1. Literature Review

2.1 Human genome and history of human Cytogenetic

This is an especially exciting time in the medical and human genetics. Medical genetics has achieved a recognized role as the specialty of medicine that deals with the diagnosis, treatment and management of hereditary disorders (Gogate, 2006). The idea that medical genetics is concerned only with the inheritance of trivial, superficial, and rare characteristics has given way to an understating of the fundamental role of the gene in basic life processes. Medical and human geneticists are at the forefront of investigations into human variability and human heredity while also participating in and benefiting from rapid progress in molecular biology, biochemistry, and cell biology. In particular, the last decade of the 20th century and the beginning of the 21st century have seen the international effort to determine the complete content of the human genome, defined simply as the sum total of the genetic information of our species, encoded within each nucleated cell of the body (Salder, 2006). In partnership with all the other discipline of modern biology, the Human genome project is already revolutionizing human and medical genetics by providing fundamental insight into many diseases and promoting the development of far better diagnostic tools, preventive measures, and therapeutic methods in the near future. Completed, the Human Genome project makes available the complete sequence of all human DNA, knowledge of the complete sequence, in turn, allow the identification of all human genes and, ultimately, make it possible to determine how variation in these genes contributes to health and disease (Thompson, 1991).

The word chromosome was first coined by Waldeyer in 1888. The first observations of chromosomes from plant materials was made by E. Strausburger in 1875 and those from animal tissue by W. Fleming in 1879. Painter first published the human diploid number in 1923 as being 48. This believed to be true for almost three decades since chromosomes were difficult to spread out in the cell and because of difference between primary constrictions (Centromere) and secondary constrictions e.g. the heterochromatic region of 1,9,16 and a stalk region of the acrocentrics were obscure. Even today it may be difficult to distinguish between a translocation
and closely aligned telomeres or stretched heterochromatic area, without some experience (Purandarey, 2000).

Progress in human cytogenetic has occurred due to improvement in the laboratory technique. Tissue culturing made it possible due to obtain dividing cells, which could be used to study the chromosomes. Due to improvement in techniques, the correct chromosome number was discovered to by 46 by Tjio and Levan in 1956 (Tjio and Levan, 1956). This marked the beginning of modern human cytogenetic. The cytogenetic technique went through many stages before today’s refined chromosome spreads could be obtained.

2.2 **Prenatal diagnosis methodologies: A brief history**

In 1966, amniocites from an amniotic fluid sample were cultivated and karyotype, providing for the first time fetal diagnosis of chromosomal abnormalities (Steel and Berg, 1966). In 1968, trisomy 21 prenatal diagnosis on amniocites confirmed on fetal tissue was first published.

The possibility of amniotic fluid sample in a time when ultrasound was not yet available had been tested, experimented, and used in erythroblastosis fetalis diagnosis and therapy, and this blind sampling technique was also the standard approach for prenatal diagnosis on amniotic fluid in the sixties (Gogate, 2006).

In the 1970s the introduction of ultrasound (Table 2.1) visualization in the clinical field allowed a much less invasive approach to fetal tissue, and fetal sampling under ultrasound visualization thus became widely and safely available. The fetal tissues that were and are of maximum interest for fetal diagnosis are chorionic villi, amniotic fluid and fetal blood.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Year</th>
<th>Embryo-fetal tissue</th>
<th>Week of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniocentesis</td>
<td>1967</td>
<td>Amniotic fluid</td>
<td>15+</td>
</tr>
</tbody>
</table>
In more recent times genetic investigation has been successfully performed on blastomeres after in vitro fertilizations as well as on fetal DNA in maternal blood. (Above table).

In the early 1970s, direct endoscopy visualization of the fetus made possible mid-trimester diagnosis of severe fetal malformation like spina bifida, even though the procedure risk for the pregnancy was high (Scrimgeour, 1973). The diagnosis of these malformations has been later replaced by higher resolution ultrasound visualization. On the other end, the use of the optical fibers and the use of thinner instruments reduced the risk and this methodology was thus applied for fetal blood sampling from chorionic plate or umbilical cord (Hobbins, 1974 and Rodeck, 1978), and also for skin and liver biopsy (Rodeck, 1980). Late on the DNA methodology made these uses of fetoscopy obsolete.

Between the late 1970s and early 1980s a future improvement of ultrasound technology made amniocentesis safer and allowed chorionic villus sampling by a thin spinal needle. In December 1982, the first cases of molecular diagnosis of hemoglobinopathies on chorionic tissue were published (Old, 1982) and in March 1983 the first trisomy 21 case was also published (Brambatti, 1983). In the very same year fetal blood sampling by trans abdominal needling under guidance was also reported in cases at risk of toxoplasmosis (Daffos, 1983). Moreover, the expanded use of DNA technology and the progress in human genome investigation made first trimester CVS the principle tool for single gene disease diagnosis.
In the late 1980s, genomic amplification by polymerase chain reaction showed to produce a sufficient DNA amount for testing a single blastomere obtained at the cleavage stage as well as a single first polar body; the first reported cases of preimplantation and preconception diagnosis have reported in 1990 for X-linked and recessive autosomal disease, respectively (Verlinsky, 2004).

However, since prenatal diagnosis has available, the target of obstetrician has been to reduce the risks of invasive procedures; this wish led to investigate fetal cells in maternal blood, previously detected in 1969 (Walknowska, 1969), Monoclonal antibodies and cell sorters which appeared in the 1990s as well as recombinant DNA technology greatly contributed to feel the success approaching. Nevertheless, nucleated fetal red blood cells are still not available for current use in prenatal diagnosis. Meanwhile, free fetal DNA present in the maternal plasma seems to be a cheaper, quicker and more successful way for non-invasive fetal testing (Lo, 1997). The first diagnosis experience of the Rh(D) sequence in Rh(D) negative mothers (Lo, 1998).

Medical intervention in prenatal diagnosis followed a path toward a better accuracy and a broader diagnostic range, a lower invasiveness and an overall better compliance of the mother in front of selective abortion and even to avoid pregnancy interruption. All these effort have been finalized to let the couple choose among different diagnostic opportunities, to lower reproductive anxiety about congenital defects, and to allow an informed and responsible family planning, and in selective cases to be able to cure congenital abnormalities in utero or soon after delivery.

2.3 **Chemicals, Cytogenetic methodology by using different procedures**

Use of agent such as colchicines enable arresting of cells in metaphase and hypotonic treatment helped swell the cells to a larger volume. The swollen cells aided separation of chromosome, thus making them easier to count.
Squashing the preparation between the slide and the cover slip did earlier spreading of chromosomes; this required harsh acetic acid and fixatives and created preoperational artifacts. A new air drying technique developed by Nowell (Nowell, 1960) for spreading blood lymphocyte chromosomes made it possible to examine human chromosomes with much less technical artifacts than ever before. The discovery of the abnormal chromosomes complement of Down’s syndrome (Lejeune, 1959), Turner syndrome (Ford, 1959), Klinefelter’s syndrome (Kilnufelter, 1942) and Philadelphia chromosome in chronic myelogenous leukemia led to further development in this science increasing its clinical application.

Metaphase chromosomes were stained using Geimsa or aceto-orcein. These unbanded chromosomes referred to as solid stained chromosomes could be grouped by size and shape but could not be identified individually (Except chromosomes 1,2,9,16 and Y, If they were normal).

