1. **Introduction**

Genetics is playing an increasingly important role in the practice of clinical medicine. This is because genetic disease composes a large proportion of total disease, burden both in the pediatric and adult populations. This proportion will continue to grow as our understanding of the genetic basis of disease grows. Genetic diseases are not curable but testing can offer early prevention and increase surveillance (Kaur, 2010). Prenatal diagnosis gives clear picture of the clinical diagnosis to reduce uncertainty and anxiety of the parents.

**Problem in hand?**

The population of India consists of people from different cultural, religious, and social background. India is a country with many communities where there is high load of genetic variation and disorders. This is because of the following reason,

- Consanguineous marriage between close relatives in certain communities.
- High birth rate.
- Poor diagnostic.
- Illiteracy and blind faith.
- Lack of expertise in genetic counseling.

Rapid advancements in the genetics discoveries have increased the knowledge about the role of genes in health, from conception until death. Due to complexity of genetic disorders, affected babies and children cause multisystem involvement to family and the individual. Additionally the burden of genetic disorder falls on the health service of every country. It is now possible for people to take advantage of genetics testing which can identify at risk families and individual through various carries testing programs. As most of genetic disorders do not have pre OR post treatment, early diagnosis helps in prevention of a serious outcome (Verma, 2001).

Human genetic material can be studies in various tissues like bone marrow, peripheral blood, amniotic fluid, chorionic villi and product of conception. This is constitutional and remains same in all the tissue throughout life, except in neoplastic condition. Genetics has two components one
which deals with heredity and variation and other deals with medical implication and disease with alter genes.

Chromosomal abnormalities occur shortly after the conception. When an egg OR sperm carries abnormal number of chromosomes units with normal egg OR sperm, the zygote form will have abnormal number of chromosomes. In this condition the zygote which form is divides incorrectly, the phenomena called “Non-disjunction”. Most of the faulty zygote aborts before placenta formation. If the zygote survives and develop in the fetus, the chromosomal abnormality is transmitted in all of it cells. The child which will bear with the error of chromosome, numerical or structural, will have the symptom for the same error chromosome (Broke, 1992).

**Need of study!**
Prenatal diagnosis deals with management and correction of a defect when possible. Prenatal diagnosis forms only a small part of day to day life, but the techniques are of critical importance to couple at risk of having a child affected by genetic disorder. Its deal with decision of a selective termination, when not treatable and post birth management, if couple decides to deal with handicapped child.

Counseling for prenatal diagnosis is the role play suggestion and decision for patient. The information about the fetus condition, feeling about the pregnancies, life of fetus if abnormal, complication of abnormal results, impact of parents decision making and long term outcomes of such decision are the important fact which are consider while talking to the patient.

Various invasive and non- invasive techniques are available for such diagnosis. Non- invasive tests are Ultrasound screening and maternal serum screening (MSS). Invasive procedures are Chorionic villi sampling, amniocentesis and cordocentesis (Abramsky, 1994 ). A non invasive procedure gives the risk incidence but not the confirmatory results and consider as a high risk factor. If the chance of developing chromosomal abnormality increases then such factor refers as high risk factors for that pregnancy. Invasive procedure gives the confirmatory result of chromosomal abnormalities.
1.1- **Our hereditary material and genetic information**  
(DNA, Chromosomes, Cell Cycle and Mitosis)

To understand and study the development process and expression of character breeding experiment are to be performed. All breeding experiments are performed with looking at naturally existing genetic differences in a species. Mendel’s experiments are well known. The anatomical structure of DNA carries the chemical information that allows the exact transmission of genetic information from one cell to its daughter cell and from one generation to next. Some alteration in the DNA modifies its gene function and error may take place which is express phenotypically. Let’s talk more about DNA and genetic information.

1.1.1- **DNA**
DNA stands for De-oxy-ribose Nucleic Acid. It is the polymeric nucleic acid. DNA is composed of three types of units,

1. A five carbon sugar
2. A nitrogen containing bases
3. A Phosphate group.

The bases are of two types, purines and pyrimidines. In DNA there are two purines bases and two pyrimidine bases. The purine bases are adenine (A) and guanine (G) while pyrimidine bases are thymine (T) and cytosine (C). The unit of DNA, nucleotide composed of a base (any one), a phosphate group and sugar moiety (Figure 1.1).
Nitrogenous bases (Alberts, 1994) (Figure 1.1)

It polymerizes into long polynucleotide chains by 5’-3’ phosphodiester bonds formed between adjacent deoxyribose units (Figure 1.2 and 1.3). In human genome, these polynucleotide chains are hundreds of millions of nucleotide long. The size of the chains nucleotide is ranging from 50 millions base pairs to 250 millions base pairs.

