyte recruitment. *J Clin Endocrinol Metab*.2009;**94**(11):4567-74
12. Shi QJ, Lei ZM, Rao CV, Lin J. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. *Endocrinology*.1993;**132**(3):1387-95
13. Gitlin D, Boesman M. Serum alpha-fetoprotein, albumin, and gamma-G-globulin in the human conceptus. *J Clin Invest*.1966;**45**(11):1826-38
14. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. *Exp Biol Med (Maywood)*.2001;**226**(5):377-408
15. Gitlin D, Perricelli A, Gitlin GM. Synthesis of -fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. *Cancer Res*.1972;**32**(5):979-82
16. Jones EA, Clement-Jones M, James OF, Wilson DI. Differences between human and mouse alpha-fetoprotein expression during early development. *J Anat*.2001;**198**(Pt 5):555-9
17. Mizejewski GJ. Levels of alpha-fetoprotein during pregnancy and early infancy in normal and disease states. *Obstet Gynecol Surv*.2003;**58**(12):804-26
18. Los FJ, De Bruijn HW, van Beek Calkoen-Carpay T, Huisjes HJ. AFP transport across the fetal membranes in the human. *Prenat Diagn*.1985;**5**(4):277-81
19. Mizejewski GJ. Physiology of alpha-fetoprotein as a biomarker for perinatal distress: relevance to adverse pregnancy outcome. *Exp Biol Med (Maywood)*.2007;**232**(8):993-1004
20. Hui D, Okun N, Murphy K, Kingdom J, Uleryk E, Shah PS. Combinations of maternal serum markers to predict preeclampsia, small for gestational age, and stillbirth: a systematic review. *J Obstet Gynaecol Can*.2012;**34**(2):142-53
21. Androutsopoulos G, Gkogkos P, Papadopoulos V, Adonakis G, Tsapanos V, Vassilakos P, et al. Mid-trimester maternal serum markers in predicting adverse pregnancy outcome. *Clin Exp Obstet Gynecol*.2009;**36**(4):237-40
22. Androutsopoulos G, Gkogkos P, Vassilakos P, Panayiotakis G, Decavalas G. Mid-trimester maternal serum hCG levels in predicting adverse pregnancy outcome. *Clin Exp Obstet Gynecol*.2009;**36**(3):173-5
23. Gagnon A, Wilson RD, Audibert F, Allen VM, Blight C, Brock JA, et al. Obstetrical complications associated with abnormal maternal serum markers analytes. *J Obstet Gynaecol Can*.2008;**30**(10):918-49
24. Dugoff L, Hobbins JC, Malone FD, Vidaver J, Sullivan L, Canick JA, et al. Quad screen as a predictor of adverse pregnancy outcome. *Obstet Gynecol*.2005;**106**(2):260-7
25. Chandra S, Scott H, Dodds L, Watts C, Blight C, Van den Hof M. Un-

explained elevated maternal serum α-fetoprotein and/or human chorionic gonadotropin and the risk of adverse outcomes. *Am J Obstet Gynecol*.2003;**189**(3):775-781
26. Gonen R, Perez R, David M, Dar H, Merksamer R, Sharf M. The association between unexplained second-trimester maternal serum hCG elevation and pregnancy complications. *Obstet Gynecol*.1992;**80**(1):83-6
27. Lepage N, Chitayat D, Kingdom J, Huang T. Association between second-trimester isolated high maternal serum maternal serum human chorionic gonadotropin levels and obstetric complications in singleton and twin pregnancies. *Am J Obstet Gynecol*.2003;**188**(5):1354-9
28. Morssink LP, de Wolf BT, Kornman LH, Beekhuis JR, van der Hall TP, Mantingh A. The relation between serum markers in the second trimester and placental pathology. A study on extremely small for gestational age fetuses. *Br J Obstet Gynaecol*.1996;**103**(8):779-83
29. Shenhav S, Gemer O, Sassoon E, Volodarsky M, Peled R, Segal S. Mid-trimester triple test levels in early and late onset severe pre-eclampsia. *Prenat Diagn*.2002;**22**(7):579-82
30. Liu DF, Dickerman LH, Redline RW. Pathologic findings in pregnancies with unexplained increases in midtrimester maternal serum human chorionic gonadotropin levels. *Am J Clin Pathol*.1999;**111**(2):209-15
31. Fox H. Effect of hypoxia on trophoblast in organ culture. A morphologic and autoradiographic study. *Am J Obstet Gynecol*.1970;**107**(7):1058-64
32. Heinonen S, Ryynanen M, Kirkinen P, Saarikoski S. Velamentous umbilical cord insertion may be suspected from maternal serum alpha-fetoprotein and hCG. *Br J Obstet Gynaecol*.1996;**103**(3):209-13
33. Morssink LP, Sikkema-Raddatz B, Beekhuis JR, De Wolf BT, Mantingh A. Placental mosaicism is associated with unexplained second-trimester elevation of MShCG levels, but not with elevation of MSAFP levels. *Prenat Diagn*.1996;**16**(9):845-51
34. Endres LK, Krotz S, Grobman WA. Isolated low second-trimester maternal serum beta-human chorionic gonadotropin is not associated with adverse pregnancy outcome. *Am J Obstet Gynecol*.2003;**189**(3):755-7
