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Comparative study between 1st and 2nd trimester screening for neural tube defects using US markers and maternal serum alpha fetoportein

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***Abstract*---**Background; Neural tube defects, the commonest central nervous system congenital anomalies, are a heterogeneous group of malformations resulting from failure of normal neural tube closure. Aim and objectives; to assess the potential value of combined maternal serum alpha-fetoprotein and ultrasound markers at 11-13weeks of gestation in early screening for fetal neural tube defects in comparison to the standard second trimester screening of neural tube defects. Subjects and methods; This prospective study will be conducted at the Prenatal Diagnosis and Fetal Medicine Department – National Research Centre and Obstetrics and Gynecology Department in Kasr Al Ainy Teaching Hospital. Result; Number of patients with MSAFP ≥ 2.0 MoM in the study population was 50 (25%). Number of patients with MSAFP < 2.0 MoM in the study population was 150 (75%). Conclusion; Combined maternal serum alpha-fetoprotein and ultrasound markers at 11-13 weeks of gestation can be used as early screening for fetal neural tube defects as well as the standard second trimester screening of neural tube defects, the second trimester ultrasound is more sensitive than that of the first trimester for the diagnoses of neural tube defects.

***Keywords*---**alpha fetoportein, maternal serum, neural tube, trimester screening, US markers.

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Introduction

Congenital malformations are multifactorial disorders that affect about 1 in 33 infants per year. The global prevalence of congenital malformations is about 2-3% yearly, resulting in 3.2 million birth defect- related disabilities and about 270,000 newborns deaths every year.[1] Congenital heart diseases represent the first common congenital malformations and central nervous system is the second common anomaly world widely.[2] In Egypt, congenital anomalies represent 3.17% per total live births every year. Central nervous system anomalies are the commonest and representing near 30% of all the other congenital anomalies.[3] Central nervous system malformations cause up to 75% of fetal deaths and 40% of deaths in infancy.[4] Neural tube defects, the commonest central nervous system congenital anomalies, are a heterogeneous group of malformations resulting from failure of normal neural tube closure.[5] Anencephaly, encephalocele and spina bifida are the most common forms of neural tube defects.[6]

Screening for aneuploidies has shifted from second – trimester to first trimester combined test. In addition to aneuploides, the 11-13 weeks scan can identify the major fetal abnormalities.[7] The typical cutoff for MSAFP screening is 2 to 2.5 MoM. Using a cutoff of 2 MoM can increase detection of open spina bifida by approximately 10%. The detection rate for open spina bifida using a2- MoM cutoff is 90%, whereas virtually all cases of anencephaly can be detected In the last few years the introduction of successful second – trimester sonographic diagnosis of neural tube defects and the improved detection of aneuploidies by combined nuchal translucency thickness and maternal serum free hCG and PAPP-A at 11-13 weeks gestation have reduced the use of serum AFP.[8]

However, recent evidence suggest that in trisomy 21 pregnancies , the maternal serum level of AFP is reduced not only in the secondtrimester but also in the first trimester, and it is possible that measurement of serum AFP may improve the performance of the combined test at 11- 13weeks.[9] The measurement of maternal serum alpha- fetoprotein in neural tube defect pregnancies became possible during the first trimester at (10– 13^{th+6}) weeks of gestation and can improve the screening programs and early detection of neural tube defects.[9]So, introducing of both ultrasound and biochemical markers in the first trimester screening can improve the detection rate not only of chromosomal anomalies but also neural tube defects in early pregnancy.

Patients and Methods

This prospective study had been conducted at the Prenatal Diagnosis and Fetal Medicine Department – National Research Centre and Obstetrics and Gynecology Department in Kasr Al Ainy Teaching Hospital. It included 205 patients attended for her routine 1^{st} trimester screening between (10 -13^{th+6}) weeks of gestation. The study will be conducted after the approval of Ethics Committee of the National Research Centre.

Inclusion criteria

Pregnant female in her 1st trimester (10– 13^{th+6}) weeks of gestation and sure of her dates, couple with history of neural tube defects or family history of neural tube defects and pregnancy with neural tube defects or part of syndrome associated with neural tube defects.

