2. REVIEW OF LITERATURE

Epidemiological data on cancers have revealed a two to three fold increase in the risk of developing cancer among first degree relatives of cancer patients. Among the high risk group, two types of cancer susceptibility genes might be distinguished at the molecular level (i) the inherited mutation in tumor suppressor genes which are directly involved in tumorigenesis (ii) predisposition to cancer as a secondary effect as seen in DNA - repair disorders like Fanconi anemia.

Down syndrome is one of the congenital disorders that predisposes the affected individuals to leukemia. These individuals are twenty times more at risk to develop leukemia than normal individuals.

All Fanconi anemia individuals are at increased risk of developing leukemia or carcinomas. 5-10% of the total Fanconi anemia population are known to die from leukemia. The DNA of Fanconi anemia individuals are extremely sensitive to bifunctional alkylating agents and they are also highly susceptible to transformation by the SV 40 virus (Swift, 1976). A deficiency in the repair enzymes which are necessary to repair cross-links formed by the bifunctional alkylating agents, leads to multiple chromosomal aberrations.

Myelodysplasia is an acquired preleukemic syndrome which represents an advanced step in development towards overt leukemia. It consists of an initial phase where genetic variations accumulate followed by haematological symptoms and clinical preleukemia (Bartram, 1992).
An attempt is made in this chapter to provide a brief account of the developments on the cytogenetic aspects of DS, FA and MDS and on molecular aspects of MDS.

2.1 DOWN SYNDROME (DS)

Down syndrome, referred to earlier as ‘mongolism’ was first described by Dr. J. L. H. Down, a London-based physician in 1866. It is the most common chromosomal abnormality found in newborns. It is also known to be the most common genetic cause of mental retardation. It is reported to occur as one in 600-800 live births.

It is reported to be more common in children born to older mothers, especially in women over the age of 35 years. The infants are reported to be susceptible to develop infectious diseases and the majority of them die in infancy. Presently with the advent of the use of antibiotics for chemotherapy, the number of deaths in DS children is reported to be on the decline. Possibility of prenatal diagnosis allows elimination of mongoloid foetus in utero, thus reducing the incidence in general population.

The first report on the involvement of chromosomes in DS appeared in 1959, when Lejeune and co-workers demonstrated the presence of an extra chromosome in their cells. Over 90% of the DS cases exhibit trisomy 21. Less than 10% of the cases may be instances of translocation or mosaicism, involving chromosome 21.
Clinical features observed in DS individuals include: dysmorphic mongolian type of face, namely flat facial profile, dermatoglyphic change namely, single palmar crease, hypotonia, loose skin on the posterior part of the neck, congenital heart defects, duodenal atresia, Hirschsprung disease and acute leukemia (Korenberg, 1993). Other phenotypic features observed are: mongolian like eyes, cataract, snub nose, high cheek bones, protruding large tongue, small skull, short and thick hands and feet, dysplastic pelvis, clinodactyly of fifth finger, short ears with overhanging helices, upslanting palpebral fissures, hyper extensibility of joints, poor reflex, dementia and premature ageing.

Features observed in DS infants also include mental retardation, brachycephaly, short stature, epicanthic folds, flat occiput, Brushfield spots, open mouth, wide gap between first and second toes, distally placed axial triradii and tibial arch pattern (Lewinson, 1955; Smith, 1976). Both prenatal and postnatal growth deficiencies are evident in them. A tendency towards premature birth is reported. There is delay in brain growth and the infants are microcephalic at birth (Benda, 1960). A mongoloid child may not exhibit all the features mentioned above, though most of them may be present.

Mental retardation is considered to be a hallmark of this trisomy 21 syndrome. The IQ is known to vary between 30 and 50 (Dunsdon et al., 1960). Cardiovascular anomalies appear in 40% of DS patients and is considered to be one of the important causes for their death in infancy. Gastrointestinal malformations occur, so also congenital haematological disorders are common.
Higher risk for developing neoplasia particularly leukemia, increased susceptibility to infection, autoantibodies against thyroid antigens and premature ageing seen in DS children are related to observed immunodeficiency (Gorlin et al., 1990).

The association of maternal age with nondisjunction was discussed by Hook and Lindsjo (1978). Parental mosaicism may lead to the production of a child with DS (Warburton, 1985). Couples who have already produced a child with DS, stand a greater risk of recurrence as compared to the general population (Chauhan, 1988).

Less than 5% of DS infants exhibit translocation involving chromosome 21, which may have been formed either de novo, or transmitted to the child from one of the parents (Giraud et al., 1975).

Environmental factors may also play important role in giving rise to trisomy 21 (Chauhan, 1988).

