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Clinical Research

CYTOGENETIC FINDINGS IN PRENATAL DIAGNOSIS (PND) FROM KING GEORGE HOSPITAL, VISAKHAPATNAM

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ABSTRACT The present study was to diagnose the fetal chromosomal composition has provided medical cytogenetics with one of its major areas of application. A total of 64 amniotic fluid samples were collected from King George Hospital and Padmasri clinic, Visakhapatnam and cells were cultured using international standards. Out of 64 samples, 28 (43.75%) samples showed structural chromosomal abnormalities that is deletions and translocations, ring chromosomes and other abnormalities, whereas 36 (56.25%) samples showed aneuploidy (monosomies, traploidies and polyploides). The discovery of an abnormality allows the option of termination of the pregnancy or, later in gestation, a more suitable obstetric management. The main indications for prenatal cytogenetic diagnosis are the following: (1) the pregnant woman being of advanced childbearing age, (2) parental heterozygosity for a chromosome rearrangement, (3) the birth of a previous child with a chromosome defect, (4) abnormal maternal blood biochemistry, and (5) fetal anomaly detected on Ultrasonography. A useful source for the lay person is Prenatal Testing making choices in Pregnancy. The means to diagnose the fetal karyotype has provided medical cytogenetics with one of its major areas of application.

KEYWORDS : Prenatal Diagnosis, PND, Cytogenetics, tetraploidies, Ultrasonography

I. Introduction

While most babies are born healthy, approximately 3-5% will be affected with certain birth defects or genetic conditions (1). In all pregnancies, women are offered tests that can help tell which pregnancies are at high risk for a type of genetic condition called chromosome abnormality(2).

The chance for a chromosome abnormality to occur in a pregnancy increases with each year of age, and can be estimated for any woman based on how old she will be when the baby is born. This is referred to as the age-related chance (3). For example, a 20-year-old woman has less than a 1/1000 chance of having a baby with Down syndrome; a 35-year-old woman has a 1/350 chance of having a baby with Down syndrome; a 40-year-old woman has a 1/100 chance of having a baby with Down syndrome. So even though older women have a higher chance for these abnormalities, these abnormalities can occur in any pregnancy regardless of the mother's age.

With the rapidly increasing use of prenatal chromosome analysis, it has become apparent that the interpretation of the cytogenetic observations, which is generally assumed to be straightforward, may not always be a simple matter. In particular, the presence of mosaicism in amniotic fluid cultures, involving tetraploidy, aneuploidy, or translocation, has been the cause of some dilemma [10,11] and controversy [4]. In this paper, the occurrence of chromosomal mosaicism in a small series of diagnostic amniotic fluid cell cultures were documented and discussed. Trisomies 13 and 18 (and monosomies X) have high rates of fetal lethality, with the majority of pregnancies aborting. For XXX and XXY, in contrast, there appears to be very little selective loss in the latter part of pregnancy (5).

Generally, people who chose to have prenatal diagnosis (PND) are concerned about some specific chromosomal condition, the most common of which is Down syndrome in the context of older child-bearing age or of an increased-risk abnormal screening test. The major categories of unexpected chromosomal abnormality are (1) an autosomal trisomy other than trisomy 21, (2) a sex chromosome aneuploidy, (3) a structural rearrangement, (4) an extra structurally abnormal chromosome, (5) polyploidy, and (6), for each of the foregoing, mosaicism (1 &2).

Since the early 1970s, prenatal diagnosis (PND) of chromosome disorders has been done by culture of amniotic fluid cells obtained by amniocentesis at about 16 weeks of pregnancy (6). A number of other approaches to PND have since been developed, ranging from preimplantation diagnosis (following in vitro fertilization), through chorion villus sampling (CVS), to fetal blood sampling, and some more experimental procedures (7). Naturally, parents-to-be are anxious to have results as early as possible. A desire for an early result needs to be balanced against a number of considerations which can include complexity of the procedure, both clinically and in the laboratory, procedural trauma and risks, reliability of results, cost, and the prior risk for a fetal abnormality. A useful source for the lay person is Prenatal Testing making choices in Pregnancy (6, 8).

Amniocentesis is, therefore, a procedure that samples cells having origin from the epiblast of the inner cell mass, and these cells rather closely reflect the true constitution of the embryo. Chorionic villus sampling, by contrast, samples more distantly related cells: trophoblast cells (direct and short-term culture), which were the first lineage to differentiate from totipotent cells of the morula, and villus core cells (long-term culture), which reflect the more recently separated lineage of the extraembryonic mesoderm.(6-12).

II. Materials and Methods

Amniotic fluid for prenatal chromosome analysis was obtained by transabdominal amniocentesis between 14 and 20 weeks gestation. (13). Total 64 of Amniotic fluid samples were collected from the women,who attended to the Obg and Gynaec department of King George Hospital and Padmasri clinic, Visakhapatnam with previous child genetic abnormality, history of spontaneous abortions and infections, infertility and older maternal age in the duration, from January 2016 to December 2016.