Nucleotide (Alberts B, 1994) (Figure 1.2)
A portion of a DNA polynucleotide chain (Lewin B, 2000)
(Figure 1.3)
1.1.2 **Chromosome**

The compact DNA forming a chromosome is composed of acidic chromosomal protein called histone, and other heterogeneous proteins, non-histones. This DNA and protein complex is called chromatin. Histones are of five major types, and are termed as H1, H2A, H2B, H3 and H4. These histones help in proper packing of the chromatin. Two copies of these four histones form an octamer around which DNA winds. Each histone is associated with 140 base pairs, making two turns (Alberts, 1994). Each DNA core compels spaces by 20-60 base pairs. Thus the appearance of chromatin is like a beaded string. The compels of DNA and histone is called a nucleosome (Figure 1.4). Out of the five histone mention above, amino acid sequence of H1 varies more between species, while the other four show a conversion of amino acid sequence (Claussen, 1995). The helical structure of the nucleosome is compacted into secondary chromatin structure, called solenoid. Under electron microscope the chromatin structure appears three times thicker than nucleosome fiber. Each turn of solenoid contains six nucleosomes. The solenoids are packed into loops, which are attached to non-histone protein. The light and dark bands seen in prophase and metaphase chromosomes reflect the folding of cluster of loops and also define functioning regions of the genome (Purandarey, 2009).
Apart from the germ line cells, cells that contribute to the formation of body are called somatic cells. Somatic cells contain 46 human chromosomes constitute 23 pairs. Out of 23 pairs, 22 pairs are autosomes, classify in decreasing order from the largest chromosomes 1 to the smallest number 21. The remaining pair is sex chromosomes: XX in female and XY in male (Figure 1.5). The genes which code for specific protein are encoded on the DNA. Each chromosome carries a different subset of genes (Thompson, 1991). In each pair of chromosomes the member of chromosome carries the same matching genetic information that is they have the same gene. However they may have the same form or slightly different form of genes called alleles. One
member of each chromosome is inherited from the father and another is from mother. The members of a pair of chromosomes are microscopically indistinguishable from each other. In female the sex chromosome, the two X chromosomes, are likewise largely indistinguishable. In male however the sex chromosomes differ. One is X which is identical to the Xs of the female, inherited by a male from his mother and transmitted to his daughter; other the y chromosome which id inherited from his father and transmitted to his son(Purandarey, 2009).

Normal Male Karyotype
(Lab Report, SRL Diagnostic)
1.2 **Cell cycle**

Life of human begins as a fertilized ovum is formed from Ova and sperm. A diploid cell from which all the cells of the body, estimate at about 100 trillion in number, are derived by a series of dozens or even hundreds of mitoses. Mitosis is obviously crucial for growth and differentiation, but it takes up only a small part of the life cycle of a cell. There are four phase of the cell cycle and are (Alberts, 1994) (Purandarey, 2001)

1. G1- Phase
2. S - Phase
3. G2 - Phase
4. M – Phase

Immediately after mitosis the cell enters a phase called G1 in which there is no DNA synthesis. Some cells spend a very long time, days or even years in G1 phase. Others pass through this stage in hours. Although the molecular mechanism controlling cell cycle progression are
incompletely understood. The cell cycle is governed by a series of checkpoint that determine the timing of each step in mitosis. In addition the checkpoint monitors and controls the accuracy of DNA synthesis as well as the assembly and attachment of an elaborated network of microtubules that facilitate chromosomes movement. If damage to the genome is detected these mitotic checkpoints halt cell-cycle progression until repairs are made. If the damage is excessive the cell is instructed to die by programmed cell death the process called apoptosis (Thompson, 1991).

Cell Cycle (Figure 1.6)
(http://www.centergrove.k12.in.us/Page/2571)
G1 is followed by the S phase, the stage of DNA synthesis. During this stage each chromosome which in G1 has been a single DNA molecule replicates to become bipartite chromosomes consisting of two sister chromatids (Figure 1.6). Each of which contains an identical copy of the original linear DNA molecule. The two sister chromatid are held together physically by centromere. Centromere is a region of DNA that associated with a number specific protein to form the kinetochore. This complex structure serves to attach each chromosome to the microtubules of the mitotic spindle and to govern chromosome movement or even within a single chromosome. Individual chromosome segments have their own characteristic time of replication during the 6 to 8 hours S-Phase (Salder, 2006).

By the end of S-phase the cell content has double and cell enters a brief next stage called G2 phase. Through the whole cell cycle ribonucleic acids and protein produced and the cell gradually enlarges, eventually doubling its total mass before the next mitosis. G2 is ended by mitosis, which begin when individual chromosomes begin to condense and become visible under microscope. The G1, S and G2 phase together constitute interphase. In typical dividing human cells, the three phases take a total 16 to 24 hours, whereas mitosis lasts for only 1 to 2 hours.

1.3 Mitosis
Mitosis is a continuous process, and is subdivided into 4 stages,

1. Prophase
2. Metaphase
3. Anaphase
4. Telophase

Between cell division, cells are said to be in interphase. The type of tissue, temperature, and nutritional health of cell determine the relative length of each cycle (Figure 1.7).

**Interphase:** In the late interphase, cells prepare to undergo mitosis. The nucleus assumes a reticulate appearance due to the maximally extended, uncoiled chromosomes. There is often a single nucleolus at this stage. A centrosome encompassed by astral rays and containing a medium centriol is seen at the surface of the nuclear envelope (Purandarey, 2009).
**Prophase:** Until prophase begin, it is usually not apparent that a cell is about to divide. Generally the cell enlarges relative to the neighboring cells. During the early prophase divided chromosomes separates and take their positions at opposite poles. The chromosomes now coil into compact structure and appear shorter and thicker. The Nucleoli disperse. In late prophase stage chromosomes become clearly visible and Nucleoli disappear.

