American Journal of Obstetrics and Gynecology (2006) **195**, 1623–8

ELSEVIER

American Journal of Obstetrics & Gynecology

www.ajog.org

Alpha-fetoprotein detection of neural tube defects and the impact of standard ultrasound

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Received for publication December 21, 2005; revised March 17, 2006; accepted March 28, 2006

KEY WORDS
Standard ultrasound
Neural tube defects

Objective: The purpose of this study was to evaluate neural tube defect (NTD) detection according to whether serum alpha-fetoprotein (AFP) screening or standard ultrasound are performed.
Study design: Prenatal and neonatal datasets were reviewed to identify pregnancies with NTDs from 1 institution between January 2000 and December 2003. AFP screening was offered <21 weeks and considered elevated if ≥ 2.50 multiples of the median. Standard ultrasound was performed for specific indications in low-risk pregnancies.
Results: There were 66 NTDs, 1 per 950 deliveries. AFP sensitivity was 65%. If the gestational age used for AFP calculation was confirmed with ultrasound, sensitivity improved to 86%. The sensitivity of standard ultrasound was 100%, $P < .001$ compared with AFP screening. NTDs detected with standard ultrasound were identified later in gestation, as examinations were performed for other indications.
Conclusion: Standard ultrasound improved NTD detection over AFP screening alone, by improving AFP test sensitivity and identifying NTDs in low-risk pregnancies.

Maternal serum screening for open neural tube defects using alpha-fetoprotein (AFP) has been routinely offered in the United States for nearly 2 decades. In 1977, a landmark trial from the United Kingdom found that serum AFP was greater than or equal to 2.50 MOM in 88% with fetal anencephaly and 79% with spina bifida when tested at 16 to 18 weeks' gestation.[1] Other investigators subsequently confirmed these findings,[2-4] all in the era before routine ultrasound was commonplace. The AFP test is performed between 15 and 20 or 22 weeks, typically using the threshold of 2.50 MOM, and the anticipated sensitivity for neural tube defect (NTD) detection is 85%.[5,6] The test has a screen-positive rate of 5% or less and a positive predictive value of about 2%.[4,6] Interestingly, Norem et al recently reported that serum AFP screening failed to detect 25% of NTDs in their population, including 38% of spina bifida cases.[7]

When serum AFP is elevated, many centers now offer targeted ultrasound examination as the initial diagnostic test, either in addition to or in place of amniocentesis.[6,8,9] Experts have argued that a normal targeted

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doi:10.1016/j.ajog.2006.03.097

Table I Maternal demographic characteristics for pregnancies with NTD

	Overall group (n = 66)	Anencephaly (n = 32)	Spina bifida (n = 27)
Maternal age	24.1 ± 6.2	24.1 ± 5.8	23.6 ± 6.2
Age ≥35	7 (11)	2 (6)	4 (15)
Age <20	15 (23)	8 (25)	6 (22)
Ethnicity			
Hispanic	60 (91)	31 (97)	22 (81)
Black	6 (8)	1 (3)	5 (19)
Pregestational diabetes	2 (3)	0	2 (7)

Data presented as mean ± SD and n (%).

examination may confer a reduction in risk for NTD of at least 95%.[8-10] However, targeted ultrasound is impractical for population screening, and the serum AFP screening test is only valid over a 5- to 7-week period of gestation. It follows that from a population screening perspective, some or perhaps many neural tube defects might be identified during the course of a standard or routine ultrasound examination.

Approximately 67% of pregnant women in the United States receive at least 1 standard ultrasound.[11] Despite widespread use, the utility of standard ultrasound as an adjunct to serum screening or as a screening tool for neural tube defects has not been determined. We sought to compare the sensitivity of standard ultrasound with that of AFP in our population. As technology has improved, we hypothesized that standard ultrasound might perform comparably with AFP screening, and that such information could be useful for counseling patients. We were also interested in the "real-world" application of these screening methods, namely, what percentage of neural tube defects would be identified, and at what point in gestation, among women who did and did not receive AFP screening or ultrasound evaluation.