Exclusion criteria

Multiple pregnancies, molar pregnancy or non-viable pregnancies. For all subjects the following will be performed: Informed written consent and received counseling before and after each procedure, detailed history taking (personal, present, obstetric, past and family history and pedigree analysis), complete blood picture, fasting blood glucose & post prandial and urine analysis will be performed as part of routine investigations, general examination (weight measurement, height measurement, blood pressure measurement.

Ultrasonographic screening

Ultrasound scans had been carried out transabdominally using General Electric Voluson 730 and Samsung medisonaccuvix v20 real- time scan system with a capacity of simultaneous B-mode and M-mode scanning. The carrier frequency had been obtained using a broad-band probe which covers range of frequencies from 5 to 7.5 megahertz (MHz). All measurement will be done using hadlock formula all scans will be performed under supervision of senior staff who have extensive experience in anomaly scan and genetic ultrasound scans.

First trimester ultrasonographic scan (10- 13^{th+6}) weeks

Crown rump length, Assessment of the intracranial translucency of the fourth ventricle: in the same mid-sagittal view of the fetal face which used for measurement of nuchal translucency and assessment of the nasal bone, the brain stem and fourth cerebral ventricle are easily visible. Fourth ventricle presents as an intracranial translucency (IT) parallel to the NT and is delineated by two echogenic borders; the dorsal part of the brain stem anteriorly and the choroid plexus of the fourth ventricle posteriorly. Between the fourth ventricle and the occiput there is another thinner translucency generated by the developing cisterna cerebellomedullaris. At 11–13 weeks' gestation the fourth cerebral ventricle is easily recognizable as an intracranial translucency in the standard mid-sagittal view of the face used routinely in screening for chromosomal abnormalities, in cases of open spina bifida the intra cranial translucency not seen,Posterior cranial fossa examination, Fetal spine in both: Lateral view and cronalview, Bi pareital diameter measurement, nuchal translucency and Nasal bone prescience or abscence.

Second trimester ultrasonographic scan (18th – 22^{nd}) weeks

All subjects will be re-examined at their (18th – 22^{nd}) weeks of gestation for Biometric assessment and detailed cranial anomalies scan to confirm the diagnosis of central nervous system congenital anomalies by checking the

following soft tissue markers: Biparaital diameter, head circumference, Fronto-occipital diameter, cavum septum pellucid, posterior fossa scan: Cerebellum and cesterna magna, cerebral ventricles and fetal Spine.

Biochemical screening

First trimester maternal serum alpha feto protein measurement: 5ml of venous blood samples will be collected from the pregnant women in their First trimester between (10th and 13^{th+6} weeks) of gestation. The blood samples will be centrifuged at 3000 rpm for 3 minutes, and the serum will be separated and frozen at -20°C. Alpha feto protein measurement will be performed by using DELFIA XPRESS IMMUNE ANALYZER MACHINE using fluorescent immune assay technique; the concentrations were expressed as Multiples of Median (MOM).

Second trimester maternal serum alpha feto protein measurement: Another blood samples will be withdrawn for second trimester measurement of alpha feto protein as the same technique of first trimester biochemical screening. All the patients followed by the ultrasound till the time of the delivery and we contact them to ensure that all of them had normal outcome. The results of first trimester screening using combined ultrasound and biochemical markers will be compared to the second trimester screening to evaluate the role of combined first trimester screening in early detection of neural tube defects in the first trimester.

Ethical consideration

Study protocol was submitted for approval by the Ethical Committee of faculty of medicine - AL Azhar University – Cairo, Informed verbal consent was obtained from each participant sharing in the study after explanation of the purpose and procedures of the study. Confidentiality and personal privacy was respected in all levels of the study.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 26.0 for windows (SPSS Inc., Chicago, IL, USA). Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The used diagnostic test parameters were: Sensitivity (True Positive Rate): the probability of a positive test, conditioned on truly being positive. Specificity (True Negative Rate): the probability of a negative test, conditioned on truly being negative. Positive predictive value (PPV): the probability that following a positive test result, that individual will truly have that specific disease. Negative predictive value (NPV): the probability that following a negative test result, that individual will truly not have that specific disease.