In 1968, Caspersonn (Caspersson et al, 1968) developed a florescence method using quinacrine mustard stain, which allowed identification of a distinct pattern in each human chromosome. Visualization of this requires more sophisticated and expensive fluorescence microscope.

G banding using Trypsin and Geimsa became popular since its introduction in 1971 by Sumener (Sumener et al, 1971) the pattern of light and dark region or bands observable by this staining method enable identification of individual chromosomes and small parts on each chromosome. This allowed the detection and characterization of many more cytogenetics abnormalities than did solid staining.

With the advent of amniocentesis, cytogenetic prenatal diagnosis became possible. In 1976, Yunis (Yunis, 1976) revolutionized the field by demonstrating that elongate chromosomes with nearly twice the number of bands cold be used to detect smaller and subtler chromosomal abnormalities. These methods made the detection of micro deletion such as those seen in prade-Willi, Angelman del, Di-George syndrome and Miller Dieker syndromes as well other smaller rearrangement possible. Chorionic villus biopsy allowed earlier detection than by amniocentesis. Diagnosis and prognosis of disease became much better defined with improvement knowledge of neoplasm cytogenetics as these could be correlated (Purandarey, 2000).
With the development of molecular cytogenetics in 1980s, the nature of cryptic rearrangement was uncovered. These were often visible only at the molecular level. The new technique of fluorescent in situ hybridization (FISH) in which whole chromosome probe, single copy probes and centromere specific probes have aided in answering many different kinds of question, sometimes even in the absence of metaphases. This technique has helped resolve previously baffling problem and identify disorder at the molecular level.

With this advancement cytogenetics technique capability of diagnosing various chromosomal syndromes has increased immensely and has now become an important tool for diagnosis, prognosis and management of various chromosome based disorder.

2.4 **MILESTONES IN HUMAN CYTOGENETICS:**

1888: Waldeyer coined Term chromosome.
1875: First observations of chromosomes from plant material by E. Strausburger.
1879: First observations of chromosomes from animal tissues by W. Fleming.
1923: Painter published human diploid number as being 48.
1956: Tijo and Levan discovered number of chromosomes as being 46.
1959: The discovery of the abnormal chromosome complement of Down's syndrome.
1959: The discovery of the abnormal chromosome complement of Turner's syndrome
1942: Discovery of abnormal chromosome complement of Klinefelter's syndrome.
1968: Caspersson, developed fluorescence method using quinacrine mustard stain.
1976: Yunis discovered possibility of microdeletions in elongated chromosomes.
1980 (Late) Fluorescent in situ hybridization technique was developed.

Genetics is plying an increasingly important role in the practice of clinical medicine. Improvement hygiene, better health care and awareness of good nutritional standard have resulted in an overall decrease in the incidence of infectious disease. Additionally the role of genetic factors in the underlying pathology of disease is being better understood, the importance in medicine has increased (Warburton, 1991).

The life time frequency of genetic disorder is estimated to be 7 per thousand, and this number includes cardiovascular disease, which result from complex interaction of genes and environment and cancers, which result from accumulation f mutation in somatic cells. Genetic disease is responsible for 10 % of adult and 30-40% of pediatric hospital admissions. Congenital malformations when caused by genetic factors constitute a major cause in infant mortality (Wieacker, 2010).

Following table list the burden of genetic disease and their frequency in the general population. These figures necessitate today’s physicians and healthcare professionals to understand the fundamentals and principles of genetic science in order to accurately counsel patient and their families. Patterns of genetic disorders vary in their occurrence, mode of inheritance and recurrence risk estimates (Verma, 2001). In addition, environmental factors also play a role in modifying both the risk factors and severity of the disease. Many birth defects caused by environmental factors and teratogens tend to mimic genetic disease, making it mandatory to tae the role of these factors in human embryonic and adult development into consideration before making a final diagnosis.

<table>
<thead>
<tr>
<th>Burden of genetic disorders</th>
<th>Frequency in population.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte aneupoidy</td>
<td>18%</td>
</tr>
<tr>
<td>Sperm aneuploidy</td>
<td>4%</td>
</tr>
<tr>
<td>1st trimester spontaneous abortion</td>
<td>50%</td>
</tr>
</tbody>
</table>
Perinatal deaths | 30%
---|---
Stillbirth | 5.6%
Chromosomal carriers | 0.2%
Congenital malformation | 3.6%
Neonatal deaths | 11.5%
Monogenic disorder | 0.36%

### Burden of genetic disorders in population (Gogate, 2006)

(Table 2.2)

Various measures reflect the population burden of genetic disorder and congenital anomalies. These include the incidence or prevalence of these disorders, associated morbidity and mortality, life expectancy and the economic burden on the family and society. Oocytes and sperm show aneuploidies in 18-19 percent and 3-5 percent respectively, as a result 1 in 13 conception show chromosomal anomalies. Fifty percent of first trimester abortions are due to chromosomal disorder. Still births and neonatal deaths show chromosomal defects in 5.6-11.5 percent cases. The exact incidence of various categories of genetic disorder is not known, the following table shows the incidence of these disorders from a very large study by Baird et. al. in 1988 (Table 2.3).

<table>
<thead>
<tr>
<th>Category</th>
<th>Rates per million live births</th>
<th>% of total births</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal recessive</td>
<td>1395.4</td>
<td>0.14</td>
</tr>
<tr>
<td>Autosomal dominant</td>
<td>1665.3</td>
<td>0.17</td>
</tr>
<tr>
<td>X- linked</td>
<td>532.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Chromosomal</td>
<td>1845.4</td>
<td>0.18</td>
</tr>
<tr>
<td>Multifactorial</td>
<td>46582.6</td>
<td>4.64</td>
</tr>
<tr>
<td>Genetic Unknown</td>
<td>1164.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Total</td>
<td>53175.3</td>
<td>5.32</td>
</tr>
<tr>
<td>All congenital anomalies</td>
<td>52808.2</td>
<td>5.28</td>
</tr>
</tbody>
</table>

### The frequency of genetic disorders in 1,169,873 births
Developmental abnormalities presenting as unusual single or multiple anatomic alteration are not restricted to any ethnic human population. Every year about 3% of all children are born in any hospital or in any country will have a significant congenital abnormality (Caron, 1999). It may be more than of cosmetic concern and which, if uncorrected, will interfere with normal functioning. Such anomalies occur in only a small fraction of all newborn. However, these collectively attribute to about 30% of all neonatal and infant deaths, and children born with birth defects make up about 30% of all admissions in pediatric hospital (Bui, 2007). Furthermore, these children present with a range of problems requiring medical support from various specialists. These problems usually start from an early life and may require chronic care for decades. The burdens imposed on these children and their families, and society at large, may be enormous. The great majority of birth defects are neither detectable by prenatal diagnosis nor preventable. Thus the impact of these problems hasn’t decreased despite advances in medicine.

2.5 **Burden of genetic disorders in India**

India has a population of more than a billion people, with almost 25 millions annual births (Verma, 2001). Combine this with high rates of consanguinity in many communities, endogamous marriages in various ethnic groups, poor nutritional status (Low folate levels) of the mothers and high incidence of infections, and the stage is set for a high frequency of genetic disorders and birth defects. We review the burden of genetic and genetically related disorders that are relevant for preventive intervention.

Genetic disease occurs in two waves--- one at birth and one later in adult life. In the current research we discuss the disorders that occur in early life.