Study design

This was a retrospective hospital-based study of singleton pregnancies complicated by open fetal neural tube defects. Pregnancies were included regardless of whether serum screening or ultrasound were performed, or whether the NTD was diagnosed before or after delivery. Cases were identified from review of 2 sources: (1) a comprehensive log of prenatally diagnosed fetal anomalies, maintained for patient care; and (2) an obstetric database that contains anomaly information about all liveborn and stillborn infants delivered at our hospital, maintained for operations including quality assurance. In order to focus on the AFP screening component, we included all singleton pregnancies that had been 15 to 21 weeks of gestation between January 1, 2000 and December 31, 2003. We are not a referral center for pregnancies with anomalies. The study was population-based in that it involved all those cared for at our hospital, a tax-supported county institution serving the medically indigent. This enabled us to evaluate the detection of neural tube defects and to estimate their incidence in our population.

All women who receive prenatal care in our system are offered multiple marker serum screening at 15 to 20 weeks. Ninety-six percent of women who deliver at our hospital receive prenatal care, and approximately 67% enroll for care by 20 weeks of gestation and are eligible to receive AFP screening.[12] Of women offered the AFP screening test, approximately 75% or 8000 per year elect it.[13] Alpha-fetoprotein levels are determined by immunochemiluminometric assay using an ADVIA Centaur (Bayer Diagnostics, Norwood, MA). As is commonly done, AFP MOM values have been derived from our population, and multiple regression analyses are used to adjust for gestational age, maternal weight, ethnicity, and pregestational insulin-dependent diabetes mellitus.

During the study period, ultrasound was performed in low-risk pregnancies as indications arose, per guidelines established by the American College of Obstetricians and Gynecologists (ACOG) and the American Institute of Ultrasound in Medicine (AIUM).[14,15] When calculating the AFP multiples of the median (MOM), if the ultrasound measurement of the crown-rump length or biparietal diameter was not within 7 days of that expected based on last menstrual period (LMP), the gestational age corresponding to the ultrasound measurement was used; otherwise, LMP dating was used. Our protocol was also to perform standard ultrasound to confirm gestational age if the AFP result was between 2.00 MOM and 2.49 MOM. If the AFP result was 2.50 MOM or greater, it was considered *elevated*. All women with serum AFP elevation were offered targeted ultrasound. The ultrasound unit is accredited by the AIUM, and all of our sonographers are certified by the American Registry of Diagnostic Medical Sonographers. Targeted examinations were performed by faculty with board certification and expertise in this area. For study purposes, open neural tube defects were grouped into the categories anencephaly, spina bifida, cephalocele, and iniencephaly.

After obtaining approval of the Institutional Review Board of the University of Texas Southwestern Medical Center, we compiled a list of singleton fetuses and newborns with NTDs. Laboratory records were reviewed to obtain AFP MOM results and determine if ultrasound had been used to either confirm or modify the gestational age based on LMP. Ultrasound reports and images were evaluated to determine the type of examination, indication, and findings. The sensitivity of AFP was defined as the percentage with NTDs who had

