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ORIGINAL ARTICLE

Down Syndrome Screening: Evidence that Test Results Differ According to Phenotype

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Abstract The purpose of the present study was to examine screening marker levels in Down syndrome (DS) pregnancies with and without cardiac defects and in euploid pregnancies. Retrospective series in two centers with one or more markers—ultrasound nuchal translucency (NT), first trimester maternal serum pregnancy associated plasma protein (PAPP-A), free-β human chorionic gonadotrophin (free β-hCG), and second trimester serum α-fetoprotein (AFP), unconjugated estriol (uE_3), hCG, and free-β hCG. Levels were expressed as multiples of the gestation-specific median (MoM). Differences were assessed by the Wilcoxon rank sum test and 95 % confidence intervals. There were 318 DS pregnancies including 53 (17 %) with cardiac defects. Median NT was higher in cardiac defects (1.82 compared with 1.62 MoM), but not statistically significant ($P = 0.17$). Median free β-hCG was significantly highly reduced in the first trimester (1.14 and 2.17 MoM; $P < 0.005$) and similarly but nonsignificantly in the second trimester (1.59 and 2.32 MoM; $P = 0.14$). PAPP-A was reduced and AFP increased nonsignificantly with no material differences for uE_3 and hCG. The results on NT and free β-hCG were consistent with a series of 62 euploid pregnancies with cardiac defects screened in one of the centers. The distribution of some markers differs in DS pregnancies with cardiac defects. Depending on the screening protocol, this may affect the phenotype of DS births.

Keywords Down syndrome · Cardiac defects · Screening · Marker levels · Risk

Introduction

The widespread introduction of routine antenatal screening for Down syndrome (DS) has had a considerable impact on birth prevalence. For example, in 2006 when the Danish first trimester screening program covered the whole country, there were just 32 DS births compared with 135 expected from the maternal age distribution [1]. When evaluating screening programs, it is generally assumed that affected infants born following screening will have a similar phenotype. However, there are indications that some of the screening markers are associated with a more severe DS phenotype.

One study investigated 17 DS pregnancies in which second trimester maternal serum α-fetoprotein (AFP) levels had been measured for neural tube defect (NTD) screening only, and unconjugated estriol (uE_3) and human chorionic gonadotrophin (hCG) levels were measured, retrospectively, in frozen blood sample [2]. There was a cardiac defect in 40 % (6/15) and an anatomical abnormality in 69 % (11/16), excluding those without postmortem examinations. No statistically significant difference between

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those with and without a severe phenotype but the numbers studied was small. A second study included 30 births with DS in women who had second trimester screening with AFP, uE_3, and hCG; 50 % (15/30) had cardiac defects, and 67 % (20/30) had an anatomical abnormality [3]. The only statistically significant difference was an increased mean hCG level in those with anatomical abnormalities. The third study comprised a series of 92 DS cases detected by second trimester screening [4]. The seven cases with hydrops fetalis had statistically significantly lower AFP and uE_3, and higher hCG than nonhydropic cases. In a subset of 42 women with maternal serum inhibin levels, there was no significant difference between the five hydropic and the nonhydropic cases.

Abnormal levels of some of the screening markers are also associated with fetal cardiac defects in euploid pregnancies. In a study of 507 euploid pregnancies in women having second trimester maternal serum AFP screening for NTDs, there was an association between raised AFP and fetal cardiac abnormalities [5]. The incidence was 40 % (12/30) in those with AFP levels above 2 multiples of the normal gestation-specific median (MoM) compared with 12 % (58/477) with normal levels, a statistically significant difference.

The state-wide California Prenatal Screening Program has reported altered second trimester maternal serum screening marker levels in euploid fetuses with cardiac defects [6]. A series of 306 cases was compared with 1224 unaffected controls. There was an excess of cases when AFP was raised or uE_3 reduced, whilst an excess of cases was found both in those with raised hCG and those with reduced levels.

An increased frequency of cardiac defects was first reported among euploid pregnancies with elevated first trimester ultrasound nuchal translucency (NT) levels as early as 1997 [7]. This was subsequently confirmed in many studies and when complete series of cases were compiled, the distribution of NT levels in cardiac defects could be evaluated. In one series of 37 cases with major defects, the median NT level was 1.52 multiples of the gestation-specific median (MoM) [8]. In a meta-analysis the estimated NT, cardiac defect detection rate was 52 % for a fixed 5 % false-positive rate [9].