<table>
<thead>
<tr>
<th>Disorders</th>
<th>Incidence</th>
<th>Number per years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital malformation</td>
<td>1 in 50</td>
<td>595,096</td>
</tr>
<tr>
<td>G-6-PD deficiency</td>
<td>1 in 10-30</td>
<td>390,000</td>
</tr>
<tr>
<td>Down’s syndrome</td>
<td>1: 1139</td>
<td>21,412</td>
</tr>
<tr>
<td>Congenital hypothyroidism</td>
<td>1: 2500</td>
<td>10,400</td>
</tr>
</tbody>
</table>
Presented in the table below is the frequency of genetic disorders observed at birth in India.

<table>
<thead>
<tr>
<th>Disorders</th>
<th>Frequency</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Thalassemia</td>
<td>1:2700</td>
<td>9,000</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>---</td>
<td>5,200</td>
</tr>
<tr>
<td>Amino acid disorders</td>
<td>1:2347</td>
<td>9,760</td>
</tr>
<tr>
<td>Other metabolic disorders</td>
<td>1:2500</td>
<td>9,000</td>
</tr>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>1:5000</td>
<td>2,250</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>1:10,000</td>
<td>2,250</td>
</tr>
</tbody>
</table>

**Summarizes the frequency of genetic disorders observed at birth in India.**

(Table 2.4) (Verma, 2002)

2.6 **Newborn surveys for chromosomal diseases**

In the multicenter (Verma, 1998) study 8333 infants with Down syndrome were born among 94,610 births, giving a frequency of 0.87 per 100, or 1 per 1150. In this study every newborn was not tested cytogenetically, but in all clinically suspected cases the diagnosis was confirmed by cytogenetically analysis. A Meta-analysis of there published studies on newborn (Kaur, 2010) showed there were 82 cases of Down syndrome among 75,103 births (one per 916 births). The incidence seems to be similar to the western figure, if one keeps in mind that all the Indian studies are based on clinical examination with chromosomal studies only in selected infants, while the western data is therefore, an underestimate. The increasing incidence of Down syndrome with advanced maternal age at conception is evident also in the Indian data, and thus is similar to that observed in the west. In recent years there has been an increase in the number of pregnancies among older women, due to women increasingly going into higher education and employment, with delay in having children due to their careers. This has been matched to some extent with increasing awareness of Down syndrome among women and the obstetricians so that triple tests and ultrasongraphy are increasingly being utilized to screen pregnancies for Down syndrome.
The maternal age related risk for having a baby with Dow’s syndrome and other chromosomal abnormalities is well known (Table 2.5) and should be discussed with the woman/couple contemplating pregnancy. In addition there is an increased risk for developing pregnancy induced hypertension, gestational diabetes, placental abruption, miscarriages and even stroke. Furthermore, women should be aware of the increase in incidence of dizygotic twins.

<table>
<thead>
<tr>
<th>Maternal age at delivery</th>
<th>Risk for having a live born with Down’s syndrome</th>
<th>Risk for having a live born with any chromosomal abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1/1351</td>
<td>1/480</td>
</tr>
<tr>
<td>30</td>
<td>1/909</td>
<td>1/420</td>
</tr>
<tr>
<td>35</td>
<td>1/384</td>
<td>1/190</td>
</tr>
<tr>
<td>36</td>
<td>1/307</td>
<td>1/160</td>
</tr>
<tr>
<td>37</td>
<td>1/242</td>
<td>1/130</td>
</tr>
<tr>
<td>38</td>
<td>1/189</td>
<td>1/110</td>
</tr>
<tr>
<td>39</td>
<td>1/146</td>
<td>1/88</td>
</tr>
<tr>
<td>40</td>
<td>1/112</td>
<td>1/70</td>
</tr>
<tr>
<td>41</td>
<td>1/85</td>
<td>1/55</td>
</tr>
<tr>
<td>42</td>
<td>1/65</td>
<td>1/43</td>
</tr>
<tr>
<td>43</td>
<td>1/49</td>
<td>1/34</td>
</tr>
<tr>
<td>44</td>
<td>1/37</td>
<td>1/27</td>
</tr>
<tr>
<td>45</td>
<td>1/28</td>
<td>1/20</td>
</tr>
</tbody>
</table>

Maternal age related risk for having a baby with Down’s syndrome and other chromosomal abnormalities. (Table 2.5)(Kaur, 2010)
2.7 Genetic counseling and chromosomal abnormalities in prenatal diagnosis with high risk factors

There are a couple of facts that counselors 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 Mendelian inheritance show no chromosomal abnormality. It is well worth having the figures of chromosomal abnormality occurring in the normal population at hand, so as to advise client of their risk in the current situation (Gersen et al, 2005, Gardner et al, 1996, Kuller at al, 1996). These sources of information are available from studies of newborn population, studies of particular chromosomal disorder, studies of abortion and stillborn and prenatal diagnosis series.

Doctors advise prenatal karyotyping for many reasons. Patients who benefits from genetic counseling and diagnosis include,

- Pregnant women who will be 35 years of age or older on their due date.
- Families with a child or close relative with a chromosome problem.
- Families with a history of mental retardation, open defects of the spine and other birth defects or inherited disease.
- Couple with a history of infertility or two or ore miscarriages.
- Pregnant women who have been exposed to medication, radiation, or other agents that may be harmful the developing fetus.

Couple should meet ideally meet with a genetic counselor before having a diagnostic procedure. A careful genetic family history helps measure the risks for genetic problem in the developing baby. The counselor explains he risk, benefits and limitations of the testing procedures so the parents can choose a course of action based on their own specific family needs and goals (Verma, 2003). 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, chorionic villus sampling biopsy done at 8-10 weeks, early amniocentesis at 11-14 weeks, routine amniocentesis
at 15-20 weeks, and fetal blood sampling from 20 weeks onwards. Risks with amniocentesis are uncommon, but include fetal loss and maternal sensitization. The increase risk for fetal mortality following amniocentesis is about 0.5 percent above what would normally be expected. CVS carries a slightly higher risk of about 1 percent, which is similar to the early amniocentesis and fetal cord blood (Seed, 2004).

Frequencies of chromosomal abnormalities in the population (Table 2.6 and 2.7)

A. Chromosome abnormalities in unselected newborns

<table>
<thead>
<tr>
<th>Abnormalities</th>
<th>Frequency (per 1000 births)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All abnormalities</td>
<td>9.2</td>
</tr>
<tr>
<td>Autosomal trisomies</td>
<td>1.4</td>
</tr>
<tr>
<td>Balanced autosomal rearrangements</td>
<td>5.2</td>
</tr>
<tr>
<td>Unbalanced autosomal abnormalities</td>
<td>0.6</td>
</tr>
<tr>
<td>Sex chromosomal abnormalities in phenotypic male</td>
<td>1.2</td>
</tr>
<tr>
<td>Sex chromosomal abnormalities in phenotypic female</td>
<td>0.75</td>
</tr>
</tbody>
</table>

(Table 2.6)(Jacob et al, 1992)

B. Population frequency of specific chromosomal abnormalities

<table>
<thead>
<tr>
<th>Abnormalities</th>
<th>Per 1000 births</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>1.5</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>0.12</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>0.07</td>
</tr>
<tr>
<td>XXY (Klinefelter syndrome)</td>
<td>1.5</td>
</tr>
<tr>
<td>45,XO (Turner syndrome)</td>
<td>0.4</td>
</tr>
<tr>
<td>XYY syndrome</td>
<td>1.5</td>
</tr>
</tbody>
</table>
2.7.1 **Autosomal trisomies**

When an abnormality is actually discovered, it is necessary to discuss in detail with the couple the implication of this particular abnormality and to help them decide on a suitable course of action. The counselor has to deal with question regarding the magnitude of risk, survival chance of the clinical consequences of these abnormalities usually serves as a basis for these decisions. The counselor is obliged to be clear and accurate about the particular abnormality and to take care that a patient autonomy is not compromised in the decision making process. The difficult decision and for or continuation of pregnancy is the immediate one to be made. Two invaluable resources for viability of segment for specific deletion and duplication and viability of segment are Schinzel’s catalogue of unbalanced chromosome aberrations (2001) and Stene- Stengel, Rutkowski data (1988) from 1120 translation pedigree, determining viability numerous chromosomal segments in the partially monosomic or trisomic states.