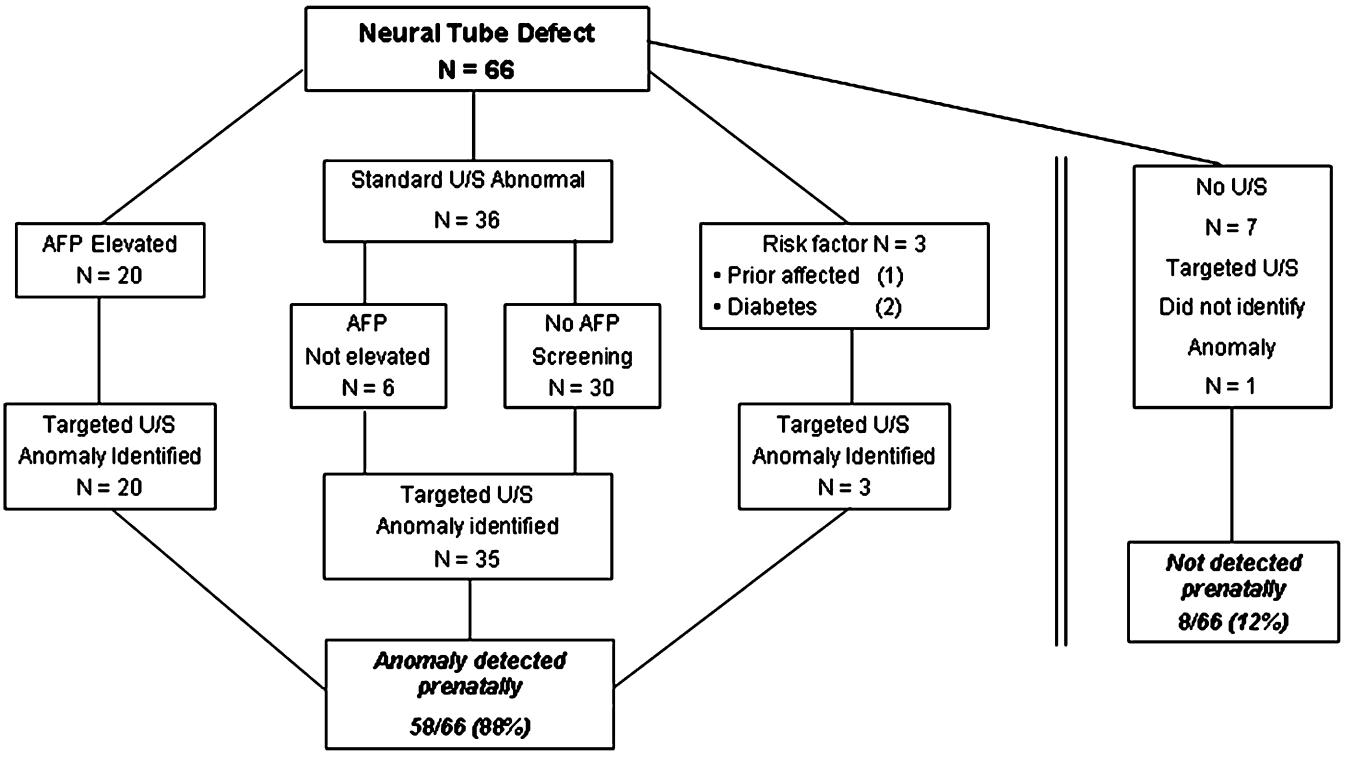

Figure How pregnancies with neural tube defects (NTDs) came to attention. In 58 cases (88%), the NTD was identified prenatally during targeted ultrasound examination performed either (1) for serum AFP elevation; (2) for an abnormality detected during standard ultrasound examination; or (3) for a maternal risk factor. These were 3 independent groups. The NTD was not identified until delivery in 8 cases, 7 of whom had no prenatal ultrasound, and 1 in whom targeted ultrasound in the third trimester identified ventriculomegaly but not the accompanying sacral spina bifida.

serum AFP elevation to 2.50 MOM or greater. The sensitivity of standard ultrasound was defined as the percentage with NTDs who had an abnormality identified on standard ultrasound performed at or beyond 15 weeks. Because women with elevated AFP and those with other a priori risk factors for NTDs were offered targeted ultrasound, they were not included in the standard ultrasound group; thus, pregnancies with NTDs identified through AFP screening were separate from those identified by standard ultrasound (independent groups). Accuracy of targeted ultrasound was verified postnatally or at pregnancy termination. Statistical analyses were performed using χ^2 test, χ^2 goodness-of-fit test, and Wilcoxon rank sum test. P values $< .05$ were considered statistically significant.

Results

During the study period, 66 singletons with neural tube defects were identified prenatally or upon delivery, including 32 with anencephaly (48%), 27 with spina bifida (41%), 5 with cephalocele (8%), and 2 with iniencephaly (3%). Approximately 62,800 singletons were delivered at our hospital over these 4 years, yielding an incidence of about 1 per 950 deliveries. Of pregnancies complicated by a neural tube defect, 45 infants were liveborn, 6 were stillborn ($\geq$500 g), and 15 pregnancies resulted in fetal demise $<$500 g or pregnancy termination. Confirmation or characterization of the neural tube defect in the immediate neonatal period or at pregnancy termination occurred in 98% of cases, with the remainder (1 case) representing an early loss of a fetus with anencephaly in which the family declined autopsy.

Maternal demographic characteristics are presented in Table I. There were no significant differences in maternal age, ethnicity, or prevalence of pregestational diabetes according to the type of neural tube defect. Women 35 years of age or older comprised 5% of our overall pregnant population[16] but made up 11% of those with NTDs. Also shown in the table is that 92% with fetal NTDs were Hispanic, as are 84% of the patients in our overall obstetric population.[16]

The Figure depicts how the pregnancies with NTDs came to attention. In 20 women (30%) the serum AFP was elevated, which prompted targeted ultrasound, and the defect was identified. Three women (5%) had a risk factor for NTD, either insulin-dependent diabetes or a previous infant with NTD, and they proceeded directly to targeted ultrasound, which also identified the

Table II Likelihood of AFP elevation and likelihood of abnormality noted during standard ultrasound among pregnancies complicated by neural tube defects

