Combined Ultrasound and Maternal Serum Beta HCG in the Detection of Down's Syndrome in Early Pregnancy

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Abstract

Objective: To assess the value of maternal serum beta HCG (human chorionic gonadotrophin) and ultrasound nuchal translucency (NT) measurement at 10-14 weeks of pregnancy in the detection of Down's syndrome.

Methods: The study conducted at Al-Yarmouk Teaching Hospital: departments of Obstetrics and Gynaecology, Neonatology, Ultrasound and Laboratories.

One hundred pregnant women in their 10-14 weeks of gestation were included. Fifty of them as a high risk group and 50 as a low risk group for the development of Down's syndrome. Maternal serum beta HCG and fetal NT by ultrasound were measured for all of them and evaluated later on for the outcome of their pregnancy regarding Down's syndrome.

Results: Beta HCG in maternal blood was found to be significantly increased in pregnancies ended with Down's syndrome. Nuchal translucency was found to be increased in pregnancies ended with Down's syndrome.

There was a highly significant correlation between a positive family history of Down's syndrome and pregnancy outcome with this chromosomal abnormality but no significant relation was found between maternal age and Down's syndrome.

Conclusion: Our data supported the relation between increased maternal serum beta HCG and increased fetal nuchal translucency at 10-14 weeks of gestation, and pregnancy outcome with Down's syndrome.

Key words: Down's syndrome, Ultrasound, Beta HCG

Introduction

Down's syndrome is the most common chromosomal abnormality among newborns, occurring in more than 1 in 600 to 1 in 700 live births [1].

The incidence of Down's syndrome among conceptions is more than twice as high as it is among live births; more than half of trisomy 21 are aborted early in pregnancy. About 95% of cases of Down's syndrome are due to regular trisomy 21 which arises as a result of non-disjunction during meiosis; normally during meiosis the two homologous chromosomes of any pair separate and pass into different gametes, occasionally an accident occurs and the chromosomes fail to separate and both members of the pair passing into the same gamete. If such a gamete is then fertilized by a normal gamete the resulting zygote will posses an additional chromosome [2].

About 4% of cases of Down's syndrome result from a phenomenon known as translocation of Robertsonian type, in which there is an exchange of segments between different chromosomes. About 1% of cases of Down's syndrome are mosaics; they possess two cell lines; one of which has a normal chromosome constitution, the other an extra chromosome 21. This is very rarely inherited [3].

The risk of fetal chromosomal abnormalities particularly trisomy 21 (Down's syndrome) rises sharply with maternal age, and the prevalence of it and other trisomies in live births don't represent the actual incidence of these conditions. Maternal specific chromosomal abnormalities rise mostly from maternal non-disjunction during the first meiotic division; in contrast, translocation trisomies are not associated with maternal age [4].

Advanced maternal age is associated with increased risk of miscarriage that is assumed to be a function of age related risk of chromosomal abnormalities since approximately 40-60% of conceptions that are aborted are chromosomally abnormal [5].

Prenatal diagnosis of Down's syndrome can be aided by means of chorion villus sampling, amniocentesis, triple test and ultrasound [6, 7, 8, 9].

HCG is a glycoprotein with a molecular weight of 40.000 Daltons and two polypeptide subunits, (alpha and beta) [10]. The beta core HCG is the major break-down product of nicked free beta subunit which is readily excreted in urine [11].

HCG is mainly biosynthesized by placental syncytiotrophoblasts [11], the plasma half life of intact HCG is 29 hours and it is detected in the plasma of pregnant women about 7.5-9.5 days after the midcycle, it is likely that HCG enters blood at the time of blastocyst implantation [12].

The level of serum HCG will steadily increase and peak during the first 3 months of pregnancy; the level then decreases until the 16th week of pregnancy when it is stabilized until term [11].

The concentration of HCG in maternal urine is closely parallel to that in plasma, which is approximately 1000 mlU/ml by 6 weeks after the last menstrual period, increases to an average value of about 100.000 mlU/ml between 60-80 days after the last menses [12].

The physiological function of HCG in pregnancy is to maintain the gestational yellow body and to foster progesterone and oestrogen production during the first 3 months of pregnancy;

it also plays a role in the differentiation of the fetal genital tract [13].

HCG assay is an essential element in the diagnosis and follow up of abnormal pregnancies such as ectopic gestation and hydatidiform mole [14]. A number of retrospective studies have found an association between serum HCG and adverse pregnancy outcome including preeclampsia [15,16], premature delivery[17] and intrauterine growth restriction[18]. HCG can also be produced by certain tumor tissues mainly those of trophoblastic origin and it represents a biological marker of clinical significance both for prognosis and therapeutic monitoring [19].