A large number of genes, about 1500 genes, are now assigned to chromosome 21 (Patterson, 1987). An overdose of genes located on this chromosome causes DS. Data recorded from instances of various translocations, have revealed that the presence of triple dose of the entire chromosome 21 is not actually necessary for the manifestation of DS phenotype. The region 21q22, present along the distal segment of the long arm is known to be involved in the pathogenesis of the disorder (Summit, 1981). Chromosome 21
is estimated to account for about 1.5% of the total DNA of the human genome (Kurnit, 1979).

Patterson (1987) reported that after the third decade of life, individuals with trisomy 21 may exhibit central neuronal degeneration similar to that seen in individuals with Alzheimer disease.

The origin of extra chromosome in DS infants has been investigated by geneticists. DNA markers and data on cytogenetic heteromorphism were employed by Hassold and Jacobs (1984). A combination of molecular and cytogenetic techniques was employed by Stewart et al. (1988) to demonstrate the feasibility of employing a comprehensive approach for the analysis of nondisjunction of chromosome 21. Antonorakis et al. (1992) employed the technique of DNA polymorphism to examine the origin of extra chromosome 21 at meiosis.

Compared to healthy individuals, DS infants are prone to develop leukemia (Schunk and Lehman, 1954; Merril and Harris, 1956; Krinit and Good, 1957; Stewart et al., 1958; Wald et al., 1961; Warkany et al., 1963). Various investigators have reported the risk rate of DS children to develop leukemia. de Alarcon (1987) observed acute nonlymphocytic leukemia (ANLL) in DS individuals. According to Krinit and Good (1957), Conen and Erkman (1966) and Shafik (1988), DS children are estimated to have 20-fold increased risk of developing leukemia than normal individuals. Ragaab et al. (1991) also estimated DS infants to be 20 times more at risk of developing leukemia compared to general population. A review of the earlier
literature on this aspect reveals that DS children are 10-30 times more likely to develop leukemia than non-DS infants (Miller, 1970; de Alarcon et al., 1987; Zipursky et al., 1992; Shen et al., 1995).

Increased incidence of leukemia in DS patients was reported also by Jackson et al., (1968). According to Sasaki and Tonomura (1969), trisomy 21 predisposes the affected persons to the development of leukemia. Compared to healthy individuals, patients with DS exhibited greater susceptibility to develop leukemia at all stages (Shunk and Lehman, 1954; Wald et al., 1961). Individuals with DS are known to have about 1% risk of developing ANLL or ALL during their life time. They are also at risk of developing a transient myeloproliferative syndrome indistinguishable from ANLL except by its eventual clinical recovery (de Alarcon, 1987).

The association of DS with leukemia provided several possibilities to understand the biological intricacies underlying the development of cancer. It is suggested that there may exist a change in sensitivity of cells to the toxic effects of the external environment, probably an increased sensitivity to the oncogenic viruses and ionizing radiation. Results obtained by Sasaki and Tonomura (1969) suggest that the trisomic cells with chromosome 21 exhibit an increased level of radiosensitivity. The difference observed by them in mosaic (normal/trisomy 21) patients suggested that the increased chromosomal radiosensitivity observed may be attributed to the intracellular factors and not to the environment. Their study also demonstrated that trisomies for chromosomes other than chromosome 21 also showed increased radiosensitivity, thus suggesting the nonspecificity of trisomy for chromosome 21.
Various investigators have attempted to elucidate the action of different mutagens on the lymphocytes of DS individuals and compared the results with those on the lymphocytes of healthy individuals. Studies carried out in this direction include: DS lymphocytes and/or fibroblasts exposed to ionising radiation (Higurashi and Cohen, 1972; Leonard and Merz, 1987), methyl nitrosourea (Kona et al., 1977) and trenimon (Aldenhoff et al., 1980). Lymphocytes and fibroblasts of DS infants exhibited increased chromosomal aberrations on exposure to various mutagens such as X-rays and dimethyl benzanthracene (O'Brein et al., 1971). Chromosomal radiosensitivities of skin fibroblasts of trisomy 21 individuals and of normal individuals did not exhibit any difference in a study carried out by Leonard and Merz (1987). It is thus evident that the increased radiosensitivity of DS lymphocytes was not typical of all the tissues.

According to Sasaki and Tonomura (1969), altered enzyme levels in DS patients may be responsible for the enhanced radiosensitivity. Parallelism can be seen between the increased radiosensitivity of DS patients and their increased susceptibility to develop neoplasia. Exposure of trisomic DS cells to low levels of radiation or chemical mutagens resulted in the induction of site-specific breaks which initiated the development of leukemia in them (Shafik et al., 1988).