Five to 15 ml fluid was withdrawn from each patient and set up in culture the same day. After mixing the amniotic fluid thoroughly, 1-ml aliquots were pipetted into a series of 60-mm plastic petri dishes each containing 3 ml McCoy's 5a modified tissue culture medium.supplemented with 10% fetal bovine serum and 5% human cord serum. The dishes were agitated and then incubated at 37" in a 100% humid atmosphere of 5% C02. The cultures were viewed routinely at 8 days and thereafter for colony formation. Culture time ranged from 8 to 18 days with a mean of 10 days. When sufficient growth had occurred, Colcemid (14) was added to each dish to give a final concentration of 0.1 pg/ml and the dishes were incubated for a further 4 hours for the accumulation of metaphase cells. Chromosome spreads were then prepared in situ (6). The culture medium was replaced with the same volume of a warm hypotonic solution composed of 1 part growth medium and 3 parts distilled water and the dishes were reincubated for 30 min at 37^0. Four or five drops of fixative (1 part glacial acetic acid to 3 parts methanol) were then added directly into the hypotonic medium. After 5 min, half of this medium-fixative mixture was poured off and replaced by the same volume of fresh fixative. After a further 5 min the mediumfixative mixture was discarded completely and replaced with fresh fixative. This was left for 5 min, whereupon the fixative was poured off and the cells allowed to

air dry, often with a little blowing to increase the rate of evaporation. The preparations were hydrolyzed with 5 N HC1 for 8 min at room temperature, washed in tap water, stained with 1% aqueous cresyl violet for 15 min, passed through 70%, 95%, and absolute alcohol, and air-dried. After removing the side of the plastic dish, the disc was attached with tape to a glass slide, 51 by 75 mm, and the preparations were viewed under oil immersion microscopy without a mounted coverslip. As far as possible, three metaphases were analyzed for each colony examined.

III. Results

Structural abnormalities	N(28)
Translocations: 46, XX t(10p; 12q),46,XY t(14q; 21q), 46,XX t(9q;10q)	4
ring chromosomes of 1, 2, 5 and 7	4
sex chromosomes rearrangements	12
deletions	6
Micro deletions	2
isochromosome	1
Numerical chromosomal abnormalities	**N (36)**
Monosomies(45,XO),	6
(44,X,-2,-17)	1
45, XX,-10	2
45, XY, -17	1
Triploidies : 47,XX, +21	2
47,XY, +21	4
47,XX,+18	1
Tetraploidies: 92,XXYY	8
92,XXXX	6
Polyploidies	4

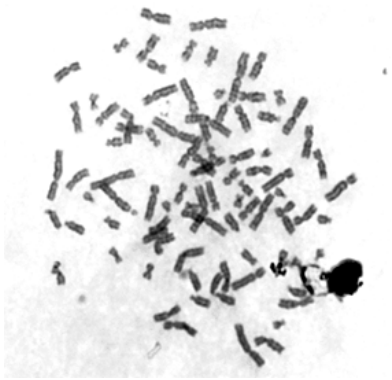

Fig-1 shows the Tetraploidy

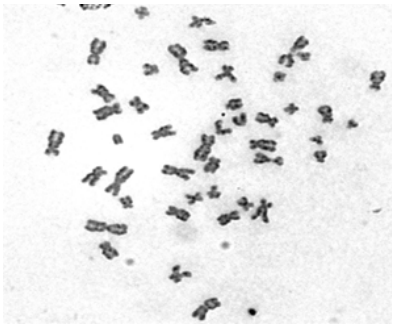

Fig – 2 shows the aneuploidy and centric fusion of acrocentric chromosomes

Fig-3: shows the karyotype with monosomies of chromosome 2 and chromosome 17

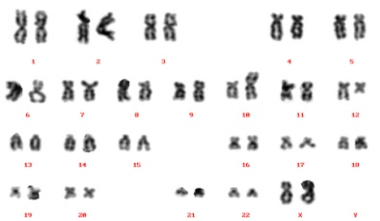

Fig-4: shows the translocation of chromosome 10 and 12(t(10p;12q))

IV. Discussion

Amniocentesis is, therefore, a procedure that samples cells having origin from the epiblast of the inner cell mass, and these cells rather closely reflect the true constitution of the embryo (15).A total of 64 amniotic fluid samples were collected from King George Hospital and Padmasri, Visakhapatnam and cells were cultured using international standards. Out of 64 samples, 28 (43.75%) samples showed structural chromosomal abnormalities that is deletions and translocations, ring chromosomes and other abnormalities, whereas 36 (56.25%) samples showed aneuploidy (monosomies, traploidies and polyploides) (12 - 16).

Unsurprisingly, the severity of the condition influences decision making. found that 93% of parents having a prenatal diagnosis with a poor prognosis (autosomal trisomy, unbalanced translocation, 45, X with major Ultrasonography defects) chose pregnancy termination, while only 27% of parents given a questionable prognosis (sex chromosome aneuploidy, 45,X with normal Ultrasonography, de novo apparently balanced translocation or inversion) took this course (17). They make the interesting observation that ultrasound visualization of fetal defects in a society dominated by the television screen can be useful in helping parents better grasp the implications of the diagnosis (18).

V. Conclusion

The means to diagnose the fetal karyotype has provided medical cytogenetics with one of its major areas of application. The discovery of an abnormality allows the option of termination of the pregnancy or, later in gestation, a more suitable obstetric management .(19 & 20).

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