The major categories of chromosomal abnormalities which may occur are,

1. An autosomal trisomy
2. A sex chromosomal aneuploidy
3. A structural rearrangements
4. An extra structurally abnormal chromosome.
5. Polyploidy
6. For all above, Mosaicism.

Down’s syndrome is among the most common of these disorders, affecting about 1 in 800-1000 live born babies (Hunter, 2005). The risk of this and other trisomies increase with the mother age. The risk of having a live-born baby with Down syndrome is about 1 in 1250 for woman at age 25, 1 in 1000 at 30, 1 in 400 at 35, and 1 in 100 at age 40. Recent studies reporting the comparison between Asian and western a population indicates that the risk in Asian population differ slightly, the risk odds between age 28 and 35 years is slightly greater but this may be due to socio-cultural trends (Sheu, 1998).
Apart from the typical facial appearance, certain health conditions and birth defects are more common in individuals with Down syndrome, including congenital heart defects, gastrointestinal problem, leukemia, Alzheimer disease, immune dysfunction, thyroid dysfunction and problems with hearing and vision. Most have mental retardation in the mild to moderates range. With early intervention and special education, many learn to read and write and participate in adverse childhood activities. A study by Kramer et al, in 1998, reported that 87 percent woman who received a positive 47, +21 elected to terminate the pregnancy. Besides having a typical karyotype of 47 chromosomes with a straightforward trisomy 21, i.e. 3 copies of chromosome 21 observed as separate entities.

There is a high possibility of spontaneous abortion after amniocentesis and even somewhat more after CVS for a fetus with trisomy 13 (43%) and trisomy 18 (68%) (Hook, 1983). These trisomies are usually much more severe than Down syndrome, but fortunately less common, each affecting about 1 in 5000 babies. Babies with trisomies 13 or 18 generally have severe mental retardation and many physical birth defects. Those who decide to maintain the pregnancy should know of the high perinatal and early infant mortality, the high likelihood of congenital malformation and the rarity of survival beyond infancy.

Almost never do other nonmosaics true fetal trisomies survive through to a stage of extra uterine viability. Schinzel (2001) catalogs a few trisomy 9 and 22 and one or two possible trisomies 7, 8 and 14 which usually result in miscarriage within 8-14 weeks gestation range. Only with trisomy 22 there might be an extremely rare possibility of limited postnatal survival.

### 2.7.2 Sex Chromosomal abnormalities

Abnormalities involving the X or Y chromosomes or portion thereof can affect sexual development and may cause infertility, growth abnormalities, and in some cases, behavioral and learning problem. The SCAs most commonly include 45, X, and 47,XXY, and 47,XXX, and 47, XYY and various forms of mosaicism.

The Turner syndrome (45,X) is a sex chromosome abnormality that affects about 1 in 2500 girls. Girls with Turner syndrome have only one X chromosome, instead of the normal two. They usually are sterile, and do not undergo normal pubertal changes unless they are treated with sex
hormones. Affected girls are short, though treatment with growth and sex hormones can help increase height. Some have other health problem, including heart defects. Girls with Turner syndrome have normal intelligence, though some have difficulties with mathematics and spatial concepts (Hamamy, 2004).

About 1 in 1000-2000 females has an extra X chromosome (47, XXX), referred to as a triple X. These girls, who tend to be tall, have no consistent pattern of physical abnormalities, undergo normal puberty, and appear to be fertile. Intelligence is normal, though learning disability is fairly common. Because these girls are healthy and have a normal appearance, parents are most likely to know their daughter has this chromosomal abnormality only of they have undergone prenatal testing by amniocentesis or CVS.

Klinefelter syndrome is a sex chromosome abnormality that affects about 1 in 600-800 boys. Boys with Klinefelter syndrome have two, or occasionally more, X chromosomes along with their Y chromosome (47,XXY). Affected boys tend to be tall with normal intelligence, though learning disabilities are common. As a group, they have more problems with judgment and impulse control than XY males (Nicolaides, 2003). As adults, they produce lower than normal amounts of the male hormone testosterone and are infertile.

The 47,XYY syndrome with an incidence of 1 in 1000 males, is another abnormality which is very difficult, where decision is concerned. Boys with XYY syndrome often are more physically active than their brother, and if this activity is channelized into play, sports or other physical activities with parent and other children, this fact is in no way negative. Boys with XYY syndrome have a tendency to a delayed mental maturation, sometimes delayed speech, increased tendency for learning problem in school which means need for early and adequate stimulation is essential. On the other hand, the 47,XYY karyotype maybe an accidental discovery in absolutely normal males.

However, most SCA affected individual live essentially normal lives making this a very difficult situation in prenatal diagnosis, both for the counselor as well as the parents. The variation associated with developmental delays in motor skills, speech/language and the risk for learning
disabilities is so wide, that the best alternative for the counselor is to give all the factual information and let the parents make an autonomous choice as is best suitable regard to their social, economic and religious consideration (Linden, 2002).

2.8 Different studies done of genetic amniocentesis for high risk pregnancies

2.8.1 Age-specific incidences of chromosome abnormalities at the second trimester amniocentesis for Japanese mothers aged 35 and older: collaborative study of 5484 cases

(Nobuo, 1998)

Maternal age is the age of women who deliver a child at the age of 35 or above 35 years. This study is known the chromosomal abnormality associated with maternal age in Japanese women. The demand for amniocentesis increased in Japanese women, this is because of the number of pregnancies in maternal age has increased. The data is collected from four genetic centres at Japan the test were performed in maternal age risk women only. Amniocentesis was done in the second trimester of the pregnancy. The method for the study was the routine standard amino culture methods and GTG banding. The chromosomal abnormalities observed were numerical, structural and mosaic cell line abnormalities. The anuploidy not only observed in autosomal but also in sex chromosomes.

This study was carried out on the 5484 pregnancies were only maternal age was considere as the high risk factor. Out 5484 cases, 117 case had the chromosomal abnormality and i.e. 2.1 %. The autosome anuploidy like trisomy 21, 13 and 18 are 62 cases and sex chromosomal anuploidy like XXY, XXX AND XYY are 15 cases. The important conclusion came after statistical method was that incidence for trisomy 21 increases with maternal age.

The sex chromosome anuploidy incidence also increased with maternal age but it was not supported with statistics. No other trisomies were detected other then listed above. There is not
much difference in the abnormality observed and those in other country. The structural abnormality observed in 27 cases. These abnormalities were in, one is unbalance Robertsonian translocation, 4 balance Robertsonian translocation and 12 inversions. The mosaic anuploidy also observed in 14 cases. No relationship of maternal age and structural abnormality and mosaic anuploidy found.