**Metaphase:** This is the portion of prophase immediately preceding metaphase. The chromosomes attain their maximum thickness and minimum length. Each chromosome that has split longitudinally for most of its length remains connected at a single point at the centromere. These separated chromosomes are called sister chromatids (Bernard, 1995). The nuclear membrane begins to break down and chromosomes are left in the cytoplasm. A mitotic apparatus begins to assemble, and chromosomes start taking their position at the equatorial plane after attachment of the centromere of each chromosome to spindle fiber. The spindle apparatus seen now, consists of centromeres, their encompassing astral ray, a gelatinous spindle made up of fibers extending between centromere and traction extending from each centromere to the chromosomal centromere. Chromosomes can be seen aligned equatorially in the mitotic apparatus and can be best studied and counted at this time (Salder, 2006).
Mitosis (Figure 1.7)

**Anaphase:** In this stage separation of chromosome begins. Each centromere divides longitudinally, thus converting two chromatids of the chromosomes into two daughter chromosomes. These daughter chromosomes disjoin and gradually move to opposite poles. This occurs due to pulling of the chromosomes by traction, in a process called karyokinesis. The longer chromosomes may still be adhered at their distal ends. Chromosomes are pulled towards the pole and as they move away from the centre and the cell membrane starts invaginating. This process is called cytokinesis.

**Telophase:** This phase begins when sister chromatids reach the poles. The cell membrane invaginates from the area opposite the spindle equator. This process, which begins in late anaphase, ends here. The nuclear membrane is formed around the chromosomes thus separating them from the centriole and the rest of the cytoplasm. Chromosomes become uncoiled and spindle fibers and astral disappear. The centriole divides as the centrosome prepare for the next mitosis (Thompson, 1991).

The sequential and purposeful actions of mitosis focus on the movement of the chromosomes to ensure that they are distributed equally. It is essential that each chromosomes of the parent cell have an identical counterpart in each of the daughter cells.

1.4 **The Central Dogma: DNA → RNA → Protein**

Genetic codes are stored in the DNA in chromosomes within the cell nucleus. Protein synthesis takes place in the cytoplasm; this is the method by which the genetic information stored in DNA is encoded. This proves that the human cells have nucleus and have DNA within surrounded by cell membrane from the cytoplasm. This compartmentalization reflects the fact that the human organism is a eukaryote.
The molecular links between these two related types of information (the DNA code of genes and the amino acid code of proteins) is ribonucleic acid (RNA). The chemical structure of RNA is similar to that of DNA, except that each nucleotide in RNA has a ribose sugar component instead of a deoxyribose in addition uracil replaces thymine as one of the primidines of RNA. In addition RNA is single strand.

The informational relationships among DNA, RNA, and protein are intertwined. DNA directs the synthesis and sequence of RNA, RNA directs the synthesis and sequence of polypeptides and specific protein are involved in the synthesis and metabolism of DNA and RNA. The flow of information is referred to as the “Central Dogma” of molecular biology (Figure 1.8). Genetic information is stored in DNA by means of a code in which the sequence of adjacent bases ultimately determines the sequence of amino acids in the encoded polypeptide. First RNA is synthesized from the DNA the process called transcription. The RNA carrying the coded information in a form called messenger RNA (mRNA) (Salder, 2006). It is then transported from the nucleus to the cytoplasm of the cell. Here the RNA sequence is decoded OR translated to determine the sequence of amino acid in the protein being synthesized. The process of translation occurs on the ribosomes. Ribosomes are cytoplasmic organelle with binding site for all interacting molecules, including the mRNA involved in the protein synthesis (Barnes, 1984).
Ribosomes are themselves made up of many different structural proteins in association with a specialized type of RNA know as ribosomal RNA (rRNA). Translation involves yet third type of RNA transfer RNA (tRNA) which provides the molecular link between the coded base sequence of the mRNA and the amino acid sequence of the protein (Figure 1.9).

Transcription and Translation in protein synthesis
(Figure 1.9)
(http://desertbruchid.net/4_GB1_LearnRes_fa10_f/4_GB1_LearnRes_Web_Ch12.html)

1.5 **Chromosomes and the disorders**
As mention earlier Human cells contain 22 pairs of chromosomes called autosomes and one pairs of sex chromosome. It was three decades the correct chromosome number in man was determined to be forty six. The technical improvement in culture methods banding techniques
and molecular cytogenetic has lead to the identification of various microscopic and sub microscopic rearrangement.

Each chromosome has number of genes located on them. Autosomal chromosomes are responsible for the structure and function of the body whereas sex chromosomes are responsible for the development and function of reproductive system. Small numbers of autosomal genes also contributes to development and function of reproductive organs. The chromosomal pattern is unique in each species, is constitutional and does not alter during lifetime, except when there are malignant changes in the tissue (Margaret, 1991).