Exposure of DS leucocytes to radiation resulted in increased incidence of chromosomal aberrations. Various types of structural aberrations such as
breaks, fragments, minutes, dicentrics and rings were encountered (Reeja et al., 1993).

Shafik et al. (1988) in their study on irradiated DS lymphocytes, recorded 16 bands or "hot spots", located on chromosomes 1,3,7,12,17,19 and X, to be preferentially involved in chromosomal breakage and rearrangements. These bands were sites of oncogenes, cancer break points and/or fragile sites. In a study carried out by Reeja et al., (1993) on similar lines, fourteen non-random break points were identified. Thirteen of them were found to be sites of cancer break points, four of them were oncogene sites and two of them were locations of heritable fragile sites. Further, all these breakpoints were found to be localised mostly to chromosomes of A, B and C groups.

Action of various chemical and physical agents and also viruses results in higher frequency of cytogenetic abnormalities (Higurashi et al., 1975; Leonard and Merz, 1983; Vijayalaxmi and Evans, 1982; Morimoto et al., 1984). The chromosomal rearrangements thus formed may get associated with leukemia (Berger et al., 1985).

There have been attempts to examine whether the frequency of SCE in DS patients differ from those of healthy individuals (Crossen and Morgan, 1980). It is found that the exchanges in DS are comparable to those in the lymphocytes of the control group of healthy individuals of the same age and sex.
In a study on SCE in DS, following measles vaccination, Knuutila
*et al.* (1979) found the level of SCE to be lower than that seen before
vaccination. They attributed this to defects in immunological properties,
interferon formation or to defects in DNA repair.

**Modifying effect of 2 deoxy-D-glucose (2-DG) on radiation - induced
cytogenetic damage.**

2-DG, an antimetabolite of glucose, functions as a potent inhibitor of
glycolysis and of ATP supply in a variety of cellular systems (Cramer and
Woodward, 1952; Woodward and Hudson, 1954). 2-DG closely resembles D-
glucose in structure except that in 2-DG the hydroxyl group on the second
carbon atom of the pyranose ring is replaced by a hydrogen atom (Fig.1).

**2-DEOXY-D-GLUCOSE**

(Figure 1)
The chemical formula of 2-DG is $C_6H_{12}O_5$ and its molecular weight is 164.2. It is readily soluble in water. Comparison of the action of 2-DG in normal and in tumor cells shows that it functions in a differential manner. It inhibits the repair of radiation-induced damage in tumor cells and enhances repair processes in normal cells.

Investigations carried out by Jain and co-workers revealed the differential mode of action of 2-DG on various cell systems. Jain et al. (1977a) examined the effect of 2-DG on irradiated Ehrlich ascites tumor cells. They found that the presence of 2-DG inhibited repair of potentially lethal damage in these cells. Experiments carried out on mice bearing sarcoma-180 revealed that administration of 2-DG enhanced survival in the mice population as it brought about tumor regression (Jain et al., 1977b). A reduction in the frequency of chromosomal aberrations was recorded by Jain et al. (1979) when 2-DG was injected into mice before exposing them to gamma rays.

Controlling cancer with the help of carbohydrate analogues was initially suggested by McDonald and Cramer (1952). Anaerobic glycolysis was considered necessary for the survival of cancer cells (Dickens and Weil-Malherbe, 1943). It was thus natural to suggest that an analogue of glucose, inhibitory to anaerobic glycolysis, preferentially inhibits the proliferation of cancer. Experiments carried out by Cramer and Woodward (1952) demonstrated that 2-DG strongly inhibited glucose fermentation by yeast cells.

The repair of X-ray induced damage by 2-DG in 'petite' mutants of yeast cells was demonstrated by Jain et al. (1977b). Further, they (Jain et al.,
studied the effect of 2-DG on normal mammalian system. It was found that in mice, treatment with 2-DG immediately before irradiation reduced the frequency of chromosomal aberrations by 20-30%. Treatment with 2-DG also increased the survival of whole body irradiated mice by about 20% compared to those irradiated mice not treated with the chemical. Gopinath et al. (1983) irradiated Chinese hamster cells and treated them with 2-DG. There was a significant reduction in the frequency of radiation-induced micronuclei.

Work carried out at this department revealed radiation-induced mitotic anomalies to be markedly reduced on treatment with 2-DG (Damodaran et al., 1982). 2-DG also reduced the frequency of chromosomal aberrations in cultured lymphocytes of patients with Down syndrome, Fanconi anemia, retinoblastoma and psoriasis (Girijamani, 1988).

