Introduction
1. INTRODUCTION

India is undergoing an epidemiological transition; communicable diseases are on the decline due to better living conditions and healthcare delivery. On the other hand, the relative increase in the prevalence of non-communicable, chronic and genetic diseases threatens to be a public health problem in India. One such group of disorders is congenital malformations (Suresh et al., 2005).

The prevalence of congenital malformation worldwide is about 2-3%. Although the nationwide prevalence estimate is not known in India, a few hospital-based studies indicate that it would be more than what meets the eye (Thangavel, 2006). The incidence of congenital anomalies is higher amongst still born than among live babies (Patel and Adhia, 2005). It is responsible for 15% of perinatal mortality and account for the third commonest cause of death after perinatal asphyxia and prematurity. Congenital malformations have considerable consequences on the mothers and the families. These children have a wide array of problems including complex medical management issues, growth abnormalities, special educational needs, behavioral, psychological problems and cosmetic concerns. Epidemiology of congenital malformations may be due to genetic, environmental or a combination of both these factors.

The improvements in the obstetric management have changed the scene with a better care of premature newborns. Birth defects are being diagnosed in an increasing number of infants in perinatal period due to improved diagnostic technology especially ultra sonography (USG) (Desai and Desai, 2006).
Obstetric sonography is the application of medical USG to obstetrics, in which sonography is used to visualize the embryo or fetus in its mother’s womb. Since its introduction in the late 1950’s USG has become a very useful diagnostic tool in obstetrics. The procedure is often a standard part of prenatal care, as it yields a variety of information regarding the health of the mother and of the fetus, as well as regarding the progress of the pregnancy. Currently used equipments are known as real-time scanners, with which a continuous picture of the moving fetus can be depicted on a monitor screen. Very high frequency sound waves of between 3.5 to 7.0 megahertz are generally used for this purpose.

High-resolution USG can detect a number of structural malformations such as those of central nervous system, gastro intestinal tract, renal, limbs, heart etc. In first/second trimester scan; each chromosomal defect has its own syndromal pattern of detectable abnormalities. Common sonographic abnormalities seen in fetus with chromosomal defects are increased nuchal translucency (NT), cardiac defect, diaphragmatic hernia, agenesis of corpus callosum, ventriculomegaly, absent nasal bone, renal defect, microcephaly, limb defects etc.

NT is the sonographic appearance of subcutaneous accumulation of fluid behind the fetal neck in the first trimester of pregnancy. The optimal gestational age for measurement of fetal NT is 11 to 13+6 wk and the fetal crown–rump length should be 45–84 mm. In normal fetuses, NT thickness increases with fetal crown-rump length. After 14 wk, increased NT usually resolves but in some cases it evolves
into nuchal edema or cystic hygromas. The prevalence of chromosomal defects increases exponentially with increase NT thickness (Nicolaides, 2004).

Congenital heart defect (CHD) is a defect in the structure of the heart and great vessels of a newborn. Most heart defects either obstruct blood flow in the heart or vessels near it or cause blood to flow through the heart in an abnormal pattern. CHD is the most common form of human birth defects accounting for about 30% of the total anomalies, which is found to affect nearly 1% of newborns, and their frequency in spontaneously aborted pregnancies is estimated to be tenfold higher (Behrman et al., 2000). Most of the CHDs are sporadic. The major genetic cause for congenital heart defects includes the following: (a) chromosomal disorders and single gene disorders constituting 8% (b) 2% of environmental teratogens and (c) 90% multifactorial disorders (Payne et al., 1995). Congenital anomalies of the heart and blood vessels arise during the first 10 wk of embryonic development and are present at birth.

According to Merck Manual of Diagnosis different types of CHDs are Atrial septal defect (ASD), Ventricular septal defect (VSD), Tetralogy of fallots (TOF), Patent ductus arteriousus (PDA), Pulmonary stenosis (PS), Aortic stenosis (AS), Coartation of aorta (COA) and Atrioventricular septal defect (AVSD) which accounts for 85% of all CHDs. The remaining 15% of rare and complex CHDs are Truncus arteriosus (TA), Tricuspid atresia, Total anomalous pulmonary venous connection (TAPVC), Hypoplsatic left heart syndrome (HLHS), Double outlet
right ventricle (DORV), Single ventricle (SV), Ebstein anomaly (EA) and Dextrocardia (Smitha et al., 2006).