The introduction of the triple test in 1988 has changed the practice of prenatal screening for Down's syndrome; it is a combination of the serum level of maternal alpha fetoprotein, unconjugated oestriol, and HCG [6, 7, 8, 9].

The beta subunits of HCG have been found to be the most discriminatory and sensitive markers for Down's syndrome screening in maternal serum [20, 21, 22].

Table (1) shows the normal values of beta HCG during pregnancy [20].

Table (1): The normal values of beta HCG during pregnancy (20).

Weeks of amenorrhoea	Mean (mlU/ml)
4-5	7400
5-6	32800
6-7	52000
7-8	74000
8-9	100000
9-10	105000
10-11	96000
11-12	75300
12-13	66700
13-14	65900
2nd trimester	31500
3rd trimester	22800

Nuchal translucency (NT) or nuchal oedema is a subcutaneous accumulation of fluid between the skin and the soft tissues overlying the cervical spine and appears as an echo free space on ultrasonic examination, this is called the NT test and is done between 11 and 14 weeks of pregnancy with the aim of determining if the pregnant woman is at high risk of having a baby with a chromosomal abnormality. NT has a diverse aetiology, including trisomies, cardiovascular and pulmonary defects, skeletal dysplasias, congenital infection, and hematological and metabolic disorders [23].

In 1990 Cullen reported the significant association of an abnormal collection of fluid behind the fetal neck (NT) in the first trimester of pregnancy and chromosomal abnormalities, particularly the trisomies 21, 18, and 13 [24]. Over the next four years, eight additional publications reported from 13-68 cases of abnormal NT wherein 53% (172 of 327) of such fetuses with increased NT had an abnormal karyotype. The definition of

abnormal NT thickness ranged from 2 to 6 mm in these studies and the incidence of chromosomal abnormalities ranged from 28 to 79%. An NT measurement of up to 2.0 mm is considered normal at about 11 weeks of pregnancy and up to about 2.8 mm by 13 weeks and 6 days. This is because the NT normally grows in proportion to the growth of the fetus [25].

Kypros Nicolaides of King's College Hospital in London, United Kingdom focused on the nuchal oedema specifically. He found this test to be highly sensitive (80%), and favorably specific (only 4.5% of chromosomally normal fetuses had NT thickness of 3 mm or greater [26].

Researches have found that 18% of fetuses with NT above 3mm had Down's syndrome. The risk rose with the thickening of the nuchal fold – a measurement of 6mm meant 36 times the base risk. So the overall risk for Down's syndrome at 35 years of maternal age is around 1 in 270. If the fetus has a nuchal fold of 6mm, then its risk becomes 1 in 7.5. [27].

Charts were constructed to drive the probabilities of a fetus being affected by a chromosomal abnormality on the basis of maternal age combined with nuchal translucency measurement. Using this method, 80% sensitivity for the detection of fetal chromosomal abnormality was predicted with a false positive rate of 5% [28]. Bewley et al. in their study reported that only two of five affected fetuses with Down's syndrome had increased nuchal translucency [29]. A more recent study showed a detection rate of 77% [30].

The aim of this study was to evaluate the role of combined maternal serum beta HCG and ultrasonographically measured fetal nuchal translucency early in pregnancy in the detection of Down's fetuses.

Methods

This was a prospective study conducted in the department of Obstetrics and Gynaecology / Al-Yarmouk Teaching Hospital for the period June 2002- October 2003.

One hundred pregnant women (10-14 weeks of gestation) were included, fifty of them were a high risk group (over 35 years of age and/or had a family history of Down's syndrome), the other fifty were considered to be a low risk group (age under 35 years with no family history of Down's syndrome).

All women had a venous blood sample taken from them and an abdominal ultrasound done for them.

The venous blood taken was centrifuged and serum taken for Immunoradiometric assay of human chorionic gonadotrophin [HCG], which is a "sandwich" type assay, the concentration of total beta-HCG in the samples is directly proportional to the radioactivity.

The followings were determined by the abdominal ultrasound:

-Gestational age

-NT for each fetus was measured more than once and the maximum measurement was recorded (3mm was taken as the cut-off for increased NT).

Statistical methods

Data were presented in simple measures of frequency, percentage, mean, range, and standard deviation (SD) using computer facility (SPSS 11.5) [Statistical package for social science-version 11.5].

Significancy test by chi square test was used for comparing different proportions. The $p< 0.05$ was used as the level of significance.

Results

The pregnancy outcome in both groups was: 4 newborns with Down's syndrome (4%) and 6 newborns with other congenital abnormalities (6%): 3 of them with trisomy 13 and the rest with multiple congenital abnormalities as shown in table (2).