	Overall group	Anencephaly	Spina bifida	Other NTD
Serum AFP elevated	20/31 (65)*†	11/14 (79)†	7/15 (47)*†	2/2 (100)
Dated by ultrasound	13/15 (87)†	6/6 (100)	5/7 (71)†	2/2 (100)
Dated by LMP, age confirmed by ultrasound	6/7 (86)†	4/4 (100)	2/3 (67)†	0
Dated by LMP, no ultrasound performed	1/9 (11)†	1/4 (25)†	0/5 (0)†	0
Abnormality on standard ultrasound	36/36 (100)	20/20 (100)	13/13 (100)	3/3 (100)

Data presented as frequency (%).

* Less than expected based on published likelihood of AFP elevation with neural tube defects overall (85%) and spina bifida in particular (80%), both $P < .05$ using goodness-of-fit χ^2.

† $P < .05$ as compared with sensitivity of standard ultrasound.

defect. In 36 pregnancies (55%) an abnormality was first noted during standard ultrasound, targeted ultrasound was performed, and in 35 of these cases the NTD was identified (97%). The 1 pregnancy in which the NTD was not identified was 34 weeks of gestation at initial ultrasound and had obvious ventriculomegaly, but the sacral spina bifida was not observed. There were 7 additional cases in which no ultrasound was performed, and the NTD was first noted at delivery.

The sensitivity of serum AFP is shown in Table II, stratified by type of defect and also by the method of determining gestational age for the AFP calculation. Thirty-one pregnancies with NTDs (47%) received AFP screening. AFP values (median [Q1, Q3]) were significantly greater in the setting of anencephaly than with spina bifida, 4.89 MOM [3.12, 7.55] versus 2.22 MOM [1.23, 3.09], respectively, $P = .005$. The AFP value was elevated in 20 pregnancies (65%) with NTDs; the sensitivity was 79% in the setting of anencephaly and 47% in the setting of spina bifida (Table II). The overall AFP sensitivity and sensitivity for spina bifida in particular were significantly less than expected based on published detection rates of 85% overall and 80% for spina bifida, both $P < .05$. Interestingly, for the 22 cases (71%) in which ultrasound measurements were available to confirm or modify the gestational age used for the AFP calculation, the overall sensitivity of the screening test improved to 86%, comparable to published reports. In the relatively small number of pregnancies in which the AFP calculation was based on LMP alone, the sensitivity was significantly less, only 11%, $P < 0.01$.

The sensitivity of standard ultrasound is also presented in Table II. As noted, ultrasound detection was evaluated only in pregnancies with no a priori indication for a targeted examination, so that we could be certain of when the abnormality was first identified. Thirty-six neural tube defects were present in women who either had AFP values below 2.50 MOM (n = 6) or who did not receive AFP screening (n = 30). In each of these cases, an abnormality was identified, a sensitivity of 100%. As shown in the table, the sensitivity of standard ultrasound was greater than that of serum AFP screening, even when the AFP MOM calculation was verified with ultrasound, all $P < .05$.

Targeted ultrasound was performed in 59 women, including the 36 already described who had an abnormality on standard examination (61%), 20 women with serum AFP elevation (34%), 2 women with pregestational diabetes mellitus, and one with a previous child who had a neural tube defect. In 58 cases (98%), the NTD was correctly identified. Other anomalies were present and correctly identified in 6 pregnancies, 4 of which had multiple malformations, including 4 infants with cardiac anomalies, 3 with renal anomalies, 1 with diaphragmatic hernia, and 1 with omphalocele. Thus, 91% of NTDs were isolated.

Because ultrasound was performed in low-risk pregnancies only as indications arose, eg, for size-date discrepancies and other concerns, the NTDs noted during standard ultrasound were frequently identified later in gestation than those identified through the AFP screening process. As shown in Table III, the *overall* prenatal detection of NTDs (at any point before delivery) was not significantly different among women who received AFP screening or did not receive AFP screening, 87% versus 91%, respectively, $P = .6$. However, those who received AFP screening were more likely to have the anomaly detected before 24 weeks, 77% versus 40%, $P < .01$, with gestational age at detection approximately 8 weeks earlier in the screened pregnancies, $P < .05$. Despite all cases having an abnormality detected with standard ultrasound, in only 42% was the NTD identified before 24 weeks, with median age at detection nearly 28 weeks—significantly later in gestation than if the AFP had been elevated, $P < .05$.