Through their work on root meristematic cells of *Trigonella foenum-graecum*, Rao and Gopinath (1988) found that 2-DG possessed mitodepressive and non-clastogenic properties. 2-DG was found to reduce the radiation-induced frequency of micronuclei and mitotic anomalies in root meristems of *Allium cepa*.

### 2.2 FANCONI ANEMIA [FA]

Fanconi anemia (FA), an eponym coined by Naegeli in 1931 is a paradigm for premalignancy, aplastic anemia and chromosome breakage disorders. According to Alter (1993), FA should be considered as a syndrome because it exhibits a spectrum of physical phenotype running from the
extremely abnormal to normal condition and a haematological spectrum which ranges from severe aplastic anemia to normal. Its malignant potential includes carcinomas, leukemia, liver tumor or no malignancy. From the perception of diagnosis any patient belonging to the above categories, who exhibits increased chromosome breakage in the presence of DNA clastogenic agents are considered to have Fanconi anemia (Auerbach et al., 1981). Due to the variability of the FA phenotype, a patient is not diagnosed until after the haematological abnormalities are ascertained. According to Auerbach (1993) more enlightenment of the clinicians on the FA phenotype is required to diagnose these patients during the preanemic phase.

Although FA cells are known to have defective DNA repair and sensitivity to oxygen damage (Moustacchi et al., 1987; Digweed et al., 1988; Joenje et al., 1981) the underlying defect remains unknown. In spite of extensive investigations carried out, the mechanism behind the bone marrow failure also remains to be elucidated (Stark et al., 1993). All the FA patients studied by Stark et al. (1992) showed impaired granulomonocytopeniesis. Further, defective haematopoiesis in vitro was observed in all the FA patients studied.

High levels of spontaneous chromosome breakage and a remarkable propensity to develop malignant tumors and leukemia have been observed in FA individuals by different groups of investigators (Swift and Hirschhorn, 1966; Dosik et al., 1970; Shroeder and Kurth, 1971; Swift, 1971; German, 1972; Swift, 1976).
Metabolic deficiencies have been observed in FA cells. The enzyme hexokinase, active in the process of glycolysis, was reduced in the erythrocytes of the FA patients. It also demonstrated a reduced substrate affinity (Lohr et al., 1965). A DNA ligase deficiency was also reported in one of the very first cell-free repair assays (Hirsch-Kaufmann, 1978).

According to Zakrzewski et al. (1983) all biochemical abnormalities associated with FA are secondary events and that the primary defect still remains obscure. Even in a recent study by Giampietro et al. (1993) the underlying mechanism(s) by which a mutation initiates the development of a multitude of congenital anomalies in FA is stated to be unknown. It has been hypothesized by these investigators that the FA gene promotes susceptibility to factors that tamper with organogenesis.

FA females are reported to have late menarche, irregular periods and early menopause. Gynecological malignancies are also observed. Fertility is not reduced (Alter et al., 1991). Males exhibit hypogonadism and hypospermia (Bargman et al., 1977). Very few patients have claimed fatherhood (Shroeder et al., 1976; Alter et al., 1991; Liu et al., 1991).

Even as early as the early sixties, an increase in chromosome breakage was observed in patients with FA by several investigators (Schroeder et al., 1964; Schmid et al., 1965; Bloom et al., 1966; German and Crippa, 1966; Swift and Hirschhorn 1966).
In a study carried out by Taylor and Duckworth (1983), lymphocytes of 8 patients out of 9 demonstrated an increase in the level of spontaneously occurring chromosome aberrations. According to a few investigators (Sasaki, 1978; Glanz and Fraser, 1982) some patients with clear cut clinical symptoms of FA failed to exhibit an increase in spontaneous or induced chromosomal aberrations. In contrast to this, some patients who did not show any clinical symptoms demonstrated high levels of spontaneous and induced chromosome breakage (Cohen et al., 1982).

It has been confirmed by different groups of researchers that the chromosomes in FA cells show remarkable hypersensitivity to DNA cross-linking agents such as mitomycin C (MMC) (Sasaki et al., 1973), nitrogen mustard (Berger et al., 1980) and diepoxybutane (Auerbach et al., 1981). This procedure is used to confirm or to exclude FA, both prenatally and postnatally (Shipley et al., 1984; Auerbach et al., 1986; 1991). This has been further substantiated by Giampietro et al. (1993) who affirm that FA is an autosomal recessive chromosomal instability syndrome with a recurrence risk of 25%, characterized by a high risk of malignancies and a unique cellular hypersensitivity to DNA cross-linking agents such as diepoxybutane. In a study conducted by Glanz and Fraser (1982) a significant decrease in the incidence of congenital anomalies was observed among the affected siblings compared to the FA patients.
FA cells grow poorly in culture. While growing the cells *in vitro*, many of the specific symptoms of FA cells can be alleviated by growing them at lower oxygen tension (Digweed and Sperling, 1996).