Chromosomal abnormalities show a visible alteration of chromosome number or structure, and are produced by either improper segregation of chromosomes during mitosis or meiosis (Figure 1.10) or during crossing over and misrepair of broken chromosomes. Routine chromosomal analysis by Giemsa banding at 450-850 band resolution can pick up majority of the numerical and structural chromosome anomalies.
It has been apparent for more than 40 years that microscopically visible changes in the number or structure of chromosomes could account for a number of clinical conditions. Today chromosomal analysis, now with dramatically improved resolution and precision is an increasingly important diagnostic procedure in numerous areas of clinical medicine. Chromosome disorders form a major category of genetic disease. They account for a large proportion of all reproductive wastage, congenital malformations, and mental retardation and play an important role in the pathogenesis of malignancy. Specific chromosomal abnormalities are responsible for more than 100 identifiable syndromes that are collectively more common than all the Mendelian single gene disorders together. Chromosomal disorders are present in nearly 1 percent of live births, in about 2 percent of pregnancies in women older than 35 years.
who undergo prenatal diagnosis and in fully half of all spontaneous first trimester abortion (Lewin, 2000).

1.6 **Chromosomal disorders and prevention**

Chromosomes are best studied in metaphase. Metaphase chromosomes are seen in spontaneously dividing cells such as progenitor stem cells in bone marrow, lymph node, testis, chronic villi of placenta, leukemic blood, solid tumor ect. Advancement in the technique of cytogenetic made it possible to study the metaphase chromosomes by culture method. In this method the cells are grown in the lab by providing nutritional supplement and by maintain the optimal physiological conditions for cell grow (Gogate, 2006). Chromosomal abnormalities are a major cause of human suffering and of all the genetic disorders, form a large of conditions responsible for pregnancy wastage, congenital malformations and infertility. Evolution of the chromosomal pattern and its interpretation therefore is crucial (Nussbaum, 2001). Almost all the constitutional chromosomal anomalies can be detected by prenatal diagnostic techniques.

It is often though, that cytogenetic studies are dead end studies with no possible treatment. Though this is a fact, cytogenetic studies can be successfully used in management of the affected, recurrence risk estimation and in offering various reproductive options to couples (Verma, 2003).

1.7 **Prenatal Diagnosis**

Genetics and environment factors have an influence on various stages of development in the zygote, the pre-embryo, the fetus and the neonate. With the growth of genetic technology and development of high resolution ultrasound in recent years (Figure 1.11), it has become possible to detect more than 5000 defects of hereditary and non-hereditary origin in the prenatal period (Abramsky, 1994). Prenatal diagnosis focuses on the diagnosis of various birth defects. Prior to development of this technology couples at risk were left with option of a risk of genetic disease or choosing other reproductive option like contraception, sterilization, or adoption. Today these at risk couples can make an informed choice about continuation or termination of pregnancy if a serious abnormality is detected, or think about effective management to improve quality of life.
for their child. Another advantage of prenatal diagnosis is that with normal test result at risk couple is reassured (Gardner, 2004).

Amniocentesis and cells culture (Figure 1.11)  
(http://www.eplantscience.com/index/genetics/human_genetics/use_of_human_genetics_in_medical_science.php)

Various invasive and non invasive techniques are now available for prenatal diagnosis. The current commonly used and reliable methods of prenatal diagnosis are ultrasound, maternal serum screening, amniocentesis and chorionic villi biopsy.

1.7.1 Non-Invasive techniques

These are the screening test which detects about 97% fetuses with trisomy 21 and other chromosomal abnormalities. This test includes combination screening of ultrasound, serum and NT. In ultrasound screening various soft markers present which can points to chromosomal abnormality. NT is nuchal translucency which is the fluid filled space behind the fetal neck. NT is present in all fetuses but increased NT is associated with trisomy 21. NT itself has the
detection rate of 70% with 5% false positive rates. Maternal serum screening is the detection of some chemical which are secreted by fetus. These chemicals are beta hCG, AFP, Free estrodiol, PAPPA and Inhabin –A. The combination of these chemical are use in 1\textsuperscript{st} and 2\textsuperscript{nd} trimester of pregnancies. The variation in these chemical values indicates high risk pregnancies for chromosomal disorders. First trimester screening can detect high rate 70% abnormality then second trimester 60%. The factors affecting these parameters are smoking, diabetes type 1, and weight gain in pregnancy.

1.7.2 \textbf{Invasive techniques}

Invasive test are the confirmatory test which detect the chromosomal abnormality. The tests are amniocentesis at 15-19 weeks of gestation, chorionic villi sampling 10-13 weeks of gestation and cordocentesis in late second trimester. Chromosomal analysis can be performed by gold standard detection, accurate and reliable GTG banding method. The accuracy rate for amniocentesis is 99.4 to 99.8 % and for CVS is 97.5 to 99.6 %. The main drawback of technique is that it is time consuming approximately 10 to 18 days. Advances in molecular technique like PCR and FISH can give results in 2 to 3 days.