We realize that many institutions provide routine ultrasound evaluation for all pregnant women. In this series 32 pregnancies with neural tube defects received ultrasound at or before 20 weeks, 21 of which were targeted examinations and 11 of which were standard examinations. In this subgroup of well-dated pregnancies,

Table III Prenatal detection of neural tube defects, detection before 24 weeks, and median gestational age at detection, according to whether serum screening or standard ultrasound was performed

	Detected prenatally	Detected <24 weeks	Gestational age at detection
AFP screening (n = 31)	27 (87)	24 (77)	18.9 [17.7, 20.9]
AFP elevated (n = 20)	20 (100)	20 (100)	18.5 [17.7, 20.3]
AFP not elevated (n = 11)	7 (64)†	4 (36)†	22.7 [18.6, 34.9]
No AFP (n = 35)	32 (91)	14 (40)*	27.0 [19.1, 33.5]*
Standard U/S (n = 36)	36 (100)	15 (42)†	27.9 [19.5, 34.5]†
Overall group (n = 66)	58 (88)	38 (58)	20.7 [18.3, 30.9]

Data presented as n (%) and median [Q_1, Q_3].
* $P < .05$ compared with AFP screening group.
† $P < .05$ compared with AFP elevated group.

we found 86% likelihood of AFP elevation, similar to other reports,[1-4] and 100% likelihood of an abnormal finding with ultrasound.

Comment

There were 2 primary findings from this review of serum AFP screening and standard ultrasound for detection of fetal neural tube defects. First, in our population the sensitivity of serum AFP screening for neural tube defects was significantly improved when the gestational age used for the AFP MOM calculation was verified by ultrasound. We did not anticipate that in a series of 66 pregnancies complicated by fetal neural tube defects, there would be 8 affected pregnancies (12%) with normal serum screening results in the setting of a "sure" last menstrual period and no size-date discrepancy on physical examination. Though ultrasound has long been considered a first step in the evaluation of AFP elevation,[9] little attention has been placed on neural tube defects undetected because of underestimation of gestational age and resultant normalization of serum AFP test results. Currently, ACOG and AIUM do not list maternal serum screening as an indication for ultrasound in pregnancy.[14,15] We would not wish to imply that serum screening is not an important tool in the detection of NTDs as well as aneuploidy. However, it may be time for studies to address whether offering ultrasound to women who desire serum screening might improve sensitivity of this test on a large scale.

The second finding was the high sensitivity of standard ultrasound, as abnormalities were identified in all fetuses with neural tube defects. Nearly 20 years ago, Nicolaides et al described cranial and cerebellar ultrasound findings present in virtually all fetuses with spina bifida.[17] Imaging of the cerebellum and cisterna magna in addition to other views of the head is considered an essential component of standard ultrasound examination.[15] Our results would support the anecdotal observation that improvements in ultrasound technique and technology have facilitated fetal anomaly detection. One potential limitation is that because our standard examinations were performed at a tertiary center, results might not be generalizable. However, Norem et al also recently reported excellent spina bifida detection of spina bifida with standard ultrasound examinations done at 40 sites throughout Northern California.[7] Another potential limitation might be that the gestational age at standard ultrasound was beyond the typical screening period, when ventriculomegaly might have been present. Countering that argument would be that the cranial and cerebellar Chiari II findings are ideally visualized during the AFP screening window, facilitating spina bifida detection.

The role of routine or standard ultrasound for anomaly detection has been debated since the publication of the routine antenatal diagnostic imaging with ultrasound (RADIUS) trial over a decade ago.[18] Well-conducted studies in the late 1990s reported detection rates for major malformations ranging from 48% to 74%.[19,20] Of note, however, sensitivity for neural tube defect detection within these screening studies was fairly high: the RADIUS trial identified 88%,[18] the trial by VanDorsten et al[19] identified all spina bifida cases, and the Eurofetus study by Grandjean et al identified 89%.[20] Norem et al also recently reported 96% sensitivity for the detection of neural tube defects using standard ultrasound.[7] What about when routine ultrasound is not performed, as in our series? Scholtenhuis et al published an audit of the Dutch policy of offering ultrasound only when indicated, reporting that only 43% of 88 prenatally diagnosed neural tube defects were detected before 24 weeks.[21] Whether ultrasound performed for this purpose would be cost-effective, either from the standpoint of pregnancy termination or delivery at a tertiary center with reduced morbidity, is an important issue that may merit consideration.