35. Gkogkos P, Androutsopoulos G, Vassilakos P, Panayiotakis G, Kourounis G, Decavalas G. Mid-trimester maternal serum AFP levels in predicting adverse pregnancy outcome. *Clin Exp Obstet Gynecol*.2008;**35**(3):208-10
36. Hung T, Shau W, Hsieh C, Chiu T, Hsu J, Hsieh T. Risk factors for placenta accreta. *Obstet Gynecol*.1999;**93**(4):545-550
37. Zelop C, Nadel A, Frigoletto FD, Jr, Pauker S, MacMillan M, Benacerraf BR. Placenta accreta/percreta/increta: a cause of elevated maternal serum alpha-fetoprotein. *Obstet Gynecol*.1992;**80**(4):693-4
38. Krause TG, Christens P, Wohlfahrt J, Lei U, Westergaard T, Nørgaard-Pedersen B, et al. Second-trimester maternal serum alpha-fetoprotein and risk of adverse pregnancy outcome. *Obstet Gynecol* .2001;**97**(2):277-282
39. McPherson E, Thomas GD, Manlick C, Zaleski CA, Reynolds KK, Rasmussen K, et al. Extreme values of maternal serum analytes in second trimester screening: looking beyond trisomy and NTD's. *J Genet Couns*.2011;**20**(4):396-403
40. Berkeley AS, Killackey MA, Cederqvist LL. Elevated maternal serum alpha-fetoprotein levels associated with breakdown in fetal-maternal-placental barrier. *Am J Obstet Gynecol*.1983;**146**(7):859-61
41. Perkes EA, Baim RS, Goodman KJ, Macri JN. Second-trimester placental changes associated with elevated maternal serum alpha-fetoprotein. *Am J Obstet Gynecol*.1982;**144**(8):935-8
42. Spong CY, Ghidini A, Walker CN, Ossandon M, Pezzullo JC. Elevated maternal serum midtrimester alpha-fetoprotein levels are associated with fetoplacental ischemia. *Am J Obstet Gynecol*.1997;**177**(5):1085-7
43. Salafia CM, Pezzullo JC, Lopez-Zeno JA, Simmens S, Minior VK, Vintzileos AM. Placental pathologic features of preterm pre-eclampsia. *Am J Obstet Gynecol*.1995;**173**(4):1097-105
44. Baschat AA, Harman CR, Farid G, Chodirker BN, Evans JA. Very low second-trimester maternal serum alpha-fetoprotein: Association with high birth weight. *Obstet Gynecol*.2002;**99**(4):531-6
45. Burton BK. Outcome of pregnancy in patients with unexplained elevated or low levels of maternal serum alpha-fetoprotein. *Obstet Gynecol*.1988;**72**(5):709-13
46. Chitayat D, Farrell SA, Huang T, Meier C, Wyatt PR, Summers AM. Double-positive maternal serum screening results for down syndrome and open neural tube defects: An indicator for fetal structural or chromosomal abnormalities and adverse obstetric outcomes. *Am J Obstet Gynecol*.2002;**187**(3):758-63
47. Huang T, Hoffman B, Meschino W, Kingdom J, Okun N. Prediction of adverse pregnancy outcomes by combinations of first and second trimester biochemistry markers used in the routine prenatal screening of Down syndrome. *Prenat Diagn*.2010;**30**(5):471-7
48. Alkazaleh F, Chaddha V, Viero S, Malik A, Anastasiades C, Sroka H, et al. Second-trimester prediction of severe placental complications in women with combined elevations in alpha-fetoprotein and human chorionic gonadotrophin. *Am J Obstet Gynecol*.2006;**194**(3):821-7
49. Androutsopoulos G. Marcadores séricos como predictores de mala evolución durante el segundo trimestre de embarazo. *Salud (i) Ciencia*.2012;**18**(8):729-31
50. Campbell S, Diaz-Recasens J, Griffin DR, Cohen-Overbeek TE, Pearce JM, Willson K, et al. New doppler technique for assessing uteroplacental blood flow. *Lancet*.1983;**1**(8326 Pt 1):675-7
51. Papageorghiou AT, Yu CK, Nicolaides KH. The role of uterine artery Doppler in predicting adverse pregnancy outcome. *Best Pract Res Clin Obstet Gynaecol*.2004;**18**(3):383-96
52. Bower S, Schuchter K, Campbell S. Doppler ultrasound screening as part of routine antenatal scanning: prediction of pre-eclampsia and intrauterine growth retardation. *Br J Obstet Gynaecol*.1993;**100**(11):989-94
53. Toal M, Chaddha V, Windrim R, Kingdom J. Ultrasound detection of placental insufficiency in women with elevated second trimester serum alpha-fetoprotein or human chorionic gonadotropin. *J Obstet Gynaecol Can*.2008;**30**(3):198-206
54. Elsandabesee D, Srinivas M, Kodakkattil S. The clinical value of combining maternal serum screening and uterine artery Doppler in prediction of adverse pregnancy outcome. *J Obstet Gynaecol*.2006;**26**(2):115-7
55. Hershkovitz R, de Swiet M, Kingdom J. Mid-trimester placentation assessment in high-risk pregnancies using maternal serum screening and uterine artery Doppler. *Hypertens Pregnancy*.2005;**24**(3):273-80
56. Papageorghiou AT, Leslie K. Uterine artery Doppler in the prediction of adverse pregnancy outcome. *Curr Opin Obstet Gynecol*.2007;**19**(2):103-9
57. Tuuli MG, Odibo AO. The role of serum markers and uterine artery Doppler in identifying at-risk pregnancies. *Clin Perinatol*.2011;**38**(1):1-19.