The increased sensitivity induced in FA lymphocytes by a variety of physical and chemical agents including alkylating agents was tested by Sasaki and Tonomura (1973). FA cells were observed to be most sensitive to cross-linking agents like nitrogen mustard, MMC, 8-methoxypsoralen and 355-nm UV light. Latt *et al.* (1975) reported that FA cells were highly sensitive to ethylmethane sulphonate (EMS), a monofunctional alkylating agent. Auerbach and Wolman (1976) have demonstrated the cultured fibroblasts from FA patients to be particularly sensitive to DEB, a bifunctional agent. Further, they observed the fibroblasts from obligate carriers to be more sensitive to DEB compared to normal cells (Auerbach *et al.*, 1978).

Experiments by Sasaki and Tonomura (1973) have revealed a 42-fold increase in chromosomal damage on treatment with MMC. MMC, a bifunctional alkylating agent, is capable of intercalating into a DNA double helix and forming covalent cross links between its two strands. Fanconi anemia cells are particularly sensitive to interstrand cross-links (Digweed and Sperling, 1996). The persistence of interstrand cross-links leads to unfavourable consequences during S phase (Moustacchi and Diatloff-Zito, 1985) and this effect is more restricted to cells of FAA (FA complementation group A) group (Moustacchi *et al.*, 1987; Digweed *et al.*, 1988).
Lymphocytes of 8 out of 9 FA patients studied by Duckworth et al. (1984) showed high levels of spontaneous and MMC- or DEB-induced chromosome breakages. However, a consistent increase in induced chromosome breakage could not be observed in obligate carriers.

According to Higurashi and Cohen (1971 and 1973) cultured skin fibroblasts and peripheral lymphocytes from FA individuals exhibited increased chromosomal aberrations on irradiation with gamma rays, than those from healthy individuals.

It has been demonstrated by researchers that even low doses of many chemical agents which did not induce chromosome aberrations caused an increase of SCE rate (Latt, 1974; Perry and Evans, 1975; Stefka and Wolff, 1976). Novotna et al. (1979) did not observe any difference between the spontaneous frequency of SCE in FA patients and control group of individuals. They observed an increase in chromosomal aberrations (CA) only after exposure of FA peripheral blood cells to high doses of mutagens while low doses of mutagens resulted only in high frequency of SCE. An increased level of SCE was produced in the presence of clastogenic agents like MMC, nitrogen mustard and mustine hydrochloride. Analysis of SCE along with CA analysis, is the most reliable means of confirming FA (Howell, 1991).

With the help of somatic cell fusion, cells from FA patients have been assigned to five groups (A to E). Five causative genes (FAA to FAE) have been identified. One gene for FAC (Fanconi anemia complementation group C) was cloned and was localized to chromosome 9q (Strathdee et al., 1992). The FAC
gene was cloned by functional complementation of FA lymphoblastoid cells. Fourteen exons were shown to make up the coding sequence (Gibson et al., 1993). The protein product of this gene is novel and does not resemble anything in the data bank. Immunofluorescence and western blot analysis have shown that the 63 kD FAC protein is cytoplasmic and that it does not have a nuclear location signal (Yamashita, et al., 1994).

Acute leukemias occur with high frequencies in FA patients. In a few instances, acute leukemic phase is preceded by a preleukemic phase (Alter et al., 1993). The types of leukemia which occur in the FA patients usually fall within the confines of a spectrum which includes all types of myeloid leukemias from M1 to M6. The leukemia observed in FA have one characteristic feature in common, that is, they are very difficult to treat and survival has been found to be very poor. An increased sensitivity to chemotherapy is observed in FA cells probably due to defect in DNA repair (Alter, 1993).

A review of all the cases of Fanconi anemia reported to the International Fanconi Anemia Registry (IFAR) indicates that preleukemia manifests in atleast 15% of them. These patients usually have karyotypically abnormal bone marrow clones, but do not exhibit chromosomal translocations involving breakpoints associated with specific oncogenes. Further, leukemia in FA is a multistep process (Auerbach, 1992).