Given that all women in the US are routinely offered AFP screening for the primary purpose of NTD detection, it would seem that prospective evaluation of standard ultrasound for this indication might also be warranted—particularly if doing so would also improve the sensitivity of the AFP MOM calculation.

References

1. Wald NJ, Cuckle H. Report of UK Collaborative Study on alpha-fetoprotein in relation to neural tube defects. Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Lancet 1977; 8026:1323-32.
2. Haddow JE, Kloza EM, Smith DE, Knight GJ. Data from an alpha-fetoprotein pilot screening program in Maine. Obstet Gynecol 1983;62:556-60.
3. Burton BK, Sowers SG, Nelson LH. Maternal serum α-fetoprotein screening in North Carolina: Experience with more than twelve thousand pregnancies. Am J Obstet Gynecol 1983; 146:439-44.
4. Milunsky A, Jick SS, Bruell CL, MacLaughlin DS, Tsung Y, Jick H, et al. Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data. Am J Obstet Gynecol 1989;161:291-7.
5. Maternal serum screening. American College of Obstetricians and Gynecologists Educational Bulletin No. 228, September 1996.
6. Neural tube defects. American College of Obstetricians and Gynecologists. Practice Bulletin No. 44, July 2003.
7. Norem CT, Schoen EJ, Walton DL, Schoen E, Krieger RC, O'Keefe J, et al. Routine ultrasonography compared with maternal serum alpha-fetoprotein for neural tube defect screening. Obstet Gynecol 2005;106:747-52.
8. Nadel AS, Green JK, Holmes LB, Frigoletto FD, Benacerraf BR. Absence of need for amniocentesis in patients with elevated levels of maternal serum alpha-fetoprotein and normal ultrasonographic examinations. N Engl J Med 1990;323:557-61.
9. Watson WJ, Chescheir NC, Katz VL, Seeds JW. The role of ultrasound in evaluation of patients with elevated maternal serum alpha-fetoprotein: a review. Obstet Gynecol 1991;78: 123-8.
10. Lennon CA, Gray DL. Sensitivity and specificity of ultrasound for the detection of neural tube and ventral wall defects in a high-risk population. Obstet Gynecol 1999;94:562-6.
11. Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Munson ML. Births: final data for 2003. Natl Vital Stat Rep 2005;54:1-116.
12. Casey BM, Dashe JS, Wells CE, McIntire DD, Byrd W, Leveno KJ, et al. Subclinical hypothyroidism and pregnancy outcomes. Obstet Gynecol 2005;105:239-45.
13. Butler EL, Dashe JS, Ramus RM. Association between maternal serum alpha-fetoprotein and adverse outcomes in pregnancies with placenta previa. Obstet Gynecol 2001;97:35-8.
14. Ultrasonography in pregnancy. American College of Obstetricians and Gynecologists. Practice Bulletin No. 58, December 2004.
15. AIUM practice guideline for the performance of an antepartum obstetric ultrasound examination. J Ultrasound Med 2003;22: 1116-25.
16. Dashe JS, Casey BM, Wells CE, McIntire DD, Byrd W, Leveno KJ, et al. Thyroid-stimulating hormone in singleton and twin pregnancy: importance of gestational age-specific reference ranges. Obstet Gynecol 2005;106:753-7.
17. Nicolaides KH, Campbell S, Gabbe SG, Guidetti R. Ultrasound screening for spina bifida: cranial and cerebellar signs. Lancet 1986;ii:71-4.
18. Crane JP, LeFevre ML, Winborn RC, Evans JK, Ewigman BG, Bain RP, et al, and the RADIUS study group. A randomized trial of prenatal ultrasonographic screening: Impact on the detection, management, and outcome of anomalous fetuses. Am J Obstet Gynecol 1994;171:393-9.
19. VanDorsten JP, Hulsey TC, Newman RB, Menard MK. Fetal anomaly detection by second-trimester ultrasonography in a tertiary center. Am J Obstet Gynecol 1998;178:742-9.
20. Grandjean H, Larroque D, Levi S, and the Eurofetus study group. The performance of routine ultrasonographic screening of pregnancies in the Eurofetus Study. Am J Obstet Gynecol 1999;181: 446-54.
21. Scholtenhuis MAGO, Cohen-Overbeek TE, Offringa M, Barth PG, Stoutenbeek PH, Gooskens RH, et al. Audit of prenatal and postnatal diagnosis of isolated open spina bifida in three university hospitals in The Netherlands. Ultrasound Obstet Gynecol 2003;21:48-52.