1.8 \textbf{Genetic Counseling}

The American college of medical genetics defines genetic counseling as a communication process, which deals with the human problem associated with occurrence or risk of occurrence of a genetics disorder in a family. This process involves an attempt by one appropriates trained persons to help the individual or the family to’(Gardner, 2004)

1. Comprehend the medical facts, including the diagnosis, probable course of the disorder and available management.
2. Appreciate the way heredity contributes to disorders and the risk of recurrence in specified relatives.
3. Understand the option for dealing with the risk of recurrence.
4. Choose the course of action, which seems appropriated to them in view of their risk and their family goals, and act accordance with that decision.

5. Make the possible adjustment to the disorder in affected member and to the risk of recurrence of the disorder.

The counselor should keep all the fact and the available option in front of the patients and let them choose the path.

Genetic Counseling is an important step in the process of genetic consultation. It is the step, which offers various option available to an individual or the family of an individual affected with a genetic disorder. Counseling is also required at every step in a diagnostic procedure and thus may require several specialists under one roof. For example in a prenatal diagnostic procedure one requires a counselor to give an overall idea of the procedure involved till the final results are obtained, and follow-up advice further on the basis of the results obtained. The obstetrician involved in the procedure should counsel before sampling is done. An expert in cytogenetic and molecular or biochemical genetics is consulted to interpret the result and a pediatric surgeon may be required if post natal surgery is planned (Park, 2003).

Any couple with a child or a family member with a genetic problem or a history of congenital defect will be keen to know more about the disorder, the risk of recurrence of the disorder and remedial measures if any. The aim of the genetic counselor is to provide these individual seeking information with an understating of the disease in question and its implications as well as the option available. A good counseling process helps families with their problem allows informed decision-making reduces possible anguish and is a step towards the final adjustment in dealing with the disorder. Accurate diagnosis is of paramount importance for meaningful genetic counseling and counseling needs to include all aspects of the condition (Gogate, 2006).

1.8.1 **Stages in genetic counseling**

- History taking and pedigree construction
- Clinical examination
There are a couple of facts that counselor dealing with chromosomal abnormalities should bear in mind right from the outset. Firstly the great majority of chromosomal disorders have an extremely low risk of recurrence in a family, especially when no abnormality is present in a parent. Secondly the great majority of disorders following the Mendelian inheritance show no chromosomal abnormality. It is well worth having the figures of chromosomal abnormalities occurring in the normal population at hand so as to advise clients of their risk in the current situation (Purandarey, 2009). These sources of information are available from studies of abortion and stillborn and prenatal diagnosis series.

Couple should ideally meet with a genetic counselor before having a diagnostic procedure. A careful genetic family history helps measure the risks for genetics problem in the developing baby. The counselor explains the risk, benefits and limitation of the testing procedures so the parents can choose a course of action based on their own specific family needs and goals. The primary screening tests through which chromosomal abnormalities are suspected are abnormal triple test values and ultrasound indicators. Depending on the gestational age of the fetus a variety of procedures for prenatal diagnosis are offered chrion villus sampling biopsy at 8-10 weeks, early amniocentesis at 11-14weeks, routine amniocentesis at 15-20 weeks and fetal blood sampling from 20weeks onwards (Arulkumaran, 1980).

1.9 **High risk factors for prenatal diagnosis (Amniocentesis)**

The indications for preconception genetics counseling should be determined at the first visit and can be consider in a few clear categories. Doctor advises prenatal karyotyping for many reasons. Patients who benefits from genetic counseling and prenatal diagnosis are mention bellow. Following are the high risk factors which include in the current study and are follows,

- Advanced Maternal age
- Positive Maternal serum screening
- Abnormal Ultrasound
- Increased Nuchal Translucency (NT)
- Bad obstetric history (BOH)
- Previous child with chromosomal abnormalities
- Carries Parents with chromosomal abnormalities

1.9.1 **Advanced Maternal Age**

It is reported and observed by various workers that there is an increasing risk of aneuploidy with advanced maternal age. (Table 1.1)

<table>
<thead>
<tr>
<th>Maternal age (At delivery)</th>
<th>Chance (At delivery)</th>
<th>Maternal age (At delivery)</th>
<th>Chance (At delivery)</th>
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<td>29</td>
<td>1:1018</td>
<td>47</td>
<td>1:15</td>
</tr>
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</table>
It must be noted that the risk associated with advanced maternal age is not restricted to Down’s syndrome only but includes other significant chromosomal anomalies. Screening of women with advanced age also shows aneuploidy for other chromosomes like 13, 18, sex chromosomes or presence of supernumerary chromosome or marker chromosomes. Data from CVS studies shows even higher frequencies of abnormalities (Brambatti, 2005). This occurs due to non disjunction at meiosis. The recurrence risk increases if parents have chromosomal rearrangement or have mosaic cell line.

The risk of trisomy 21 in women above 35 years of age is 3.5 per 1000 amniocentesis whereas for trisomies 13, 18, or supernumerary markers, sex chromosomal anomalies and autosomal mosaicism it is 5.4 per 1000 amniocentesis. This is collectively higher than the risk for Down syndrome (Cuckle, 2003).

Collaborative CVS study of 4481 cases reported 48 cases of trisomy 21 and 51 other chromosomal abnormalities (lethal and non lethal). If an abnormality is detected at amniocentesis needs urgent resolution due to advanced gestational age (Parker, 2003).