This is the study and first published data for Japanese which shows direct correlation of maternal age and incidence of trisomy 21. Incidence of trisomy 21 is not affected by the race and geographic factor. Recent study suggests that anuploidy not occur in Meiosis I but also in Meiosis II. Based on these facts inclusion is made that the nondisjunction is the only cause induced by the factor maternal age.

2.8.2 **Maternal Serum Screening for Neural Tube Defects and Fetal Chromosome Abnormalities** (Nancy, 1993)

Maternal serum screening is the biochemical noninvasive method to obtain information about fetus. The use of these test expanded from identifying fetus at risk for neural tube defects to other chromosomal abnormalities. Combination of three different markers is measured in the maternal serum screening and these are alpha feto protein (AFP), human chorionic Gonodotropin hormones (hCG), and free estriol.

**Alpha Feto Protein**

AFP in the normal condition of fetus it is produced solely. But the high and low production of AFP may indication abnormal condition of fetus. In 1972 by Brock it was first measured for anencephaly and spina bifida fetus and the values were elevated. The basic principle of the elevated AFP level in amnio is, in the neural tube defects the AFP leak from the capillaries of the fetus blood circulation. AFP also transfers from the amnio to the maternal blood through the placenta. Due to the leak, AFP value in amnio of the affected fetuses increase. Cranial end of the neural tube becomes the forebrain, midbrain and hindbrain. Failure of closure results in acrania or anencephaly. The Caudal end of the neural tube becomes the spinal cord, and the failure of closure results in spina bifida. The incidence of neural tube defects in US is 1 or 2 in 1000 birth. Most of about 85% of NTD are multifactorial. 90% to 95% NTD occurs in the couple without
any family history of this defect. The maternal AFP in combination will detect about 99% of cases of anencephaly. These biological markers are reported as multiple of median MOM in statistic manner. The MOM is a reflection of a patient’s result compared with the laboratory’s median value and is not influenced by outlying values. In analysis of AFP false positive and false negative results may also come. The common cause for false positive are incorrect gestation date, race, multiple gestation, low maternal weight and for false negative incorrect gestation, insulin, obesity. Maternal serum AFP is most correct at 16 to 18 weeks, but can also be done t 15 to 22 weeks.

Ultrasonography is performed to know the correct gestation age of the fetus, according to that the MOM is calculated for AFP. If the dating is correct the detail ultrasound is performed for structural abnormalities for NTD like spina bifida. The ultrasound itself has accuracy of detecting 90% of spina bifida. Amniocentesis is used to identify the cause of AFP level elevated. The fetal karyotype is obtained by standard protocol. The chromosomal abnormality associated with NTD is trisomy 18. Preventive theory for NTD is used like few other defects. Dietary supplement or Vitamin supplements especially folic acid can be used patient with history of NTD. 4mg per day for three month and till first trimester reduces 72% risk for NTD. In Down syndrome patient the AFP level is lower down 25% then in unaffected women.

**Human Chorionic Gonadotropin**

In 1984 chard and college suggest the elevated level of hCG, but don’t have any data to support. Later Bogard and associated did study of 11 women from 17 have Down syndrome fetus. Fetuses with Down syndrome have 2.5 or greater MOM. As the hCG level is elevate in Down syndrome when compare with normal control, it becomes the most effective biochemical marker. Hyper secretary or premature placenta may be the cause of increase level of hCG in Down syndrome patients.

**Maternal serum Estriol:** Estriol is the steroid hormone and is modulated by placenta, fetal adrenal gland and fetal liver. The pregnancies with Down syndrome fetus have 25% lower the value of estriol then in normal women.
Screening with the combination of all three markers: In 1988 Wald and associate study 77 women caring Down syndrome fetus with 385 matched controls. Using the risk for Down syndrome 1:250, they identify 67% cases of Down syndrome, with 5% false positive rates. The detection rate were better than alone AFP of 20-25%. The other deferent studies also suggest the better results for detection of abnormalities when used the biochemical marker in combination. Maternal age also is the strong indication for chromosomal abnormality. Mostly all women age 35 or above offered maternal serum screening test, as the risk for anuploidy increases with age. The limitation for screening is that they cannot detect all the chromosomal abnormalities, specially the sex chromosomal anomalies like XXY and XXX. The sex and other chromosomal abnormalities can be detected by amniocentesis or by CVS but not by the maternal serum screening test. Thus the patient must understand the benefits and the limitation of maternal serum screening test before they consent for test.

2.8.3 Fetal nuchal translucency(NT): ultrasound screening for chromosomal defects in first trimester of pregnancy (Nicolaides, 2003)

NT is nuchal translucency. Ultrasound screening in the first trimester of the screening gives the significance use of the nuchal fluid for chromosomal abnormalities. In second and third trimester nuchal cystic hydroma or nuchal oedema are the markers for chromosomal abnormalities. This is the study of 827 women referred for fetal karyotyping. The indications were maternal age, parental anxiety, and family history of chromosomal abnormality. The median maternal age was 38 years. Trans abdominal ultrasounds were done to obtain CRL of the fetus and to measure NT. Amniocentesis were performed on 433 cases and chorionic villi sampling done on 394 cases. The fetal karyotyping of 799 cases was normal and 28 cases were abnormal. Out of 28 cases 2 cases were trisomy 13, 5 cases were trisomy 18, 13 cases wee trisomy 21, one cases were trisomy 22, one case of 47,XY + fragment, one case of 47, XX, +21/ 46, XX, one case of 47, XXX, Two cases of 47, XXY. In 51 cases i.e. about 6% of fetuses with the NT 3-8mm have the chromosomal abnormality of about 35% i.e. in 18 cases. In contrast only 10 cases remaining i.e. 1% out of 776 have the chromosomal abnormality.
Follow up of the fetus with NT by the USG shows the resolution of fluid by 20 weeks in 52 fetuses with NT 1-2mm thick and 31 of 33 chromosomal fetuses the NT was 3-8mm. The study established four main facts. Firstly, fetal NT of 3 mm or more at 11 to 14 weeks of gestation detected by USG is 6%. Secondly the presence of NT is associated with 10 fold increase risk for chromosomal abnormality. Thirdly the risk for chromosomal abnormality increases as the thickness of the NT increases. And finally, the patterns of chromosomal abnormality like trisomies are similar to that observed in the second trimester with nuchal oedema rather then cystic hygroma.

Study explain the differentiation in the three term used in ultrasound and there associate abnormalities. Cystic hygromas are strongly associated with the Turner syndrome. Nuchal oedema is due to the subcutaneous accumulation of fluid and may be consider as fetal hydrops and associate with various physiological complications. The data suggest NT is the strongest ultrasound marker for chromosomal abnormalities. NT should be done for whole population in the routine ultrasound sonography. Patient come for fetal karyotyping should be taken priority if the NT is present as their risk for chromosomal abnormality.

2.8.4 **Uptake of invasive prenatal diagnostic tests in women after detection of soft markers for chromosomal abnormality on ultrasonographic evaluation.**

(Shrada, 2007)

USG soft marker refers the abnormality detected while ultrasound screening in fetus. These soft markers play an important role for the detection of chromosomal abnormalities. In addition to malformation many other USG soft markers identified those may be the risk factor for chromosomal abnormalities. The major soft markers are NT, echogenic cardiac focus, echogenic bowel, mild ventriculomegaly and nasal bone hypoplasia. The other soft markers which are not significant for the chromosomal abnormalities are choroid plexus cyst, renal pyelectasis, short femur and single umbilical cord.

