1. INTRODUCTION:

Leukemia is a type of cancer that arises in the blood and bone marrow. It can affect both myeloid and lymphoid lineages. If the cancerous mutation occurs in the marrow that makes lymphocytes, the disease is called lymphoid leukemia and if mutation occurs in myeloid stem cells, the disease is called myeloid leukemia. Each of the two major types includes both acute and chronic forms. Hence, there are four main types of leukemia: acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoid leukemia (ALL), and chronic lymphoid leukemia (CLL). In leukemia the malignant cells (blasts) are immature and incapable of performing their immune system functions. Chronic leukemia develops in more mature cells, which do not perform some of their duties. These abnormal cells increase at a slower rate, whereas in cases of acute leukemia the onset is rapid and fatal. In AML, the myeloid stem cells develop into immature abnormal cells called myeloblasts (or myeloid blasts). When too many stem cells develop into abnormal cells or blasts in the bone marrow and blood, there is less room for healthy white blood cells, red blood cells, and platelets. These cells spread outside the blood and to other parts of the body, including the central nervous system (brain and spinal cord), skin, and gums.

In acute leukemia these blast cells may be divided in to subtypes on the basis of cytochemical staining of the peripheral blood samples of the patients. A major initiative and a step forward in this respect was taken by the FAB (French-American-British) study group. In 1976 they proposed a classification of acute leukemia in which ALL and AML were separated and subdivided into three and six groups, respectively (Bennett et al; 1976). Initially, the FAB classification relied almost exclusively on morphologic criteria, but subsequent revisions included information gained from other investigations, mainly cytochemical and immunophenotypic analyses (Bennett et al; 1991). Ultimately, the FAB classification recognized the following eight subgroups: minimally differentiated AML (M0), AML without maturation (M1), AML with maturation (M2), acute promyelocytic leukemia (APL) hypergranular/typical (M3) as well as microgranular/hypogranular/atypical (M3v), acute myelomonocytic leukemia (M4) including the subtype (M4Eo) with BM eosinophilia, acute monoblastic (M5a) and monocytic (M5b) leukemia, acute erythroleukemia (M6), and acute megakaryoblastic leukemia (M7). This classification has further evolved into the present World Health Organization (WHO) classification. The rationale for the WHO classification is the incorporation of morphologic, immunophenotypic, genetic and clinical
features in an effort to define subgroups that are biologically homogeneous and have clinical relevance. Many translocations are characteristic of a particular subtype of acute leukemia for example t(15;17) is associated with FAB-M3 subtype. Transformed cells lack normal regulatory growth factors and favorable competitive advantage over normal hematopoietic cells. So, this results in the accumulation of abnormal cells with qualitative defects. A major cause of mortality is the deficiency of normal functioning mature hematopoietic cells rather than the number of malignant cells (Buccisano et al; 2009). Today with combination of morphological, cytogenetics, immunophenotyping and molecular studies the accuracy in the classification and diagnosis of leukemia has increased tremendously. WHO categorized AML into four types as (1) AML with recurrent genetic abnormalities [t(8;21)(q22;q22), 11q23 translocation, t(15,17)(q22;q21), and inv(16)(p13;q22)]; (2) AML with multilineage dysplasia; (3) Therapy related AML (t-AML) and myelodysplastic syndromes (t-MDS); (4) AML not otherwise categorized (including FAB subtypes M0–M7) (Jaffe et al; 2001).

The most common types of leukemia in children and adults are acute lymphoid leukemia (ALL) and acute myelogenous leukemia (AML). AML accounts for approximately 20% of acute leukemia in children and 80% of acute leukemia in adults. In the United States, AML and ALL both affect children and adult with an estimated 14,590 (male-7,820 and female 6,770) and 6,070 (male-3,350 and female 2,720) new cases the year 2013 (Leukemia Fact; 2013). There were reports of an estimated 10,370 (male-5,930 and female 4,440) deaths from AML and 1,430 (male-820, female-610) deaths from ALL in the year 2013 (Leukemia Fact; 2013).