At 10-13+6 wks of pregnancy abnormal ductal flow is associated with chromosomal defects, cardiac abnormalities and adverse pregnancy outcome. Prenatal studies of ultrasonographically detectable fetal cardiac abnormalities, have reported chromosomal defects in about 25% of cases. There is a high association between increased NT and cardiac defects in both chromosomally normal and abnormal fetuses. The risk of chromosomal abnormalities associated with fetal cardiac anomalies is much greater than that associated with advanced maternal age (Nicolaides, 2004).

The recent exponential increase in the understanding of genetics has transformed the understanding of CHDs during the past few decades. The recent outcome of candidate genes responsible for CHDs has provided new approach into the genetic basis of heart malformation. The association of CHDs with chromosomal anomalies varies between 4-12%. Aneuploidy associated with CHDs are Trisomy 21 (Down syndrome), Trisomy 18 (Edwards Syndrome), Trisomy 13 (Patau Syndrome), 45, X (Turner Syndrome), Tetrasomy 22q (Cat eye syndrome) and Tetrasomy 12q (Pallister-Killian syndrome). Chromosome deletion syndromes associated with CHDs are Deletion 22q11.2 syndrome, Williams syndrome, Wolf-hirschhorn syndrome and Alagile syndrome. Genetic syndromes associated with CHDs are Noonan syndrome, Holt-Oram syndrome and Ellis-van Creveld. Metabolic disorders associated with CHDs are Zellweger syndrome and Smith-
Lemli-Opitz syndrome (Burn and Goodship, 2002). At present, very few candidate genes have been recognized which cause CHDs in human beings, partly because of lack of big pedigrees segregating a well defined type of CHDs.

Congenital diaphragmatic hernia (CDH) is a congenital malformation of the diaphragm which allows the abdominal organs to push into the chest thereby impeding proper lung formation. CDH is characterized by variable defect in the diaphragm, pulmonary hypoplasia, and postnatal pulmonary hypertension. Prevalence in newborns ranges from 1 in 2,500 to 4,000, and there is a 30 to 60% mortality rate (Harrison et al., 1994; Langham et al., 1996; Nobuhara et al., 1996). The development of human diaphragm occurs between 4th and 12th wk of gestation.

There are two main types of CDH, the first one involves the posterior and lateral aspects of the diaphragm and hence named as Bochdalek Hernia or Posteriorlateral hernia. The second one is the non-Bochdalek which includes Morgagni hernia, Central hernia and other anterior hernias. CDH can be classified as isolated CDH and syndromic CDH. Additional anomalies frequently found in patients with isolated CDH are pulmonary hypoplasia, malrotation or incomplete fusion of bowel, patent ductus arteriosus, patent foramen ovale, heart hypoplasia, tricuspid or mitral valve regurgitation and undescended testes. The syndromic CDH includes Pallister-Killian syndrome, Wolf-Hirschhorn syndrome, Fryns syndrome, Donnai-Barrow syndrome, Cornelia de Lange syndrome and others (Pober, 2008). Most cases of congenital diaphragmatic hernia are sporadic. Although most cases of congenital diaphragmatic hernia are idiopathic, chromosomal abnormalities have
been implicated in approximately 15% of cases (Klaassens et al., 2005). It is still not yet understood as to what factors or genes cause CDH, but a few potential candidate genes has been reported and studied.

Agenesis of corpus callosum (ACC) is a congenital abnormality in which there is a partial or complete absence of the corpus callosum. The corpus callosum is the largest fiber tract in the central nervous system which consists of over 200 million nerve fibers that connects the two cerebral hemispheres in the brain. The disruptions to the development of the corpus callosum occur during the 6th to 20th wk of pregnancy. There is no single cause and many different factors can interfere with this development, including chromosome errors, inherited genetic factors, prenatal infections or injuries, prenatal toxic exposures, structural blockage by cysts or other brain abnormalities, and metabolic disorders. ACC is one of the most frequent malformations in brain with a reported incidence ranging between 0.5 and 70 in 10,000 births (Myrianthopolous, 1977; Jeret et al., 1986).