On the basis of genetic epidemiological studies it has been estimated that FA heterozygotes are at a greater risk for developing cancer than the
general population (Swift, 1971). All FA individuals are at risk of developing leukemia or carcinomas, particularly hepatocellular carcinoma. About 5-10% are reported to die from leukemia. It has been suggested that the hepatic carcinomas develop as a complication of therapy for the aplastic anemia but they have also been reported in patients receiving no therapy (Cohen and Levy, 1989). Multiple chromosome breaks are observed and the basic defect may be a deficiency of the repair of DNA strand cross-links. They exhibit extreme sensitivity to DNA damage from bifunctional alkylating agents, and high frequency of transformation by the oncogenic virus SV40 (Swift, 1976).

The diagnosis of FA based entirely either on clinical or on chromosomal findings alone may be inaccurate and it is necessary that data derived from both the sources have to be relied upon for the correct diagnosis of FA (Cohen et al., 1982). Prenatal diagnosis has been achieved by demonstrating increased spontaneous and induced chromosome breakage in fetal cells namely, in cultured amniocytes or chorionic villus cells (Auerbach et al., 1985).

Bone marrow or umbilical cord blood transplantation from an HLA-identical sibling is considered as the best choice of treatment for Fanconi anemia (Glukman et al., 1989).

2.3 MYELODYSPLASTIC SYNDROME (MDS)

Myelodysplastic syndromes comprise of a group of haematological disorders caused by an initial assault to a pluripotent haematopoietic stem cell
which results in abnormal control of cell proliferation and differentiation. The disease progression in these syndromes is a multistep process that involves genetic changes (Gadner, 1992). There is clonal proliferation of aberrant haematopoietic stem cells, whose progeny fail to differentiate or function normally, and appear dysplastic. This results in peripheral blood cytopenia and a hyper- or normo-cellular marrow and leads to a high premature death rate (Linman and Bagby, 1976).

Myelodysplasia, an acquired preleukemic state, can be distinguished from other such conditions by its morphologically and functionally dysplastic cells (Culligan, 1992). An abnormal clone expands either due to changes in normal growth control factors or due to insensitivity to inhibitory factors or due to suppression of normal haemopoiesis. In most MDS cases, increasing cytogenetic abnormalities, and increasingly malignant bone marrow characteristics accompany clonal evolution leading to AML (Jacobs, 1991).

Earlier MDS was considered as the earliest identifiable stage in the multistep process of leukemogenesis, characterized by morphologic, cytogenetic and haematologic abnormalities. It has recently been demonstrated that MDS represents an advanced step in development towards overt leukemia, preceded by various alterations in the genome that remained obscure till the advent of molecular technology. The most striking character of MDS is the presence of dysplastic, poorly functional cells which are produced insufficiently as a result of impaired differentiation and premature cell death in the bone marrow (Jacobs, 1989). Various sources of evidence suggest that clinical preleukemia
is preceded by a phase in which genetic variations accumulate without any haematologic change. Accumulation of the various genomic lesions characterize the multistep process of leukemogenesis (Bartram, 1992).

In a study conducted by Mecucci et al. (1986), four MDS patients developed new clones when the disease evolved into a more malignant form and this cytogenetic heterogeneity in MDS may well be related to a high selective pressure towards more malignant clones. Raskind et al. (1984) using G6PD and chromosomes as clonal markers demonstrated that even cells expressing the same G6PD isoenzyme showed variations in the karyotype, thus highlighting the fact that different karyotypes can be found in cell populations belonging to the same clone. These authors hence concluded that MDS originate as a multistep process from a genetically unstable progenitor cell and that subsequent events including chromosome anomalies seem to determine the progression of the disease. New events thus lead to the proliferation of a previously silent population and this might explain the emergence of different karyoptypes. Therefore, a tumor, although derived from a single progenitor might contain multiple cytogenetically distinct populations, which may also include karyotypically normal cell population with different growth patterns. Mecucci et al. (1986) have shown that the appearance of a new anomaly unrelated to the already persisting one was associated with a more malignant course.

At the Second International Workshop on Chromosomes in Leukemia held in 1979 (1980) 244 MDS cases were reviewed and it was found that the
patients who carried chromosomally abnormal clones, whether they totally (AA) or partially (AN) replaced the normal marrow, ran a greater risk of developing ANLL than patients exhibiting normal mitosis. Nowell (1982) in a study of 80 patients, discovered that 80% of those with cytogenetic abnormalities developed leukemia. Similar findings were recorded by Coiffier et al. (1983), Jacobs et al. (1986) and the Groupe Francais de Cytogenetique Haematologique (1986). RAWEB (refractory anemia without excess blasts) patients with 5q- as the sole abnormality had good survival prospects (Nowell et al., 1986; Yunis et al., 1986; Van Den Berghe et al., 1985; Dewald et al., 1985). According to Nowell (1982) and Yunis (1986) the patients with normal karyotypes were least likely to progress to ANLL and they also had a longer period of survival. Bloomfield (1986) found that -5 and -7 were mainly associated with previous haematological malignancies where as 5q- and specific translocations were the secondary effects of treatment.