The current study investigated these ultrasound and serum markers further in DS pregnancies with and without cardiac defects and in a series of euploid pregnancies with cardiac defects. An additional screening marker was also included, first trimester maternal serum pregnancy associated plasma protein (PAPP)-A, as well as a related marker, the free-β subunit of hCG, in both first and second trimester.

Materials and Methods

Down syndrome cases were identified, during pregnancy or after delivery, in a consecutive series of women who had antenatal screening at two centers, in Israel and Ukraine. Only singleton pregnancies were included and those with karyotype other than nonmosaic trisomy 21 were excluded. All screening tests had been carried out routinely and the results were expressed in MoMs—for NT using normal median data from Israel [10] and for the maternal serum markers using locally derived curves. For this purpose gestational age was based on menstrual dates corrected, if appropriate, by ultrasound biometry.

In the Israel center, various screening protocols had been used, including first trimester NT alone, together with first trimester maternal serum PAPP-A and free β-hCG; or with second trimester AFP, uE_3, and hCG; second trimester serum markers alone, and all marker. In the Ukraine center women had been screened with either the first trimester NT, PAPP-A, and free β-hCG or the second trimester AFP and hCG or free β-hCG.

Information on cardiac defects in DS cases was collected during prenatal ultrasound examination at the second trimester postmortem, or after delivery. In the Ukraine series, all cases were detected prenatally. In the Israel series five cases were delivered—they only had a second trimester screening test, which was positive in two cases, and all had a cardiac abnormality. In Israel, women with positive first trimester screening results are referred for second trimester amniocentesis rather than immediate invasive testing. Consequently, in the Israel series there were fewer nonviable DS pregnancies than in the Ukraine series.

For DS cases with and without cardiac defects, the median marker level was computed together with the standard deviation after $\log_{10}$ transformation considering non-normal distribution of the markers' levels expressed in MoM. The standard deviation was estimated from the 10th to 90th centile difference divided by 2.563. Differences in the distribution of marker levels according to the presence of cardiac abnormality were assessed with the Wilcoxon rank sum test.

In the Ukraine center, a series of euploid singleton pregnancies with cardiac defects was identified among women who had antenatal screening. For each marker, the median MoM and 95 % confidence interval were computed.

Results

A total of 318 DS pregnancies were available for the study, including 53 (17 %) with cardiac defects. In Israel, there were 249 cases, some included in previous publications

[11–13], with 36 (14 %) affected. In Ukraine, there were 69 with 17 being affected (25 %). Most of the cardiac abnormalities were septal defects—atrial, ventricular, or atrioventricular (75 %, 40/53); there were two cases (4 %) with tetralogy of Fallot and 11 (20 %) other complex malformations.

Table 1 shows the median and standard deviation of each screening marker in DS pregnancies with and without cardiac abnormalities. The median NT level was higher in affected pregnancies (1.82 compared with 1.62 MoM), but was not statistically significant ($P = 0.13$). Median first trimester free β-hCG was highly significantly reduced (1.14 vs. 2.17 MoM; $P < 0.005$) and PAPP-A was reduced, but not significantly (0.44 vs. 0.54 MoM; $P = 0.81$). The second trimester free β-hCG was also reduced to a similar extent as in the first trimester (1.59 vs. 2.32 MoM) though not significantly ($P = 0.14$), AFP was increased but not quite reaching statistical significance (0.80 vs. 0.72 MoM; $P = 0.06$), whilst there were no material differences for uE_3 (0.72 vs. 0.68 MoM; $P = 0.51$) and hCG (2.00 vs. 1.99 MoM; $P = 0.36$).

The finding of substantially reduced free β-hCG levels on average in DS pregnancies with cardiac abnormalities was consistent between the series and between trimesters. Table 2 shows the overall distribution of its levels. When data from both series and trimesters are combined, a greater proportion with cardiac abnormalities (27 %, 7/26) has free β-hCG levels below 1.00 MoM compared with unaffected cases (12 %, 15/125). In contrast, a smaller proportion of affected cases (23 %, 6/26) has levels above 2.0 MoM compared with 53 % (66/125) of unaffected cases.