Results

Table (1): Demographic characteristics of women carrying a fetus diagnosed with NTD

	Women carrying a fetus diagnosed with NTD (n = 15)
Maternal age (years)	
Mean ± SD.	34.13 ± 8.13
Median (IQR)	33 (26.5 - 40.5)
Range (Min-Max)	25 (22 - 47)
Parity	
Mean ± SD.	2 ± 1.89
Median (IQR)	2 (0.5 - 3)
Range (Min-Max)	6 (0 - 6)

SD: standard deviation IQR: interquartile range

Table (1) showed Demographic characteristics of women carrying a fetus diagnosed with NTD. Maternal age among women carrying a fetus diagnosed with NTD ranged from 22 to 47 with mean ± SD = 34.13 ± 8.13. Parity among women carrying a fetus diagnosed with NTD ranged from 0 to 6 with mean ± SD = 2 ± 1.89.

Table (2): NTD types among fetuses diagnosed with NTD

	Fetuses diagnosed with NTD (n = 15)	
	n	%
NTD type Encephalocelen (%)	2	13%
- Exencephalyn (%)	8	53%
- MMC – cervical n (%)	1	7%
- MMC – lumbar-sacral n (%)	2	13%
- MMC – sacral n (%)	1	7%
- MMC – thoracic n (%)	1	7%

Table (2) showed NTD types among fetuses diagnosed with NTD. Number of fetuses with Encephalocele in the study population was 2 (13%). Number of fetuses with Exencephaly in the study population was 8 (53 %). Number of fetuses with MMC – cervical in the study population was 1 (7 %). Number of fetuses with MMC – lumbar-sacral in the study population was 2 (13 %). Number of fetuses with MMC – sacral in the study population was 1 (7 %). Number of fetuses with MMC – thoracic in the study population was 1 (7 %).

Table (3): MSAFP screening tests results among the study population

	Study population (n = 205)	
	n	%
- MSAFP screening tests ≥ 2.0 MoM		

n (%)	50	25%
- < 2.0 MoM		
n (%)	150	75%

Table (3) showed MSAFP screening tests results among the study population. Number of patients with MSAFP ≥ 2.0 MoM in the study population was 50 (25%). Number of patients with MSAFP < 2.0 MoM in the study population was 150 (75%).

Table (4): Methods of detection of the NTD among the study population

	Women carrying a fetus diagnosed with NTD (n = 15)	
	n	%
Method of detection U/S n (%)	15	100%
MSAFP n (%)	0	0%

Table (4) showed Methods of detection of the NTD among the study population. All cases of NTD in the study population were detected with U/S.k

Table (5): Time of diagnosis among women carrying a fetus diagnosed with NTD

	Women carrying a fetus diagnosed with NTD (n = 15)	
	n	%
Time of diagnosisfirst trimestern (%)	12	80%
Second trimester n (%)	3	20%

Table (5) showed Time of diagnosis among women carrying a fetus diagnosed with NTD. Number of women diagnosed in first trimester in the study population was 12 (80%). Number of women diagnosed in second trimester in the study population was 3 (20%).

Table (6): Sensitivity, Specificity, Positive and Negative Predictive Values of First-trimester and Second-trimester ultrasound

	Diagnostic parameters			
	Sensitivity	Specificity	PPV	NPV
First-trimester ultrasound (%)	80%	100%	100%	98.45%
Second-trimester ultrasound (%)	100%	100%	100%	100%

PPV: Positive Predictive Value NPV: Negative Predictive Value

Table (6) showed Sensitivity, Specificity, Positive and Negative Predictive Values of First-trimester ultrasound. Regarding First-trimester ultrasound, it had sensitivity 80% , specificity 100% , positive predictive value (PPV) 100% and negative predictive value (NPV) 98.45% ,While Second-trimester ultrasound had

sensitivity 100% , specificity 100% , positive predictive value (PPV) 100% and negative predictive value (NPV) 100% .