A consensus is yet to be reached on the prognostic role of karyotypic changes in MDS. Allessandrino et al. (1985) were not able to relate the development of leukemia with chromosomal abnormalities in their retrospective study conducted on 30 CMML (chronic myelomonocytic leukemia) patients. Tricot et al. (1985) also did not find such a correlation. However, some tentative association need to be taken note of in this context. Monosomy 5 and 7 and structural changes associated with 12p are considered to be ominous prognostic signs (Heim & Mitelman 1995).
The 5q- chromosome has been associated mainly with good prognosis and stable conditions (Teerenhovi, 1981). Preleukemic patients with many aberrations within one clone offer very poor prognosis. Chromosomes 7, 17 and 3 were the most commonly aberrant chromosomes within the 5q- clones. The haematologic and clinical picture of 5q- is very well studied. 5q- is usually seen at the time of diagnosis but it can also be detected when the disease progresses. The significance of 5q- deletion at the molecular level has not yet been established. The genes present on the long arm of the chromosomes controlling growth and haemopoiesis are deleted in the case of MDS patients exhibiting 5q- (Jacobs, 1991).

In a study conducted by Suciu et al. (1990), it was found that patients with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) had a lower incidence of transformation to ANLL and better possibility for survival, while patients with RA with excess of blasts (RAEB) and RA with excess of blasts in transformation (RAEB-t) had a high incidence of transformation and reduced survival. However, Suciu et al. (1990) have reported that all patients with chromosomal anomalies had a tendency towards leukemic transformation. Various studies have indicated that acute leukemia is most likely to develop in patients who harbour multiple karyotypic changes and these patients have poor prognosis (Anderson et al., 1982; Nowell et al., 1986; Borgstrom, 1986). Structural and numerical chromosomal changes are common in MDS as well as in other malignant haemopathies (Mitelman et al., 1991; Sandberg, 1990). Specific chromosomal aberrations associated with MPD or ANLL occur only sporadically in MDS.
Certain specific associations between chromosomal anomalies and cellular manifestations have been identified. A significantly high percentage of ringed sideroblasts in the marrow was found in MDS cases with deletions of long arm of chromosome 11, and 11q24 was the region always involved (Mecucci et al., 1987). 20q- brought about prominent dyserythropoiesis in cases of MDS (Davis et al., 1984; Vila et al., 1990). When monosomy 7 appears in cases with aplastic anemia, it evolves into either MDS or ANLL (Mecucci, 1992). Rearrangement involving 17p has been associated with Pelger-Huet like anomaly (Kere et al., 1988; Kerndrup et al., 1987; Lai et al., 1990). Monosomy 7 is a frequently occurring anomaly in both primary and secondary MDS (Fourth International Workshop on Chromosomes in Leukemia, 1982). It is associated with short survival as well as predisposition to develop severe infections. Occurrence of monosomy 7 in DS or FA and familial monosomy 7 heralds the onset of leukemia (Mecucci et al., 1992).

Bone marrow of patients with MDS showed a 2-3 fold increase in the frequency of sister chromatid exchanges while the lymphocytes from these patients showed only a marginal increase (Mansoor et al., 1993).

In a study using fluorescent in situ hybridization (FISH) technique, Abruzzese et al. (1996) reported that trisomy 8 found in their cases could be identified in granulocytes, monocytes and erythroid elements but not in lymphocytes and plasma cells. They concluded that some types of MDS arise from pluripotent cells, but it is only under very rare circumstances that these progenitors are able to give rise to lymphoid malignancies.
The relationship of the MDS states to acute leukemia remains to be understood. A few investigators are of the view that when a chromosomal abnormality exists, patients have already transformed to leukemia (Sandberg, 1980). Linmann and Bagby (1976) opined that preleukemia represented a stage or stages in the evolution of AML. An epidemiologic study sponsored by Leukemia Research Foundation in 1984 estimated the incidence of MDS to be approximately 75% that of AML. MDS is primarily a disease of the elderly. Among elderly people over 60 years of age, the incidence was as high as 0.75 per 1000 per year (Hamblin et al., 1987).

In a study by Mufti et al. (1985) a high prevalence of immunological abnormalities were found in patients with MDS. All the different classes of MDS demonstrated a polyclonal rise in serum immunogloblin (Osserman and Takatsuki, 1965; Laughter et al., 1979). A large number of investigators have reported the presence of immunological abnormalities in primary MDS. The number of T cell lymphocytes are found to be reduced so also the natural killer cells (Yoshida, 1987). Humoral functions appear to be intact. Immunoglobulin production has been reported to be riddled with abnormalities (Yoshida, 1987). Abnormal CFU-GM growth was observed in 79% in a study conducted by Scoolin et al. (1990).