In the current study 939 women’s referred for ultrasound. The indication for USG was previous child with malformation, still birth, genetic disorder or past history of recurrent spontaneous abortion. All other indications were excluded from the study. Out of 939 women soft markers
detected in 54 cases (5.75%). Echogenic focus in the heart was the commonest soft marker detected in 24 cases, followed by echogenic bowel in 11 cases. Increase nuchal fold thickness was seen in 9 cases and the acceptance for amniocentesis was highest for this marker. There was only one case of nasal hypoplasia as it was done in second trimester.

A single marker was detected in 43 cases and of these 13 options for amniocentesis. Nine cases were detected with two or more markers, out them 5 cases were two markers and both the cases with three markers agreed for amniocentesis. As the number of marker increases the acceptance level for amniocentesis also increases. As all the age group population was involved in the study, no significant difference was observed in the acceptance of amniocentesis.

USG plays an important role in genetic disorders. Most fetuses with structural malformation have normal outcomes. USG has a part of routine antenatal management. A single soft marker may also be associated with variable risk of aneuploidy depending on the marker and mother age. If the USG is combining with the other factors like maternal age, maternal serum screening the detection rate increases. The obstetric history plays an important role in the decision regarding invasive procedure. About 50% of women are ready to take the risk of amniocentesis despite of having abnormal child. The single most important factor which effects the decision is the type of soft marker and association of that marker with abnormality.

2.8.5 **Ultrasound evaluation of fetal chromosome disorders** (Tamsel, 2007)

Ultrasound is the eye and ear of the radiologist and gynecologist. It is the most importance technique to predict abnormality in form of soft markers. About 90% of the chromosomal abnormalities can be predicted by USG. In this article we reviewed the most important USG marker like NT, nasal bone and skin fold thickness which have the potential to predict fetal chromosomal abnormality.

**Nuchal Translucency**
NT is the sonographic appearance of subcutaneous collection of fluid behind the neck of the fetus. It is appear in the first trimester of the pregnancy. The possible etiology for NT includes cardiac failure, secondary and structural malformation, abnormalities in extra cellular matrix, delayed development of the lymphatic system, neuromuscular disorder, fetal anemia, hypoproteinemia and congenital infection.

The ideal gestation period to measure NT is between 11 to 13.6 weeks. The minimum CRL of the fetus should be 45 mm and the maximum should be 85 mm. The magnification should as much as possible to measure better NT. NT should be measured in the neutral position of the fetus. Fetal NT thickness increased with CRL, so it is also important to measure the correct CRL and to note correct gestation period. The study of 96127 cases shows that, the median and 95 percentile at a CRL of 45mm were 1.2mm and 2.1mm respectively. The respective values of 85 mm were 1.9mm and 2.7mm. The various studies show that NT has the power full potential to detect chromosomal anuploidy. Study of 200000 pregnancies demonstrate that NT screen can identifies about 75% of fetuses with trisomy 21 and other chromosomal abnormalities. With the association of some chemical marker like PAPPa and Beta hCG with ultrasound, it is possible to identify 90 % of the chromosomal anuploidy.

Absent or hypoplasia of nasal bone
The fetal nasal bone is visualized by sonography throughout the pregnancy. In 1866 down noted that the common abnormality of trisomy 21 is small nose. A study of 105 patient shows that 49.5% patient have short nose. Recent reports have shows that absence fetal nasal bone or nasal hypoplasia is an important marker for anuploidy both in the first and second trimester. Most of the study shows that absence of the nasal bone is associated with trisomy 21 at 11 to 14 weeks of gestation. The combine data from various study shows that fetal nasal bone was absent in 1.4% of chromosomally normal fetus and 69% of fetus with trisomy 21. In second trimester more number of trisomy detected based on nasal hypoplasia.

Nuchal skin fold thickness
Nuchal skin fold thickness is measured with electronic caliper. Nuchal skin fold are present in 80% of fetus with trisomy 21. It is measured from outer skull table to the outer skin surface. The
value $\geq 6\text{mm}$ consider abnormal. The importance of nasal skin fold thickness as a screening tool was supported by two large clinical trials.

2.8.6 **Screening for Chromosomal Anomalies: First or Second Trimester, Biochemical or Ultrasound?** (Stojikovic T, 2003)

The quality of life for chromosomal affected babies is very poor. By the use of screening and detection techniques we can lower down the incidence. Screening is an important tool in the fetal medicine for prenatal diagnosis. Prenatal screening is done in first trimester (10 to 14 weeks), second trimester (15 to 22 weeks) or in both. The screening can be done by maternal age, ultrasound, maternal serum screening, NT, maternal urine test, combine test, integrated test.

Maternal age refers the age of mother 35 years or more then 35 years who gives birth to child. Maternal age is the factor by which the chances of chromosomal abnormal child increase with age of mother. In 1990 Penrose reported the association between Maternal age of mother and the increase incidence of Down’s syndrome.

**Second trimester biochemical screening**

It is carried out in 15 to 22 weeks. This is the biochemical test in which AFP (alpha feto protein), Beta hCG, free oestriol and inhabin –A are involved. The variation in these values refers the risk for different syndrome like trisomy 21, trisomy 13 and NTD. If these values are combine with maternal age the screening rate increases. The different combination of these biochemical markers refer double marker, triple marker and quadruple marker test in different trimester of pregnancies. The drawback of these screening are they can detect false positive or false negative report.

**First trimester biochemical screening**

The discoveries of chorionic villi sampling stimulates the screening of affected babies in the early weeks of pregnancies. This is done in the first trimester between 11 to 14 weeks of pregnancies. In this test AFP, Beta hCG and PAPPA biochemical are involved. Of these marker
free hCG and PAPPA are the most effective first trimester marker for detection to anomalies. If these are combine with the maternal age the detection rate can achieve to 67 % with 5 % false positive rates.

**Maternal urine marker**
Free beta hCG , core hCG and total hCG are the maternal urine biochemical markers. But the various studies indicate insufficient difference between affected and unaffected pregnancies. Hyperglcosylated hCG and invasive trophoblastic antigen current focusing factor which gives the detection rate of 85 %.

**Ultrasound marker test**
Advancement in the ultrasound gives the chance of detection by USG soft marker.

**NT**
The most importance marker in the ultrasound marker is the measurement of thickness of NT. NT is the fluid filled space between the spine and skin of the fetus. Increased NT is the strong indication for the downs baby. If the NT is combine with maternal age the detection rate is up to 73% with 5% false positive rates. Increased NT may also be associated with miscarriage and other systemic defects.

**Nasal bone**
Cicero and colleagues describes a new ultrasound marker and that is absent nasal bone. In 70% of the downs fetus the nasal bone is absent and in 0.5% of the normal fetus. If the combination of nasal bone, NT and maternal is use, the detection rate increase to 85 %.

**Combination test**
The combination of the biochemical marker, ultrasound marker and maternal age refer to combination test. If this combination used the detection rate for trisomy 21 increases to 90%. The combine screening test also detects the other 90% of the chromosomal abnormalities.
**Future direction in the chromosomal screening**

Fetal cells present in the maternal blood are the current interest of the researcher. These cells can play an important role in the non-invasive test for prenatal diagnosis. Most recently the fetal DNA in the maternal blood has identified as a screening method. Screening tests are the first indicator for the condition of fetus and plays an effective role in prenatal diagnosis. Maternal age, maternal serum screening, NT and ultrasound marker, in combination are the most important screening test for chromosomal anomalies.