Discussion

Neural tube defects (NTDs) are general birth defects of the central nervous system with a prevalence of 1.2 per 1000 live births, including a series of defects of varying severity.[10] NTDs can appear on any part of the nerve axis and have a specific level of clinical intensity; NTD subtypes are identified according to the anatomic location and severity of the defects. The most serious form of NTD is anencephaly or craniorachischisis, where the forebrain and entire central nervous system fail to convert from the neural plate to a neural tube. NTD may be affected by numerous types of genetic variation.[11]

This prospective study was conducted in the Prenatal Diagnosis and Fetal Medicine Department – National Research Centre and Obstetrics and Gynecology Department in Kasr Al Ainy Teaching Hospital. This study was conducted on 205 patients attended for her routine 1st trimester screening between (10 -13^{th+6}) weeks of gestation. Regarding the demographic characteristics of women carrying a fetus diagnosed with NTD, there were 15 women with mean maternal age among women carrying a fetus diagnosed with NTD ranged from 22 to 47 with mean ± SD = 34.13 ± 8.13. Parity among women carrying a fetus diagnosed with NTD ranged from 0 to 6 with mean ± SD = 2 ± 1.89. The study by Chen et al.[13] showed that having advanced maternal age was associated with more incidence of NTD.

Also, Khalil et al.[14] reported that maternal age was shown to be significantly and independently associated with the risk of spina bifida. Many countries now recommend that pregnant women with a maternal age of 35 years or more should undergo interventional prenatal diagnosis during the second trimester in order to avoid fetuses being born with chromosomal abnormalities and NTD.[15] BMI among women carrying a fetus diagnosed with NTD ranged from 18 to 39 with mean ± SD = 26.33 ± 5.91. Number of women carrying a fetus diagnosed with NTD with BMI ≥30 in the study population was 3 (20%), While number of women carrying a fetus diagnosed with NTD with BMI ≥25 in the study population was 12 (80%).

Our results suggested the association between high BMI and the incidence of NTD. This was supported by Khalil et al.[14] whoreported that BMI was shown to be significantly and independently associated with the risk of spina bifida. The meta-analysis by Rasmussenet al.[16] reported that maternal obesity is associated with an increased risk of an NTD-affected pregnancy. A more recent meta-analysis by Zhang et al.[17] suggested that maternal periconceptional obesity may be associated with an increased risk for spina bifida. Maternal underweight may be associated with increased risk for anencephaly.

Furthermore, Mezzasalmaet al.[18] reported that there were a positive association of maternal BMI ≥25 with nervous system anomalies and maternal underweight with orofacial clefts. As regard NTD types among fetuses diagnosed with NTD. Number of fetuses with Encephalocele in the study population was 2 (13%). Number of fetuses with Exencephaly in the study population was 8 (53 %). Number of

fetuses with MMC – cervical in the study population was 1 (7 %). Number of fetuses with MMC – lumbar-sacral in the study population was 2 (13 %). Number of fetuses with MMC – sacral in the study population was 1 (7 %). Number of fetuses with MMC – thoracic in the study population was 1 (7 %). The etiology of the vast majority of NTDs is proposed to be multifactorial, but specific abnormal chromosomal etiologies are reported in 2% to 16% of cases.[19] The most common chromosomal anomalies observed in MMCs are trisomy 13, trisomy 18, triploidy.[12]

As regard maternal Serum Alpha-Fetoprotein (MSAFP) screening tests results among the study population, the present study showed that number of patients with MSAFP ≥ 2.0 MoM in the study population was 50 (25%). Number of patients with MSAFP < 2.0 MoM in the study population was 150 (75%). The current stud also showed that there were 15 cases with NTD in the study population were detected using U/S. The study by Chen et al.[20] and Chen et al.[13] reported that Levels of serum maternal serum alpha-fetoprotein (AFP) variants (AFP-L2, AFP-L3) in pregnant women with ONTD fetuses were significantly higher than those measured in pregnant women with normal fetuses (all P<0.001).