1.9.2 **Maternal Serum Screening (MSS)**

In the past, previous Down syndrome pregnancy or advanced maternal ages were the only ways of identifying high risk pregnancies. With this approach a large number of normal pregnancies and very few affected pregnancies were identified as high risk. It is known that most children with Down syndrome are born to young women or a very small proportion of affected births occur in couple with family history (Spencer, 2003).
Now it is possible to screen at risk mothers by simple blood test which measures markers associated with pregnancy. These are the chemicals secreted by the placenta and fetal liver mainly. This when coupled with ultrasound evolution specially measurement of nuchal translucency (NT) has a pick up rate of 94 percent (Table 1.2). The markers can be used as first trimester screening (11-14 weeks) (Nicolaides, 2004). The test is dependent on combination of age, maternal serum alpha feto protein, human chorionic gonadotrophin hormones and unconjugated estriols picks up fetuses at risk for trisomy 21 and 18 (Royston, 1992). First trimester screening is now possible with an additional marker PAPP-A (Pregnancy associated plasma protein -A). Elevated levels of AFP indicate high risk for neural tube defects. Computer software’s are available to pick up high-risk pregnancies from such screening test (Figure 1.12). Screen positive pregnancies are then offered CVS sampling or amniocentesis (Crandall, 1991).

### Following table contains the brief details of these screening tests

<table>
<thead>
<tr>
<th>Screen</th>
<th>When performed (weeks of gestation)</th>
<th>Detection rate</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triple screen</td>
<td>15–20</td>
<td>75%</td>
<td>This test measures the maternal serum alpha feto protein (a fetal liver protein), estriol (a pregnancy hormone), and human chorionic gonadotropin (hCG, a pregnancy hormone).</td>
</tr>
<tr>
<td>Quadruple screen</td>
<td>15–20</td>
<td>79%</td>
<td>This test measures the maternal serum alpha feto protein (a fetal liver protein), estriol (a pregnancy hormone), human chorionic gonadotropin (hCG, a pregnancy hormone), and high inhibin-Alpha (INHA).</td>
</tr>
</tbody>
</table>
This test measures the **alpha feto protein**, produced by the fetus, and free beta hCG, produced by the **placenta**.

Uses ultrasound to measure Nuchal Translucency in addition to the freeBeta hCG and PAPPA (pregnancy-associated plasma protein A). NIH has confirmed that this first trimester test is more accurate than second trimester screening methods.

**Brief details of the screening tests**

(Table 1.2)

(Broke, 1992)
Reporting format for maternal serum screening (Lab report format)

(Figure 1.12)
1.9.3 Abnormal Ultrasound Findings

Many structural abnormalities in the fetus associated with recognizable chromosomal syndrome. Known ultrasound markers and associated anomalies form an important indication for prenatal diagnosis.

All the ultrasound markers of chromosomal disorder and pregnancies with more than one anomaly definitely need prenatal test appropriate for the gestation. Confirmation of fetal chromosome helps in pre-conceptional genetic counseling, recurrence risk estimation and reproductive option. A good four chamber view of heart in fetal echocardiography at 18-20 weeks gestation can pick up major congenital heart disease like VSD (Nyberg, 1990).

The common abnormal ultrasound markers are as follows,

1.9.3.1 Nasal Bone (NB)

This is a potentially powerful Down’s syndrome screening marker. In the combined first trimester results of seven studies there was absent NB in 69% of affected pregnancies and 1.4% unaffected pregnancies (Nicolaides, 2004). Thus absence of NB is associated with a 49 fold LR and presence risk reduces risk 3 fold. NB determination requires considerable skill and may not be suitable for mass screening. Nasal bone measurement rather than absence parse is also a marker, but not until the second trimester (Figure 1.13).

Ultrasound of Fetus with and without Nasal bone

Figure (1.13)
(http://www.fetal.com/NT%20Screening/03%20Nasal%20Bone.html)
1.9.3.2 Cleft lip and cleft palate

One of the most common congenital malformations, cleft lip and cleft palate are results from failure of fusion of the frontal process with the maxillary process at the 35th day of gestation (Figure 1.14). 60-80% affected individuals are males. The causative factor comprises of a heterogeneous group that includes single gene defects, chromosomal disorder and teratogenic exposure (Purandarey, 2009).

Cleft Lip (Figure 1.14)
(http://www.quincymedgroup.com/adam/dochtml/surgery%20and%20procedures/13/100010.htm)

1.9.3.3 Ductus venous Doppler

The use of Doppler to assess the blood flow in the fetal ductus venousus has great potential. A large proportion of Down’s syndrome fetuses have abnormal first trimester blood flow demonstrated by reduced end-diastolic velocities or absent velocities, or increases pulsatility
index for vein (Nyberg, 1988). Both impaired ductus venousus blood flow and increased NT could reflect an underlying cardiac dysfunction, in which case they may be correlated.

1.9.3.4 **Spina Bifida**
Efficiency is greater than for AFP screening. The combined result from eight prospective studied an 83% spina bifida detection rate, including closed lesion, with no reported false-positives. Another approach which is cheaper than anomaly scanning is to visualize the fetal skull and the brain when carrying out an earlier dating scan. In spina bifida there is a tendency for the frontal bones to be scalloped or their may be absence (Figure 1.15) or curvature of the cerebellum (Gogate, 2006).