The primary function of the corpus callosum is to transfer and integrate motor, sensory, and cognitive performances between the two hemispheres of the brain. The most frequent clinical findings in patients with ACC are mental retardation (60%), visual problems (33%), speech delay (29%), seizures (25%), and feeding problems (20%) (Schilmoeller and Schilmoeller, 2000). ACC is a clinically and genetically heterogeneous condition, which can be observed either as isolated condition or as a manifestation in the context of a congenital syndrome such as Aicardi syndrome, Acrocallosal syndrome, Andermann Syndrome, Marden-Walker...
syndrome and Cerebro-oculo-facio-skeletal syndrome (Blum et al., 1990). ACC can occur as an isolated condition or in combination with other cerebral abnormalities, like hydrocephalus, Arnold-Chiari malformation, Dandy-Walker malformation, Schizencephaly, and Holoprosencephaly (Schell-Apacik et al., 2008).

Prenatal diagnosis is most useful application as it offers prospective parents’ choice of testing for diseases or conditions in a fetus or embryo before it is born. The purposes of prenatal diagnosis: (1) to enable timely medical or surgical treatment of a condition before or after birth, (2) to give the parents the chance to abort a fetus with the diagnosed condition, and (3) to give parents the chance to "prepare" psychologically, socially, financially, and medically for a baby with a health problem or disability, or for the likelihood of a stillbirth. Chromosomal analysis of affected fetus is prudent to decide about the continuation of pregnancy in surgically correctable congenital malformation like duodenal atresia. A variety of tissues can be collected from the fetus for cytogenetic study depending upon gestational age like Chorionic villi (at 11th to 14th wk), Amniotic fluid (at 16th to 20th wk) or Cord blood (after 20th wk).

Recent advances in cytogenetic techniques made a valuable contribution toward the practice of modern medicine. The introduction of the banding techniques into cytogenetics has been regarded as a significant step in the identification of chromosomal anomalies which gave insight to many of the problems of health. It includes culturing of the tissue or blood and analysis of G-banded chromosome for numerical and structural aberration. Chromosome rearrangements are a notable cause of embryonic lethality and birth defects. Identifying the genes that underlie
the pathogenesis of chromosome deletion and duplication syndromes is a challenge because the affected chromosomal segment can contain several detrimental genes. The identification of these genes that are relevant to these disorders often requires the analysis of individuals that carry rare, small deletions (microdeletions), translocations or single gene mutations (Gowde and Patel, 2007).

Fluorescence in situ hybridization (FISH) is a cytogenetic technique that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses DNA probes that are labeled with different colored fluorescent tags to visualize one or more specific regions of the genome. FISH can either be performed as a direct approach to metaphase chromosomes or interphase nuclei. FISH in interphase analyses has become an integral part of prenatal diagnostics in rapid detection of most common chromosomal aneuploidies like 13, 18, 21, X and Y. In medicine, FISH can be used to form a diagnosis, to evaluate prognosis, or to evaluate remission of a disease, such as cancer. FISH can resolve submicroscopic deletions to a lower limit of approximately 3 Mb and has therefore become the method of choice for the diagnosis of microdeletion syndromes which are caused by a chromosomal deletion spanning several genes that is too small to be detected under the microscope using conventional cytogenetic methods.

In India, very few studies have been conducted in the field of prenatal diagnosis and most of the studies done were retrospective. In world literature, extensive studies have been carried out in prenatal diagnosis for chromosomal abnormalities using FISH for aneuploidies but same cannot be said for microdeletion study
Every mother carries a risk of having a baby with multiple congenital abnormalities due to a chromosomal abnormality or gene mutation and this risk increases with her age. In the presence of an abnormality detected by ultrasound study, this risk is further increased. This study aims to detect chromosomal abnormalities in the presence of a structural defect in fetus or neonates. Specific diagnosis will help in counseling and management for subsequent pregnancy. This study also aims to obtain data regarding the pattern of chromosomal abnormalities in fetus with multiple congenital abnormalities in Indian population and thus compare the type of abnormalities found in other studies abroad.
Objectives:

- Karyotyping and FISH study of fetus (prenatal) with ultrasound findings of CHD, CDH and ACC.

- Karyotyping and FISH study of children (postnatal) with CHD, CDH and ACC.

- Genotype-Phenotype correlation for all three abnormalities

- Follow Up