Proceedings S.Z.P.G.M.I vol: 14(2) 2000, pp. 53-56.

Original Articles

Antenatal Screening for Down's Syndrome in the Second Trimester

Nasim R. Khan, Muhammad Saeed and Howard S. Cuckle
National Health Research Complex, Gynae/Obs Department, Shaikh Zayed Hospital Lahore and
Department of Reproductive Epidemiology, University of Leeds, Leeds, UK

SUMMARY

Antenatal screening for Down's syndrome was undertaken at the Shaikh Zayed Hospital Lahore. Initially a total of 556 pregnant women in the second trimester were screened, using maternal serum Alpha feto-protein (AFP), Free ß human chorionic gonadotropin (FßhCG) and pelvic ultrasound. There were two positive cases in a sample population of 556 which is higher than 1.3 per 1000 (or 1 in 770) live births reported from other countries. There was a positive case in a 23 years old woman, contrary to the historic perspective that higher the age greater the possibility of a Down's baby. It is, therefore, suggested that younger pregnant women (≥ 20 years) should be screened for the diagnosis of Down's syndrome.

INTRODUCTION

Down's Syndrome (DS) is a common chromosomal aneuploidy and is the commonest cause of severe mental retardation. The infants with DS have three copies of chromosome 21, thus called Trisomy 21. The natural incidence is 1.3 per 1000 liver births (1 in 770)[1], although some 40-50% of Trisomy 21 conceptions miscarry spontaneously[2].

Mental retardation is not the only consequence of this condition. About 55% of live born infants with DS have structural congenital abnormalities. The most common are of heart, intestinal tract (notably duodenal atresia), urinary tract malformations and congenital cataract. Individuals with DS may also develop acute megakaryoblastic leukemia in the first few years of life that is almost never seen in normals[3-7].

For historical reasons, women aged 35 years and over have been labeled `high' risk and those under 35 years as `low' risk for having a DS infant. Under the age of 25, the birth prevalence of DS is about 1 in 1500, increasing to about 1 in 1000 of age 30, and 1 in 100 at age 40.[8-9] Penrose in 1933 reported the relationship between maternal age and the risk of having a pregnancy with DS.[10] In 1959 the presence of an extra chromosome 21 was shown to be diagnostic feature[11]. The chromosome analysis of human amniotic fluid was reported in 1966.[12] Two years later the first antenatal diagnosis of DS was made.[13]

During the past few years a number of workers have shown that the measurement of biochemical markers in maternal serum may be of value in identifying pregnancies affected by Trisomy 21.[14-18] Six biochemical markers have been found to be useful in DS screening between 14 and 22 weeks of pregnancy, namely alpha-fetoprotein (AFP), unconjugated esteriol (UE3), total human chorionic gonadotropin (hCG), free beta hCG (FßhCG), free alpha hCG (FαhCG) and inhibin-A.[19] AFP and FßhCG are the most important and widely used markers, complemented with ultrasound, and are sufficient for screening the pregnant population.

MATERIALS AND METHODS

All the pregnant women of 13-21 weeks gestation, registered in the Gynae & Obs Outpatient Department of Shaikh Zayed Hospital, Lahore were included in the study. A detailed proforma was filled and three cc venous blood was drawn. Serum was separated and stored at -20°C. Samples were

Table 1: Distribution of maternal age against maternal weight.

Maternal age (yrs)	Maternal weight (kg)							Total
	<50	50-54	55-59	60-64	65-69	70-74	75+	
< 25	42	36	31	25	17	19	8	178
25 - 27	19	25	28	20	20	16	17	145
28 - 30	10	20	20	21	20	9	17	117
31 - 33	2	2	7	18	11	8	10	68
34 - 36	3	1	6	8	11	6	7	42
37 +	1	1	2	2	2	4	4	16
Total	**77**	**85**	**94**	**94**	**81**	**62**	**63**	**556**

Table 2: Distribution of gestational age against maternal age.

Gestational age (wks)	Maternal age (yrs)						Total
	<25	25-27	28-30	31-33	34-36	37+	
13	-	2	1	-	-	-	3
14	35	24	22	11	9	4	105
15	31	31	18	5	8	2	95
16	38	34	24	16	12	2	126
17	47	35	25	15	9	4	135
18	24	16	19	6	2	3	70
19+	3	3	8	5	2	1	22
Total	178	145	117	58	42	16	556

*Eg. 13=13 weeks and 0 days to 13 weeks and 6 days

prospectively tested, within 3-7 days of collection for AFP and FßhCG.

AFP and FßhCG enzyme-immunoassay (EIA) kits were supplied by CIS (UK) Limited. The assays were performed according to manufacturers instructions provided with the kits. The principle was based on the solid phase "sandwich" technique between the two monoclonal antibodies. The molecules (AFP or FßhCG) present in the standards or samples to be assayed were sandwiched between the two antibodies. Excess of conjugate was easily removed during washing step, and only the coated ab/ag/conj antibody complex remained on the coated tube. The enzymatic reaction produced a colour whose intensity was proportional to the amount of AFP/FßhCG present in the assay. Controls were also used in the assays.