Different leukemia subtypes have shown important differences in geographic, racial/ethnic, age and trend patterns (Kulshrestha and Sah; 2009). The incidence of leukemia is highest among whites (12.8 per 100,000) and lowest among American Indians/Alaskan natives (7.0 per 100,000), Asian and Pacific Islander populations (7.3 per 100,000) (Leukemia Facts; 2013). The incidence of M3 subtype i.e. acute promyelocytic leukemia (APL) is slightly increased in the Hispanic population. High incidence rates of AML occur in the areas of the world includes Shanghai, New Zealand, and parts of Japan (Taylor et al; 2007).

In India, AML is the most common type of acute leukemia occurring in adults, comprising approximately 80 to 85% of cases diagnosed in individuals greater than 20 years of age (Jemal et al; 2008). AML is generally a disease of older individuals, the median age being 65 years. However, younger individuals seem to be afflicted by this disease (median age 30 yrs) (Scheinberg et al; 2001). Bhutani et al have reported that all hematological
malignancies are commonest in Delhi followed by Mumbai. Rural areas have least incidence of these malignancies (Bhutani et al; 2002). Incidence rates for all types of leukemia are higher among males than females (Ghosh et al; 2003, Kumar et al; 2014, Rathee et al; 2014).

The pathological characteristics of peripheral white blood cell count of AML patients may be varied. Granulocytopenia is very common; approximately 1/2 of all patients may have granulocyte counts <1,000/uL. Thrombocytopenia is frequently observed and platelet counts <20,000/uL are common. The hematocrit is generally low but severe anemia is uncommon. Circulating blast cells are absent from the peripheral blood in approximately 15% of AML patients initially, and in 1/2 of patients presenting with leucopenia (Miller and Daonst; 2000).

The sign and symptoms of leukemia results from the lack of normal and healthy blood cells because they are crowded out by malignant and immature leukocytes (white blood cells or blasts). Low numbers of red blood cells lead to anemia feeling tired or weak, being short of breath and looking pale. Low numbers of white blood cells lead to fever and frequent infections that are hard to treat. Low numbers of platelets lead to cuts that heal slowly, easy bruising or bleeding and tiny red spots under the skin (petechiae). High numbers of leukemia cells cause pain in the bones or joints, lack of appetite, headache or vomiting. If the leukemic cells invade the central nervous system, then neurological symptoms (notably headaches) occurs. People with AML experience symptoms like bleeding from the gums, fever, frequent infections, frequent or severe nosebleeds, loss of appetite, lumps caused by swollen lymph nodes in and around the neck, swollen gland in the neck, underarm and groin, pale skin, shortness of breath, weight loss, weakness, fatigue or a general decrease in energy and night sweats (Kasthuri et al; 1991). As the disease progresses, signs and symptoms of anemia, thrombocytopenia, and neutropenia increase. Leukemic cells may infiltrate other bodily tissues, causing many clinically significant comp lic actions including CNS involvement, pulmonary dysfunction, or skin and gingival infiltration. Fever, shortness of breath, easy bruising or bleeding, petechiae (flat, pinpoint spots under the skin caused by bleeding), weakness or feeling tired and weight loss or loss of appetite are some of the common symptoms of AML (Belson et al; 2007, Pauls et al; 2012).

There is no definite way of determining the cause of leukemia. Possible risk factors for AML include the ‘Pre-leukemic’ blood disorders such as myelodysplastic or myeloproliferative syndromes but the exact risk depends on the type of MDS/MPS. Smoking and alcohol consumption may affect the risk of developing adult AML (Miller and Daonst; 2000). Pesticides have been shown to increased risk of leukemia. X-ray examinations of
pregnant women may be associated with increased risk of subsequent childhood leukemia (Doll and Wakeford; 1997, Shu et al; 1999). Certain cytostatic compounds, like alkylating agents and inhibitors of DNA topoisomerase II enzyme, increase the risk of leukemia both in children and adults (Rubison et al; 1994, 1999). Among adults, the known causes are natural and artificial ionizing radiation, and some chemicals, notably benzene and alkylating chemotherapy agents. The risk is also higher for those exposed at an earlier age (Miller; 1995; Kim et al; 2006) and secondary leukemia may develop in the individuals treated by radiotherapy (Greaves; 1997). Radiation from nuclear power plants is also a known cause for both kinds of leukemia i.e. prenatal and postnatal (Meinert et al; 1999, McKinney et al; 2003, Kasim et al; 2005; Belson et al; 2007). Ionizing radiation has been found to predispose to acute leukemia (Hjalgrim et al; 2004, Bartley et al; 2010). This was proved by the high incidence of leukemia in Japanese survivors from the explosion area of nuclear bombs and secondary leukemia in the individuals treated by radiotherapy.