![Spina Bifida (Open Defect)](http://en.wikipedia.org/wiki/Spina_bifida)

Figure (1.15)

**Spina Bifida** (Warburton, 1991)

(http://en.wikipedia.org/wiki/Spina_bifida)

1.9.3.5 **Long Bone**
The short stature associated with children with Down syndrome is reflected in utero by smaller then average long bones measured by ultrasound. There have been proposals to incorporate into serum screening protocols either humerus length (HL) or femur length (FL) (Miskin, 1974).

1.9.3.6 **Anencephaly**
Absent of skull during embryonic development is called anencephaly. It is the neural tube defect which occurs when the neural tube fails to close. Second trimester AFP testing at 16-19 weeks gestation will detect about nine-tenth of cases at the 2.5 MM cut-off level (Nicolaides, 2003).

1.9.3.7 **Abdominal wall defects**
AFP screening will also detect the majority of open anterior abdominal wall defects although proportionally more cases of gastroschisis are detected than exomphalos (Gogate, 2006). Generally such pregnancies are not terminated but abdominal wall defects are associated with chromosomal anomalies and the next step is to carry out chromosomal karyotype of fetus (Heydanus, 1996).

1.9.3.8 **Cardiac defects**
Several studies have reported increased NT in pregnancies with major defects and no chromosomal abnormalities. This can be regarded as a substantial benefits of screening protocol using NT, although some are concerned that anxiety created by a raised NT cannot be fully allayed for several weeks until the fetus is larger enough to complete echo cardiology studies (Garder, 2004).

1.9.4 **Nuchal Translucency (NT)**
Nuchal translucency (NT scan) is the area just under the skin at the back of the fetal neck, which can be measured at 10-14 weeks of gestation. It is the black area lying between the subcutaneous fascia and skin (Figure 1.16). It increase with gestational age, and great care must be taken to measure it correctly to avoid measurement errors. The optimal time to perform a nuchal translucency scan, with a high end machines with expertise, would be around 13-14 weeks. If this scan at 13-14 weeks is normal, then the risk of chromosomal abnormalities would be decreased by 85 %. Hence even at 40 years of age if the nuchal translucency scan together with
MSS at 14 weeks is both normal the risk of the fetus to be chromosomally abnormal would be less than 0.1% (Nicolaides, 2004).

![Image of ultrasound](http://www.newkidscenter.com/Nuchal-Translucency-Screening.html)

**NT(Nuchal Translucency)** (Figure 1.16)

(http://www.newkidscenter.com/Nuchal-Translucency-Screening.html)

1.9.5 **Bad Obstetric History (BOH)**

BOH is the case history of couple, who have repeated abortions. There is an increase risk of abnormal child in couple with the history of repeated abortion. The usual clinical consequence of chromosomal abnormalities is abortion. Only 0.5 % of live born infants possess chromosomal abnormalities, yet 50 % of clinical recognized abortus and 5% of stillborn infants are abnormal (Anandkumar, 1990).
There are many potential explanations for early losses, but of interest to us are those which are chromosomal abnormal. Indeed it has been know that early embryos are often morphologically abnormal; their nuclei and thus probably their chromosomes are abnormal. The principal cause of genetic factor is probably a lethal genes. Such genes may be present in mutation, translocation, deletions and inversion. They may be sex-linked so that only one sex of fetus is lost (Medda, 1996). The frequency of chromosomal abnormalities before 6 to 7 weeks is difficult to establish as abortive tissue is hard to obtain and study, and also a false picture of embryonic death may be obtain as some pregnancies persist beyond 7 weeks, even though the fetus is non-viable. Finally there may be genetic effects of cousin-cousin marriages which result in the appearance of shred genes and resultant early embryonic mortality.

1.9.6 **Previous affected child**
Couples having a previous child with suspected chromosomal disorder are subjected for karyotyping to estimate the recurrence risk. If a child’s karyotype is established, probability of parents having a chromosomal rearrangement as translocation, inversion or mosaicism is higher (Gogate, 2006). The recurrence risk depends on the type of rearrangement and chromosome involvement. Chromosomal abnormality in the fetus aborted is often seen in the form of triploidy or polyploidy which occurs due to post zygotic error. In prenatal sample observation of such an abnormality can be dangerous to interpret due to culture artifact which occurs due endo-reduplication. Further testing with other fetal tissue is suggested to resolve the problem. If it true polyploidy, the pregnancies do not go up to term.

If the abnormality in the sample is (De-novo) this can rise due to maternal or paternal meiotic error or a post zygotic error (Warburton, 1991).

1.9.7 **Parents with a chromosomal anomaly (Carrier parents)**
Fetal chromosomal anomalies are known to occur due to maternal or paternal non dysfunctional or post zygotic errors. The frequency of such errors occurs due to the different procedure of meiosis in male and females. Male carriers have a very small chance of children having
unbalanced forms. Female carriers of balanced translocation have a higher chance of having a child with an unbalanced chromosomal pattern (Rao, 1996).