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**2.8.7 Chromosomal Abnormalities: Genetic Disease Burden in India** (Kaur, 2010)

Genetics plays an important role in the clinical medical. Genetics and congenital abnormality are the second and the most common cause of the fetal mortality. Approximately 7.6 million children are born every year with genetic and congenital malformation. The effect of the affected individual not only falls on the family but also on society and the country. In developing country like Indian these disease play a role of health burden. India is a county with religious and cultural diversity. In India, difficulty arises in the management of genetic disease because of consanguineous marriage between close relatives in certain communities, high birth rate, poor diagnostic facilities, and lacks of expertise in genetic counseling, illiteracy and blind faith. The most common abnormalities are,

**Mental retardation (MR)**

The mental retardation affects 2.5 to 3% of the total population. Chromosomal abnormality is the important cause of mental retardation. Various study carried out in India to find cause of mental retardation. In most of the study chromosomal abnormality plays an important role for mental retardation. The most common abnormality which was observed in mental retardation is Down’s syndrome.

**Down’s syndrome:** The chromosome associated with Down’s syndrome is chromosome number 21. In majority cases classical Down’s syndrome is observed where free three copies of chromosome no. 21 are present. Other type’s are associated with the chromosomal translocation of 21 and mosaic cell line. The general incidence of Down’s syndrome is 1:800. From various
study in India the incidence of down’s is, in Delhi its 0.81/1000, in Hyderabad its 1.17/1000, in Mumbai 1/1200, in Gujarat 1.04 /1000. The frequency of Down’s syndrome increases with maternal age. Extra chromosome present in Down’s syndrome is due to the non disjunction in the mitosis or meiosis at the time of cell division. Other abnormalities associates with Down’s syndrome are congenital heart disease, osteoarticular malformation, eye anomalies, and gastroenterological malformation. The features of the Down’s syndrome observed in various studies are Mongolian face, ear abnormality, epicanthic fold, flat facial profile, hypotonia, sandal sing, bilateral simian crease, clinodactyly, brachydacyly, hypertelorism, nystagmus, Brushfield spot and cataract.

a. **Microcephaly**: It is characterized by a reduce brain volume and small skull. Microcephaly is associated with mental retardation and other neurological syndrome. The cause of microcephaly is the injury during delivery or due to infection like Rubella, cytomegalovirus, toxoplasmosis during early phase of pregnancy. Microcephaly may also be a part of other chromosomal or genetic disease. Cytogenetic finding of microcephaly are various chromosomal aberration. The study done by Kaur in 2003 of total 143 cases of mental retardation. Out of 143 cases 13 cases showed Microcephaly and that is 9%. If the chromosomal results come normal for microcephaly patient it should referred for FISH to detect the micro aberration of chromosomes.

b. **Fragile ‘X’**: In this disorder, CGG base repeat in the FMR1 genes of X chromosomes. Fragile site is present on the Xq27 region. From most the study carried out the incidence of fragile X is low as compare to other disorder. This is X linked disorder, but may present in both sexes. Severity of the mental retardation is depend on the frequency of the CGG repeat or Full FMR1 gene mutation

**Sex Anomalies**: Apart from the X and Y other autosomal chromosomes plays an important role in sex determination. Any alteration in the genes or sex chromosomes refers to abnormality of sexual development. Primary amenorrhea, Turner syndrome (45, XO), ambiguous genitelia
Hypogonadism, Klinefelter’s syndrome (47,XXY) and Hermaphrodite are the common sex abnormality observed. In some cases mosaic chromosomal abnormality may present. Male infertility is associated with the deletion or mutation of AZFc region on Y chromosome. Y micro deletion is detected by GTG banding, FISH and PCR techniques. The common symptom of this disease is Azoospermia. Abnormality may associate with SRY gene.

**Congenital anomalies:** Incident for the congenital abnormality is approx. 15/1000 births, as per Mishra and Baveja, 1989. The pattern of congenital anomalies includes anomalies of central nervous system anomalies of skin and appendages, cardiovascular malformation, musculoskeleton and genitourinary abnormality. Stillbirths are associated with higher rate of malformation as compare to live birth.

2.8.8 **Prenatal diagnosis of sex chromosomal condition** (Biesecker, 2001)

Counseling for parental diagnosis is the role play suggestion and decision for patient. The information about the fetus condition, feeling about the pregnancies, life of the fetus if abnormal, complication of abnormal results, impacts of parents decision making and long term outcomes of such decision are the important facts which should be consider while talking to the patient. If the fetus is abnormal in prenatal diagnosis the decision of the parent may alter the life of the both partner. Prenatal service and counseling varies for different centers.

Service and counseling for prenatal diagnosis is important when it comes for the sex chromosomal abnormality. Abramsky et al studies the way of talking to parents in sex chromosomal condition. Accurate descriptions about the sex chromosomal differences are critical. If the abnormalities of sex chromosomes are comparing with Down’s syndrome patient, makeable difference will be observed. A child of Down’s syndrome born with the severe heart problem has different prognosis as compared to the unaffected child. A girl with Turner syndrome is short otherwise appear normal may have different life experience in terms of social stigmatization.

Reproductive decisions are complex and multifaceted. The data shows that how the reproductive decision made; clinical practice and theoretical model suggests that they are influenced by women values and beliefs as well as their hopes and dreams for children and family. In this
particular attitude towards abortion, desire for biological children, religious beliefs, attitude towards disability, social norms about prenatal testing and outcomes are likely influence. Money and the social support are the two main particle issues.

If the patients are not prepared for the possibility they may react strongly and react emotionally with stress. Individual under such condition may become hyper vigilant, making ill decisions as an escape from the medical condition. At the time of prenatal diagnosis parents suffers a loss of not only about fetus but also the hoped they carried. The grief is profound but does not preclude a women ability to welcome an affected fetus.

Women deserve the accurate information, convey, respects, honesty and compassion from the health provider as they suffer loss and regardless of their ultimate decision about whether or not to continue the pregnancy.

2.8.9 **Frequency of fetal chromosomal abnormalities at prenatal diagnosis: 10 year experience in a single institute** (Park, 2001)

Prenatal diagnosis deals with the condition or disease of the unborn fetus or embryo. It is the most importance technique to rule out genetic abnormality in high risk pregnancies and give choice to parents. Amniocentesis is the safe and reliable method by which we can detect the chromosomal abnormality in the high risk population. Chorionic villi sampling is done in the first trimester whereas amniocentesis done in the second trimester. Developments in the research in late 1980’s and early 1990’s have made it possible for early amniocentesis. Recently the cordocentesis made it possible to draw blood from cord and can detect chromosomal abnormality.

This study represents the use of above techniques and reports the frequency of chromosomal abnormality in 10 years. The data reveals of 4907 high risk patient who underwent amniocentesis and chorionic villi sampling. Out of total 4907 case amniocentesis done for 3913 cases and
choionic villi sampling done for 800 case. With the help of Brdu, a high resolution technique was used.

The high risk indications for cytogenetic analysis were maternal age, positive screening test, abnormal ultrasound report, genetic abnormal child, bad obstetric history. Out of 4907 case 4757 cases were reported normal i.e. 96.9%. Pericentric inversion as a cytogenetic polymorphism observed in 125 cases i.e. 2.5% and abnormal fetal kryotype were observed in 150 cases i.e. 3.1%.