Sixty-two euploid pregnancies with cardiac defects were available for study. The most common abnormalities were septal defects (13, 21 %), tetralogy of Fallot (13, 21 %), transposition of the great vessels (10, 16 %), truncus arteriosus (9, 14 %), and hypoplastic left or right heart syndrome (8, 13 %). Table 3 shows the median and 95 % confidence interval for each marker. The confidence interval for the median NT excluded 1.0 MoM, and the interval for free β-hCG, including both first and second trimester results also excluded 1.0 MoM.

Discussion

The distribution of some DS screening markers differed in affected pregnancies with cardiac defects. A substantial and highly statistically significant reduction in maternal serum free β-hCG levels was found ($P < 0.002$) consistent with our results from euploid pregnancies with cardiac defects. No other marker was statistically significantly different in DS pregnancies with cardiac defects, but there was an increase in the median NT level as well as in euploid pregnancies, as expected from the literature. There were insufficient data to examine these observations further by breaking down results according to the type of cardiac abnormality.

Any study of screening marker levels in DS pregnancies with cardiac abnormalities is potentially subject to two competing biases. Firstly, those with screen-positive results are likely to have more careful ultrasound examination prior to the performance of invasive prenatal diagnosis, which would bias towards higher NT, free β-hCG, and hCG, and towards lower PAPP-A, AFP, and uE_3. Secondly, pregnancies with screen negative results are more likely to continue, at least, until the late second trimester, when a cardiac abnormality might be detected on a routine anomaly scan or at term. The latter would bias in the opposite direction, but given the high detection rate of DS,

Table 1 Median marker levels in Down syndrome pregnancies, with and without cardiac abnormalities

Marker	Center	Median MoM [log_{10}SD] (cases)		*P* value*
		Cardiac abnormality	None	
First trimester				
NT	Both	1.822 [0.216] (34)	1.615 [0.203] (198)	0.13
PAPP-A	Both	0.435 [0.337] (22)	0.535 [0.307] (116)	0.81
Free β-hCG	Both	1.140 [0.234] (21)	2.170 [0.289] (109)	<0.005
Second trimester				
AFP	Both	0.810 [0.174] (31)	0.720 [0.164] (143)	0.06
uE_3	Israel	0.715 [0.114] (26)	0.680 [0.153] (127)	0.51
Free β-hCG	Ukraine	1.590 [0.154] (5)	2.325 [0.342] (16)	0.14
hCG	Israel	2.000 [0.225] (26)	1.990 [0.226] (127)	0.36
Both trimesters				
Free β-hCG	Both	1.235 [0.234] (26)	2.240 [0.303] (125)	<0.002

* 2-tail test

Table 2 Distribution of free β-hCG levels in Down syndrome pregnancies with and without cardiac abnormalities, according to series and trimester

Series	Free β-hCG MoM					
	<0.5	0.5–	1.0–	1.5–	2.0–	≥2.5
Israel, first trimester						
Cardiac abnormality	0 (0 %)	2 (22 %)	3 (33 %)	1 (11 %)	0 (0 %)	3 (33 %)
None	2 (3 %)	8 (11 %)	13 (18 %)	19 (26 %)	8 (11 %)	23 (32 %)
Ukraine, first trimester						
Cardiac abnormality	0 (0 %)	4 (33 %)	4 (33 %)	3 (25 %)	0 (0 %)	1 (8 %)
None	1 (3 %)	2 (6 %)	3 (8 %)	5 (14 %)	5 (14 %)	20 (56 %)
Ukraine, second trimester						
Cardiac abnormality	0 (0 %)	1 (20 %)	0 (0 %)	2 (40 %)	2 (40 %)	0 (0 %)
None	0 (0 %)	2 (12 %)	2 (12 %)	2 (12 %)	3 (19 %)	7 (44 %)
All						
Cardiac abnormality	0 (0 %)	7 (27 %)	7 (27 %)	6 (23 %)	2 (8 %)	4 (15 %)
None	3 (2 %)	12 (10 %)	18 (14 %)	26 (21 %)	16 (13 %)	50 (40 %)

Table 3 Median marker levels in euploid pregnancies, with cardiac abnormalities

Marker	Median MoM [$\log_{10}$SD] (cases)	95 % confidence interval
First trimester		
NT	1.376 [0.096] (49)	1.29–1.46
PAPP-A	1.016 [0.308] (49)	0.83–1.24
Free β-hCG	0.844 [0.290] (49)	0.70–1.02
Second trimester		
AFP	0.880 [0.116] (13)	0.76–1.02
Free β-hCG	0.690 [0.399] (13)	0.42–1.14
Both trimesters		
Free β-hCG	0.820 [0.291] (62)	0.69–0.97

especially with first trimester screening protocols, the former bias is likely to outweigh the latter.