As shown in table (3) Down's syndrome was shown to be increased in the high risk group; 3 out of 4 newborns with Down's syndrome were in the high risk group, but this increase did not reach to the level of significance ($p>0.05$).

Table (2): The outcome of the pregnant women under study

Outcome	No.	%
Congenital anomaly	6	6.0%
Down's syndrome	4	4.0%
Normal	90	90%
Total	100	100%

Table (3): The risk group distribution of sample under study by outcome.

Risk group	Total	Congenital anomaly		Down's syndrome		Normal	
		No.	%	No.	%	No.	%
High risk group	50	4	8.0	3	6.0	43	86.0
Low risk group	50	2	4.0	1	2.0	47	94.0
Total	100	6	6.0	4	4.0	90	90.0

As shown in table (4), nuchal translucency measurement showed significant increase ($p<0.0001$) in the outcome with Down's syndrome. Nuchal translucency of 4.0-4.99 was seen in seven pregnancies, four of their outcomes were with Down's syndrome, two with other trisomies, and one was normal. This increase in NT measurement showed a detection rate for abnormal fetuses of 80% with a false positive rate of 5%.

As shown in table (5) the beta HCG level was increased significantly in patients with a Down's syndrome outcome ($p<0.001$). 13 cases showed increased beta HCG, 4 of them (30.8%) ended with Down's babies. Depending on the increased level of maternal serum beta HCG, a detection rate for abnormal fetuses of 70% and a false positive rate of 6% were found in this study.

Table (4): The nuchal translucency distribution of the sample under study by the outcome.

Nuchal translucency	Total	Congenital anomaly		Down's syndrome		Normal	
		No.	%	No.	%	No.	%
0.0-0.99	84	2	2.4	-	-	82	97.6
1.0-1.99	2	-	-	-	-	2	100
2.0-2.99	1	-	-	-	-	1	100
3.0-3.99	6	2	33.3	-	-	4	66.7
4.0-4.99	7	2	28.6	4	57.1	1	14.3
Total	100	6	6.0	4	4.0	90	90.0
Mean's (Range)		2.9±1.97 (0.4-4.8)		4.4±0.27 (4-4.6)		0.5±0.84 (0.2-4.2)	

Table (5): The percentage of beta HCG increase according to the corresponding gestational age distributed by the outcome.

Beta HCG	Total	Congenital anomaly		Down's syndrome		Normal	
		No.	%	No.	%	No.	%
Increased	13	3	23.1	4	30.8	6	46.2
Normal	87	3	3.4	-	-	84	96.6
Total	100	6	6.0	4	4.0	90	90.0

Table (6) shows that no relation was found between pregnancy with a positive family history for other congenital anomalies and outcome with Down's syndrome, but a significant relation ($p<0.0001$) was found between pregnancy with a positive family history for Down's syndrome and pregnancy outcome with Down's syndrome

As shown in table (7), no significant relation was found between maternal age and pregnancy outcome in both groups ($p>0.05$).

Combined maternal serum beta HCG and fetal NT showed a detection rate for abnormal fetuses of 82% with a false positive result of 5%.

During this data collection, retrospective analysis of records of the Neonatal Care Unit – Al-Yarmouk Teaching Hospital revealed that during the period July 2001- July 2002 there were 6000 births in different age groups, 24 of them were with Down's syndrome, that means 4/1000 of births were Down's and this finding is higher than most of the recent studies done elsewhere in the world.

Table (6): The family history of the sample under study by outcome.

Family history	Total	Congenital anomaly		Down's syndrome		Normal	
		No.	%	No.	%	No.	%
Congenital anomaly	1	-	-	-	-	1	100
Down's syndrome	6	2	33.3	2	33.3	2	33.3
Normal	93	4	4.3	2	2.2	87	93.5
Total	100	6	6.0	4	4.0	90	90.0

Table (7): The age distribution of the sample under study by outcome.

Age group (years)	Total	Congenital anomaly		Down's syndrome		Normal	
		No.	%	No.	%	No.	%
15-24	24	2	8.3	-	-	22	91.7
25-34	30	2	6.7	2	6.7	26	86.7
35-44	46	2	4.3	2	4.3	42	91.3
Total	100	6	6.0	4	4.0	90	90.0
***Mean ±SD (Range)**		29.17 ±8.21 (18-39)		34.5 ±8.58 (25-44)		31.17 ±7.45 (16-44)	

*p>0.05

Discussion:

There have been significant advances in prenatal screening and detection of Down's syndrome in the last decades.