While sonography is the primary screening tool, second-trimester maternal serum alpha fetoprotein (MSAFP) detects 71% to 90% of NTDs, with a false-positive rate of 1% to 3%. Elevated levels of MSAFP can be associated with other conditions, such as fetal skin disorders, abdominal wall defects, fetal death, fetal nephrosis, and pregnancies at an increased risk for placenta-related adverse events. By contrast, first-trimester MSAFP screening detects 50% of NTDs, with a false-positive rate of 10%, and is not recommended for NTD screening.[21]

Furthermore, Norem et al.[22] identified 189 NTD cases, 102 of which had received MSAFP screening. Results of MSAFP testing were negative in 25 (25%) of these 102 cases. Without other testing, these 25 NTD diagnoses would have been missed. These included 15 (38%) of the 40 spina bifida cases screened, 6 (67%) of the 9 encephalocele cases screened, and 4 (8%) of the 53 anencephaly cases screened. Of the 186 NTD cases diagnosed prenatally, 115 (62%) were initially detected by routine ultrasonography administered during the second trimester without knowledge of MSAFP values; 69 (37%) were diagnosed by targeted ultrasonography after MSAFP screening indicated a higher risk for NTD; and 2 (1%) were diagnosed by pathology examination after miscarriage.

The current study revealed that Gestational age among fetuses diagnosed with NTD ranged from 11 to 17 with mean ± SD = 12.73 ± 1.94. Number of women diagnosed in first trimester in the study population was 12 (80%). Number of women diagnosed in second trimester in the study population was 3 (20%). In a study performed by Norem et al.[22], the best timeframe for NTD screening was suggested to be the 2nd trimester (92.1%). The percentage goes down to 83% if the screening is performed during the first trimester. It is also recommended to use serum markers such as MSAFP (Maternal Serum Alpha Fetoprotein) which leads to a diagnosis rate of 98%. Also, Huanget al.[23] revealed that the median gestational age at the time of detection for cranial dysraphism was 13.3 weeks, open spinal dysraphism was 22.0 weeks, and closed spinal dysraphism was 22.6 weeks.

In the present study Sensitivity, Specificity, Positive and Negative Predictive Values for NTD screening showed that at First-trimester ultrasound, it had sensitivity 80%, specificity 100%, positive predictive value (PPV) 100% and negative predictive value (NPV) 98.45%, while Second-trimester ultrasound had sensitivity 100%, specificity 100%, positive predictive value (PPV) 100% and negative predictive value (NPV) 100%.

In agreement with our findings Sepúlveda-González et al.[24] stated that screening was first performed by second-trimester ultrasound (US) scan with sensitivity close to 100% in detecting NTDs when performed by experienced physicians. Also, the study by Lennon & Gray, [25] reported that Sonography alone was 97% (66 of 68) (95% confidence interval [CI] 0.898, 0.996) sensitive and 100% (n = 2189) (CI 0.998, 1.0) specific in diagnosing open neural tube defect, and was 100% sensitive (n = 17) (CI 0.805, 1.0) and specific (n = 2240) (CI 0.998, 1.0) in diagnosing ventral wall defect. In two cases of neural tube defect, there were other suspicious findings on sonography, and amniocentesis was performed for confirmation.

Furthermore, the meta-analysis by Mace et al.[26] reported that at the first trimester the pooled sensitivity and specificity for qualitative assessment were 76.5% and 99.6%, and for quantitative assessment were 84.5% and 96.3%, respectively; specificity for the qualitative ultrasound signs was significantly higher (P = 0.001). The overall sensitivity of cranial sonographic markers for the screening of other posterior brain defects was 76.7% and specificity was 97.5%.

Conclusion

Combined maternal serum alpha-fetoprotein and ultrasound markers at 11-13 weeks of gestation can be used as early screening for fetal neural tube defects as well as the standard second trimester screening of neural tube defects, the second trimester ultrasound is more sensitive than that of the first trimester for the diagnoses of neural tube defects. Prompt diagnosis of a fetal neural tube defect is important for treatment decision making, especially with the recent availability of fetal surgical repair for myelomeningocele (open spina bifida). Further studies with larger sample size and longer follow-up are needed to confirm our results and to identify risk factors of adverse events.

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