The incidence of cardiac defects differs between the two centers in this study. This difference is partly a consequence of invasive prenatal diagnosis referral patterns in Israel. Delaying diagnosis until the second trimester will reduce the number of recognized DS cases by about one-third due to miscarriage of affected fetuses. If the fetal losses are more likely to have cardiac defects, the proportion of known DS cases with these abnormalities will be smaller.

The study is limited in size and consequently, has low statistical power to detect a difference in the distribution of markers of a magnitude reported in euploid pregnancies. Nevertheless, the observed median NT MoM in DS pregnancies with cardiac defects is 1.13 times of that in those without a cardiac defect with 95 % confidence interval 0.90–1.41. This is consistent with the confidence interval on the median value in euploid pregnancies with cardiac defects in one published study, which is 1.07–2.16 MoM [3] and in our series, it is 1.29–1.46 MoM. The observed increase in median maternal serum AFP in DS pregnancies with cardiac defects of 1.14 times, was not statistically significant. Whilst this is consistent with the reported 1.8-fold increased incidence of fetal cardiac defects in euploid pregnancies with AFP levels above the 95th centile [6], there was no increase in our series of euploid pregnancies.

There are no other published reports of reduced maternal serum free β-hCG levels in pregnancies with cardiac defects. In view of the highly statistically significant effect observed here for DS pregnancies and the results in euploid pregnancies, there is a need to investigate this marker further. Free β-hCG is a placental product, which in euploid pregnancies, is a predictor of fetal viability. Extremely high or low maternal serum levels are found in the presence of fetal demise [14]. The observation of relatively reduced levels, on average, in DS pregnancies with cardiac defects might be related to the reduced fetal viability in aneuploidy. Alternatively, this could be related to cardiac abnormalities, per se, which is supported by our results in euploid pregnancies with cardiac defects.

There are two observations in the current study of DS that are not consistent with reporting findings in euploid pregnancies. Here in the current study, the median maternal

serum uE_3 in pregnancies with cardiac defects was slightly *raised* compared with the reported 2.1-fold increased incidence of fetal cardiac defects in euploid pregnancies with uE_3 levels below the 5th centile [6]. Here, there was no material difference in the median maternal serum hCG level according to the presence or absence of a cardiac abnormality. Moreover, closer examination of the data did not reveal a bimodal distribution of MoMs, whilst in euploid pregnancies, there was a doubling of cardiac defect incidence, both in those with results below the 5th and above the 95th centile [6].

Until further studies are carried out, it is not possible to assess whether screening tests, which include ultrasound NT and maternal serum free β-hCG, such as some versions of the combined, contingent, integrated, and serum integrated tests, will detect more or less DS pregnancies with cardiac defects. This will depend on the relative magnitude of the increase in NT compared with the reduction in free β-hCG.

The results of the present study may have implications for DS risk assessment in screening programs. Some women with screen-positive or borderline screening results will delay a decision over invasive prenatal diagnosis until a second trimester anomaly scan has been carried out. The scan results can be used to revise the DS screening risk using published likelihood ratios for the presence or absence of each ultrasound marker [15]. If there was evidence of a cardiac defect at the time of the scan, the screening risk might be further revised using cardiac defect-specific parameters for the NT, and if measured, free β-hCG, as well as altering the prior risk.

In centers carrying out routine first trimester anomaly scanning, the diagnosis of a cardiac defect may be made close to the time of DS screening. In that case, it would be possible to adjust marker levels, and consequently risks, for the presence or absence of a cardiac defect. Whilst such scanning is not currently widespread, rapid progress is being made in that direction with the introduction of more advanced equipment and more improved training of sonographers. Thus, whilst the full clinical relevance of our findings may be limited at present, it is anticipated that they will be of greater value in the near future.

Compliance with Ethical Standards

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Conflict of interest None.

Ethics Approved by local committee.

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