In our study Down's syndrome was detected more in the high risk group, 3 out of 4 fetuses with this chromosomal abnormality were in the high risk group. 6 mothers had a family history of Down's syndrome, and two of them (33.3%) gave birth to an affected newborn. The explanation for recurrence of an affected child is not known. In some families it may represent a random risk, in others unknown genetic or environmental factors may be operating to cause repeated non-disjunction [31].

South Australian prenatal data from the year 1982 to 1996 showed that maternal serum screening for Down's syndrome increased from 17% when introduced in 1991 to 76% of women who gave birth in 1996. Between 1982 and 1996 termination of pregnancy with fetal Down's syndrome increased from 7.1% to 75% and the birth prevalence of Down's syndrome fell by 60%, from 1.05 to 0.042 per 1000 against the background of an increase in the total prevalence due to increase maternal age [32].

The difference in the birth prevalence of Down's syndrome in the above study (0.042 per 1000) from what was found in our study (4 per 1000) is probably due to perfect antenatal screening and diagnosis of Down's syndrome and early pregnancy termination in the area of that study.

Different studies by Drugan et al [33], Chan et.al[34], and Haddaw et al[35] showed significant increase in Down's syndrome with advanced maternal age. Until biochemical and ultrasonic

screening became available the most frequent method for selecting women at increased risk for fetal Down's syndrome was advanced maternal age, and the risk continues to increase with age for women of 35 years and older. Wilson (1996) reported that trisomy 21 infants occur less frequently among live births to mothers at age 20 (1/1600) than at age 35 (1/370), but more infants are born to younger mothers and therefore, most (75-80%) Down syndrome children are born to young women [36].

In our study there was no significant relation between maternal age and pregnancy outcome with Down's syndrome, this was mostly due to the small sample size enrolled here.

Investigators have recently focused their attention on first trimester nuchal translucency for fetal screening [37]. In the most recent studies they have found a false positive rate between 4% and 5% and reported sensitivity ranging between 63% and 78% which was even higher (80%) in our study[38,39].

In our study nuchal translucency was increased significantly in fetuses delivered with Down's syndrome. Measurements of 4- 4.99 mm where found in 7 fetuses, 4 of them (57.1%) were with Down's syndrome. No cases of Down's syndrome were found with a nuchal translucency below 4mm.

The percentage of Down's syndrome with nuchal translucency > 6 mm after 16 weeks of gestation varied from 69% (Benacerraf 1991) to as low as 3.8% (Grandjean 1995) in different reports[40,41].

The percentage of Down's syndrome with nuchal translucency equal or greater than 3 mm before 14 weeks of gestation ranges between 1.5% (Rodeck 1995) to 18% (Nicolaides 1994) and 45% (Salvesen 1995)[28] .In this study the percentage was 57.1%.

Nicolaides 1999[42] arrived at the following risk estimates basing on his findings in 1015 fetuses with nuchal translucency greater than 3 mm at 10-13 weeks of gestation:

3 mm-------3 times
4 mm-------18 times
5 mm-------28 times
6 mm-------36 times

The ultrasonic marker (nuchal translucency) is now available to alter the incidence of chromosomal abnormalities among livebirths [41, 42].

In this study beta HCG was found to be significantly increased in pregnancy with Down's syndrome corresponding with gestational age, 13 cases showed increased beta HCG, 4 of them (30.8%) were with Down's syndrome . In 1987 Bogart and Co workers showed that maternal serum levels of HCG were surprisingly on average higher in pregnancies affected with fetal Down's syndrome than normal unaffected pregnancies [43].

Rafaul et al (1996), Found that the mean level of beta HCG in mothers with Down's fetuses was significantly elevated compared with normal control [44].

Several attempts have been made to assess the combined performance of ultrasound nuchal translucency and serum markers measurement in first trimester. Spencer et al (1999) in a retrospective study found that beta HCG in stored sera from 210 Down's syndrome pregnancies were increased and when those data were combined with nuchal translucency, a detection rate of 89% with a 5% false positive result was found [45]. Similar results have been reported in other retrospective studies [46]. The beneficial effect of combined measurement of fetal nuchal translucency and maternal serum beta HCG in early pregnancy to detect pregnancies with Down's syndrome was also shown in our study with a detection rate for abnormal fetuses of 82% and a false positive result of 5%.

Conclusion:

From this study it was shown that first trimester and early second trimester sonography using the transient soft tissue marker (fetal nuchal translucency) combined with beta HCG in maternal blood could help early detection of Down's syndrome fetuses and those women found to have a high risk from these tests can be counseled about performing some invasive tests like amniocentesis or chorion villus sampling that will give a definitive answer to determine whether the fetus is a Down or not.

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