In reciprocal translocation the risk for maternal or paternal translocation is same. Individual carry rings a chromosomal rearrangement do not have any phenotypic changes. They have normal chromosomal material, which is only rearranged. When transmitted to progeny the affect depends on the size of loss or gain of the chromosomal material and more are the chances of fetal anomaly.

The consequences are: (Hassold, 1996)

1. A normal chromosome complements leading to normal offspring.
2. Balanced chromosomes complement i.e. a translocation chromosome as in the carrier parent.
3. An unbalanced chromosome complement with a normal chromosome and a missing chromosome. This will result in monosomy for a specified chromosome.
4. Unbalanced chromosomes complement possessing both the translocation chromosome and a normal chromosome. This will result in the fertilized embryo having trisomy for a specified chromosome.

The last two combinations will result in monosomies and trisomies.

1.10 Invasive method to confirm the anomaly suspected in the indications

1.10.1 Amniocentesis

Amniocentesis is performed through the maternal abdominal wall by an 18 to 22 gauge and 9-12cm in length spinal needle. The needle insertion site on the abdominal surface should respect the following criteria (Margaret, 1991),

A: - It should be as close as possible to the midline fundal area (to avoid puncturing large vessels) and on the fetal limb side where an optimal pocket of AF can be visualized (to avoid damage to the major fetal structure).

B: - It should be directed perpendicular to the inner uterine surface and inserted by a determined and well controlled movement (to avoid pushing back amniotic membrane).
C: Placenta should be avoided (because of the potential damage before pulling out the needle).

D: The syringe should be removed before pulling out the needle (to avoid any maternal contamination).

E: All the previous steps should be monitored under continues ultrasound control.

After assurance that the needle is in its proper location, the stylet is moved a 10ml disposable syringe attached and 15-20 ml aspirated (Figure 1.17), AF is then transferred in a labeled sterile flask or test tube at room temperature to be sent to the laboratory within 24-48 hrs (Purandarey, 2000).

**Amniocentesis** (Figure 1.17)

(http://www.eplantscience.com/index/genetics/human_genetics/use_of_human_genetics_in_medical_science.php)
The dispute between pro ultrasound continuous needle guidance and pro amniocentesis immediately after ultrasound visualization does not seem concluded as no randomized after studies are up to now available, there is a general consensus in the major societies of obstetrics and gynecology to recommended a continuous ultrasound monitoring of the needle. Moreover, the apparent safety of amniocentesis by placenta transfixion claimed in some published experience has never been confirmed by randomized studies, and fetal hemorrhage should be regarded as potential complication (Gogate, 2006).

No medications are recommended before or after sampling and thereafter the patient can resume her active life. The patient should be advised on the possibility of amniotic fluid leakage within few hours and intrauterine infection 24-36 hours after sampling. A daily control of the body temperature would detected septic in both cases the patient should urgently be referred to clinical centre.

1.10.2 **Timing of Amniocentesis**

Amniocentesis has traditionally been performed after 15 weeks gestation. The amniotic membrane is adhering to the chorionic sac, the volume of AF allows an easy sampling approach, the specimen of AF withdrawn does not significant reduce the total amount of fluid. The ratio of viable and non viable cells is very favorable. In the mid 1980s, shortly after CVS has become part of the clinical practice, some centre in Europe and USA, which does not offer CVS, viewed early amniocentesis as an alternative (Tan, 2000). These expectations were apparently confirmed by the results reported in many of the polished observational studies. However definite conclusion could not be drawn because of the small number of vases in each series the skewed timing of the procedure to the latter weeks of the early gestational interval, the lack of control cases and selection bias. The results of the previous clinical experience were clearly refuted by the four available randomized studies comparing early amniocentesis to first trimester (TA-CVS), and than first trimester and mid trimester amniocentesis. Fetal loss rate was significantly higher for early amniocentesis than for TA-CVS, and than mid trimester amniocentesis. Moreover early amniocentesis had a significantly higher rate of AF leakage than TA-CVS and mid trimester amniocentesis (Purandarey, 2009).
1.10.3 The cellular contents

The cells of the amniotic fluid are shed from the skin, amnion, genitor urinary and gastro intestinal tracts. At 15-18 weeks gestation about 80% of the cells are viable and 20% are dead. The number of amniotic fluid cell content increases with advanced gestation but the percentage of viable cells is greatly reduced. At 24 weeks only 10-15% cells are viable (Broke, 1992). Amniotic fluid contains three major cell types. They differ in cellular and clonal morphology and in their potential. 1 ml of amniotic fluid has about 3 to 10 colony forming cells. The three classes of amniotic fluid cells are fibroblast like cells, epitheliod cells and amniotic fluid cells or amniocytes. It is amniocytes, which predominate in most culture and are used for chromosomal diagnosis. In addition to three classes of fetal derived cells, maternal and fetal RBC’s and maternal macrophage are present in the fluid. There is another group of cells called ‘Rapidly adhering’ cells. This is seen in fetuses with neural tube defects. These cells are attached for 22-48 hours and fail to divide. The RA cells are mostly of neural origin. At any time amniotic fluid can also have RBC’s (Verma, 1995).