In a large number of studies, a greater percentage of patients (55-84%) with secondary MDS progressed to acute leukemia in comparison to patients with primary MDS (Cheson, 1990).
The advanced technology in molecular biology has provided the
weapon to characterize several steps that lead to malignant transformation
and to analyse and understand the multistep process of leukemogenesis - the
activation of oncogenes and/or the inactivation of some tumor-suppressor genes
(Bishop, 1991; Hunter, 1991; Layton et al., 1988). In some neoplasms different
genes mutually contribute to malignancy (Fearon, 1990). MDS provides a rare
opportunity to lay bare the molecular mechanisms underlying malignancy.

Loss of function of the tumor suppressor genes due to mutations in
them result in neoplasia. The normal functions of these gene products is to
regulate growth and differentiation in a negative fashion and thus to check
malignant transformation. Among these tumor-suppressor genes, p53 plays a
very important role, because mutations in this gene are the most common
genetic change involved in human neoplasia (Hollstein et al., 1991; Levine
et al., 1991). The p53 gene located on chromosome 17p codes for a nuclear
binding protein. Studies on p53 mutations in a variety of human cancers
suggest that p53 mutation is recessive and contributes to tumorigenesis only
when both p53 alleles are inactivated (Levine et al., 1991). However, early
studies on p53 suggested that in some instances, it could function as an
oncogene and promote cellular transformation and proliferation. This confusion
appears to have been caused because some mutant p53 forms encode a protein,
which inhibits the function of the remaining wild-type allele and so they
promote tumorigenesis by a dominant-negative effect.
A study conducted by Mori et al. (1992) had revealed the involvement of p53 mutations in haematologic neoplasms, although the frequency of these mutations was low. p53 mutations were detected in 5 of the 24 patients studied. A study by Jonveaux et al. (1991) revealed that only 5 out of 151 cases of MDS demonstrated point mutations in p53 (covering exons 5-8) using SSCP technique. Three of these five cases showed abnormalities in the short arm of chromosome 17. In another study, Wattel et al. (1994), detected p53 mutations in 11% of MDS patients with the help of PCR-SSCP.

p53 mutations are mainly seen in advanced or relapsing haematologic malignancies and this suggests that these mutations are associated with poor response to chemotherapy. In MDS too the presence of a p53 mutation indicated a significantly low response to chemotherapy. p53 protein has been implicated in DNA repair and inactivation of this gene through a mutation results in mutation of other genes (Wattel et al., 1994).

The ras gene family, H, K and N-ras coding for 21 kD proteins and having GTPase activity, has been implicated in cell proliferation and control (Barbacid, 1987; Bos, 1988). Mutation in codons 12/13 or 61 of N-ras gene has been particularly implicated in AML and CML. MDS patients with mutant ras gene show a high rate of leukemic transformation. Ras gene mutations have also been detected in healthy populations which proves that ras gene mutation alone is not enough to produce preleukemic changes (Jacobs, 1991).

Both mutant ras and mutant p53 genes in all probability provide a proliferative advantage in comparison to the cells containing wild type alleles.
Mutant p53 gene collaborates with ras genes to transform primary rat embryo fibroblasts to malignant cells (Parada et al., 1984). K-ras mutations along with mutant p53 played a vital role in colon carcinoma (Kern and Vogelstein, 1991).

In most of the human cancers, altered p53 activity is evident (Hollstein et al., 1991). p53 gene plays a vital role in damage control within the cells. It exercises G1 checkpoint control by switching off replication in response to DNA damage. It brings about apoptosis in those cells which have been irreparably damaged (Lane, 1992). Normal p53 function is therefore crucial to control damage brought about by exposure to radiation.

Earlier studies have shown SSCP analysis to be a rapid, sensitive and specific method to detect point mutations especially in p53 mutations (Cheng and Haas, 1990; Fenaux et al., 1992).

Adamson et al. (1995) screened 26 MDS patients for mutations in exons 5-8 of the p53 gene and identified four point mutations. Missense mutations and nonsense mutations were confirmed by direct sequencing of the appropriate exons. This study also suggests that p53 mutations in MDS is a terminal genetic event rather than a primary event, as only advanced cases showed p53 mutation. Sequential sampling too did not reveal any p53 mutation, thus proving the fact that p53 mutation did not bring about transformation to a more malignant form.