Both numerical and structural abnormality observed in 150 cases. Numerical abnormality observed in 87 cases are the autosomal trisomies of 21, 18. Trisomy 21 is the highest frequency of chromosomal abnormality. Sex aneuploidy observed some cases were turner syndrome was the common. Structural abnormality found in 63 cases were inversion of chromosome number 2,7,17 and Y, balanced and unbalanced translocation, addition, deletion, Robertsonian translocation. Chromosomal abnormality observed in fetus in prenatal diagnosis parental karyotype is high recommended.

This study reveals the importance of prenatal diagnosis in the high risk population and to improve genetic counseling by providing the data. As it provide the useful information to the physician for high risk population then in general population. Prenatal diagnosis is the safe and reliable technique used in the first, second and late second trimester. It is used worldwide. The current study data are similar to those of various previous reports. Some of the abnormalities are inherited from the parents as parents karyotype were done in the study.

2.8.10 The Importance of Screening and Prenatal Diagnosis in the Identification of the numerical Chromosomal Abnormalities (Neagos, 2011)

The most common chromosomal abnormalities observed are trisomy 21, 18, 13, turner and other sex aneuploidy. These abnormalities cover approximately 95 % of the total chromosomal abnormalities. Prenatal diagnosis provides the information about the unborn fetus. There are two methods of prenatal diagnosis i.e. Non invasive and invasive method.
1. **Non-invasive technique:** These are the screening test which detects about 97% fetuses with trisomy 21 and other chromosomal abnormality. 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, PAPPB and Inhabin –A. The combination of these chemical are use in 1st and 2nd trimester of pregnancies. The variation in these chemical values indicates high risk pregnancies for chromosomal disorder. 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.

2. **Invasive tests:** Invasive test are the confirmatory test which detect the chromosomal abnormality. The tests are amniocentesis in 15-19 weeks of gestation, chorionic villi sampling 10-13 weeks of gestation and cordocenthesi in late second trimester. Chromosomal analysis can be performed by gold standard detection, accurate and reliable GTG banding method. The accuracy rate fro 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 to18 days. Advances in molecular technique like PCR and FISH can give results in 2 to 3 days.

This study aims different aspect for chromosomal abnormalities like screening, analysis issues correlation with indication and results. The common high risk factors for the study were abnormal maternal serum screening, maternal age and abnormal ultrasound. Investigation for chromosomal abnormality was done by cytogenetic and FISH technique. Amniocentesis was done in gestation period of 13 to 25 weeks with the pick period of 16 to 20 weeks. The patient ages were between 25 to 45year old.

In study total 1159 patient involved. Out of 1159, 131 cases of them selected to go for convention karyotype, 181 for FISH and 847 for both the test. The result of convention
Karyotype shows 92.94% normal karyotypes, 2.56% numerical abnormality, and 4.50% structural abnormality. In numerical abnormality, 17 cases were trisomy 21 (1.74%), 2 cases of trisomy 18 (0.2%), 3 cases of trisomy X (0.31%), one case of trisomy XXY (0.1%), one case of trisomy XYY (0.1%). The structural abnormalities were translocations and inversions.

Fish result shows 97.47% normal result and 2.53% abnormal result. The abnormal results were of numerical abnormality. The autosomal aneuploidy observed in 19 cases (1.85%) and abnormality of sex chromosomes observed in 7 cases (0.68%).

The correlation is made between chromosomal abnormalities observed and the high risk factors. The result of correlation shows that non-invasive techniques are the first step of investigation. This technique identifies the fetus on high risk and further investigated by invasive procedure like amniocentesis, chorionic villi sampling and cordocentesis to confirm the abnormality.

2.8.11 Chromosomes preparation and Banding (Moore, 2001)
Chromosome preparation for analysis is very important factor in cytogenetic. Chromosomal morphology, spread, mitotic index are the important factor in preparation of chromosomes. GTG Banding is done to get the dark and light band on the chromosomes, which will help in identification and classification of the chromosomes. There are different types of banding available for different purpose in research and clinical medicine.

Chromosome spread
Good chromosome spread is the factor which can save lots of time in analysis. Chromosome preparation includes various steps. These are tissue culture, harvesting, banding and slide preparation. Chromosomes are visible only in metaphase and to get this stage cells should divide. PHA is used as a stimulant for lymphocytes. After sufficient cell division cycle colchicines is added to stop the cell cycle in metaphase. In harvesting first step is Hypotonic for 10 to 30 min, were the chromosome get spread and volume of the cells will increases. After hypotonic fixative treatment given to fix the morphology of the cells and at the end slide preparation is done. In above processing there are various factors which affect the chromosome spread. The key element
of the spread is degree of dispersion of the chromosomes. Humidity, temperature, and air blow are the factor which affects the spreads.

**Standard Banding methods**
The common standard methods are GTG banding, Q- Banding, R- banding, and C- Banding

**Q Banding**
Quinacrine banding was first demonstrated in 1968 using quinacrine mustard. The mechanism of Q-banding imagined by Caspersson is that the AT rich region of chromosome have tendency to produce fluorescence and GC rich region are weak tendency to produce florescence. Quinacrine banding is simple to perform and visualization of chromosome requires fluoresce microscope and photo-microscope.

**GTG Banding**
This is the standard and common form of banding used in all over the world. Banding is done with the help of enzymatic and chemical treatment of the chromosomes. Trypsin is the enzyme which digests the protein on chromosomes. Dark and light bands are only possible by trypsin treatment. Geimsa is the stains which stain darkly at AT rich region and light at GC rich region. Chromosomal classification is based on the light and dark bands and the length of the chromosomes.

**R- Banding**
This banding technique is exactly opposite to GTG banding. In this dye applies to stain the GC rich region of the chromosomes. Chromomycin A3, olivomycin and mithramycin are most common useful dye in Q-banding. R-Bands have the tendency of staining the gene-rich chromatin, thus enhancing the ability to visualize small structural rearrangements

**Advanced Banding method**
These are HRB- Banding, Sister chromatin exchange banding and Restriction enzyme digestion.

**HRB Banding**
This banding technique used in the detection of microdeletion and other structural rearrangement. In normal G-banding the band level is only 350 to 550, in which detection of microdeletion is sometime not possible and required band level more then 650. With HRB banding 650-1400 band resolution achieved. The mechanism of this technique is to synchronize the chromosome in prophase. The length of chromosome is more in prophase as compare in metaphase. Various synchronization agents like MTX are used in HRB banding.

**Sister Chromatid Exchange (SCE)**
This is the technique in which the one chromatid strand is treated with Brdu. As the DNA follows semi conservative nature in replication, one strand will take the Brdu and other will remain as original strand. In the next division the exchange will take and can be visualized by exposing in UV light. The one strand which was the original parent strand will remain light while another treated with Brdu will become dark. The exchanges can analysis in comparison with control sample. SCE is used to check the genotoxicity.

**Molecular Technique**

**Fluorescent in-situ hybridization (FISH):** Labeled DNA probe are used in various technique to detect the abnormality in molecular cytogenetic. This is the specific technique and used for specific chromosomal abnormality. The host DNA is denatured and labeled DNA is use to hybridize. Labeled DNA gives color signal which is visualized under fluorescent microscope and the abnormality is detected.

**Multicolor FISH**
In this technique the labeled DNA is used for all the chromosomes to detect abnormality for all chromosomes. There are three types of multicolor FISH is available ie. SKY FISH, RX- FISH and M-FISH.

The importances of these various banding techniques are in detection of the different types of chromosomal abnormality.