RESULTS

A total of 556 women were screened at 13-21 weeks gestation, 531 (96%) of them at 14-18 weeks. The distribution of maternal age is tabulated against maternal weight and presented in Table 1. The average weight was 61.7 kg with an inter-quartile range of 53.0-68.5 kg.

The distribution of maternal and gestational ages at which they were tested is shown in Table 2.

Most were tested at 16-17 weeks, but a large number were tested as early as 14 weeks gestation. The average maternal age was 27 years.

The risk of Down's syndrome (or Trisomy 21) in relation to maternal age at delivery has been calculated from unknown population incidence statistics and can be presented as a risk ratio. A ratio of 1:n implies that for n attempts there is 1 chance of a specified event occurring. Risks are calculated either at the Term or a second trimester risk. In our study , the risk of a Down's syndrome birth was computed for each screened woman on the basis of her age and marker levels. The distribution of risks is shown in Table 3. The proportion of women with risks above 1 in 300 was in line with the proportion reported from prospective studies of screening in countries using this cut-off.

The median levels for AFP and FßhCG were calculated for each completed week of gestation and are presented in Table 4.

There were two affected births in women with positive screening results. The details for these pregnancies are shown in Table 5.

Table 3: Distribution of Down's syndrome risks.

Risk	*Number*	*Proportion(%)*
Under 1 in 50000	8	1.4
1 in 50000-1 in 10000	76	13.6
1 in 10000-1 in 1000	345	61.8
1 in 1000-1 in 500	58	10.4
1 in 500-1 in 300	29	5.2
1 in 300-1 in 50	29	5.2
Over 1 in 50	11	2.0

Table 4: Marker medians according to gestational age.

Gestational age (wks)	*AFP*	*Free ßhCG*
13	23.50	19.10
14	26.10	15.50
15	31.00	11.90
16	34.25	10.55
17	36.40	8.60
18	40.50	5.40

Table 5: Details of Down's syndrome cases.

Details	*Case 1*	*Case 2*
Maternal age (yrs)	23.6	45.0
Gestation (wks)	18+4	16+0
Maternal weight (kg)	45	54
AFP MoM	0.21	0.83
Free ßhCG MoM	5.50	0.87
Risk	1 in 9	1 in 89

MoM: multiples of the normal median

DISCUSSION

Biochemical markers have been used for Down's syndrome screening in a number of countries.[18-22] In Pakistan this is the first comprehensive study on DS using AFP and FßhCG biochemical markers and ultrasound for identifying high risk pregnancies in addition to maternal age.

We have estimated the number of Down's syndrome births that should occur in women with the maternal age distribution in Table 1. To do this we multiplied the number of women at each maternal age by the age-specific birth prevalence of Down's syndrome from a published curve.[19] The curve had been derived from a meta-analysis of all single year of age studies in the literature. We have attempted to identify all births with Down's syndrome in the study population. The obstetric unit at Shaikh Zayed Hospital Lahore reported all abnormal outcomes to the study team. This included two cases of Down's syndrome. All women in the study had registered to deliver in this hospital and so we believe that it would extremely unlikely for there to be other unknown cases. Two positive cases out of 556 women screened is quite high a rate as compared to 1.3 case per 1000 live births.

As stated earlier, and supported by the literature the risk of Down's syndrome increases with the enhancing age.[8-9] In the present study, maternal age in one of the positive cases was only 23.6 years (Table 5), suggesting to include women of age 20 years and over in the screening programme. Large number of cases should be screened to identify unexpected cases and to establish reference values for Pakistani population.

Choice is an essential element in screening.

There is a need to educate our population. In other countries about 70 per cent of women take up the offer of screening, and about 70 per cent of those with positive screening results decide to have diagnostic sampling procedures. Over 90 per cent with positive diagnostic results opt for a termination of pregnancy.[20]

The screening for genetic diseases may be carried out as early as possible preferably in the first trimester of gestation so that an early decision is taken regarding termination of pregnancy for the safety of the mother. The development of new techniques, with some added serum markers, can estimate the positivity of the genetic diseases in the first trimester preferably within 10 weeks of gestation. This is the ideal situation for the termination of the pregnancy, if the need be.

ACKNOWLEDGEMENTS

Dr. Shaheen Khan reviewed the paper and offered valuable suggestions, Razzaq Chohan performed the AFP and FßhCG EIA assays. Higher Education Links (HEL) UK provided the funds, through British Council, Lahore, for this study.

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The Authors:

Nasim R. Khan
Principal Research Officer & Head
National Health Research Complex
Shaikh Zayed Hospital, **Lahore.**

Muhammad Saeed
Professor of Gynae/Obs
Shaikh Zayed Hospital, **Lahore.**

Howard S. Cuckle
Professor
Department of Reproductive Epidemiology
University of Leeds
Leeds, UK