Immunophenotyping of leukemia patients has been used for the diagnosis and classification of both AML and ALL (Orfao et al; 1995, Khoury et al; 2003). Early myeloblasts express CD34 and HLA-DR but these are lost by the promyelocyte stage. Other cell specific associated antigens may be expressed after commitment of an early CD34 hematopoietic precursor into the lymphoid or myeloid lineage. Lineage specific CD marker expressed for B-cell antigens are CD19, CD10, CD20, CD22 and surface (SIg) and cytoplasmic (CyIg) immunoglobulins. Antigen expressions for T-cell are CD2, CD7, surface and cytoplasmic CD3 and CD5. For the myeloid lineage antigen expressions are CD13, CD14, CD33, CD65w, CD11b MPO and CD15 (Orfao et al; 2004, Jiang et al; 2010). These CD markers may be used as diagnostic tool in establishing the diagnosis and classification of acute leukemia. The characterization of AML by immunophenotyping is particularly helpful when the morphology is difficult to interpret among different subtypes i.e. M0-M7. It is useful in the early detection of minimal residual disease (MRD) and is also reported to have prognostic value.

Karyotype is widely recognized as one of the most important prognostic (favorable, intermediate and unfavorable risk groups) factors in acute myeloid leukemia (AML). Along with pathology and immunophenotyping, cytogenetic analysis has played an important role in prognosis of AML. Cytogenetic data are being increasingly used to assign patients into distinct prognostic groups in the context of modern risk-adapted treatment protocols. The detection of recurrent non-random chromosomal abnormalities resulted in a new understanding of AML, as according to WHO classification subdividing the disease into true
**de novo** (TDN)-AML including patients with t(8;21), t(15;17), inv(16), t(9;11), t(11;17), t(6;9), t(1;22) and t(8;16), and myelodysplastic syndrome related (MDR)-AML including patients with −5, 5q−, −7, 7q−, +8, 11q−, inv(3) and translocations involving 3q (Head; 1996, Langabeer et al; 1997, Look; 2002). Certain cytogenetic abnormalities like, t(15;17) in acute promyelocytic leukemia (APL) showing FAB-M3 morphology are associated with very good outcomes. A number of other cytogenetic abnormalities (3q−, 5q− and monosomies) are known to be associated with a poor prognosis and a high risk of relapse after treatment (Byrd et al; 1999). Some leukemia patients characterized by specific chromosomal abnormalities i.e. trisomy of chromosome 21 carry a worse prognosis (Vardiman et al; 2002). AML patients with ‘normal’ cytogenetics fall into an intermediate risk group (Wheatley et al; 1999, Slovak et al; 2000, Ley et al; 2008).

There has been enormous progress in the treatment of leukemia in the developed world because the epidemiology in these countries is well described. The treatment of AML includes chemotherapy, radiotherapy, consolidated and maintenance therapy. The aim of remission induction is to rapidly kill most tumor cells and get the patient into remission. Once remission is achieved, additional therapy is needed to avoid relapse called consolidated or maintenance therapy. The form and intensity of the treatment are determined based on the risk group. Patients with good or standard risk may be given less intensive conventional chemotherapy in order to minimize the side effects of the treatment, whereas patients with high risk may receive intensive treatment including stem cell transplantation.

Molecularly targeted therapies may ultimately overcome the poor prognosis associated with specific cytogenetic abnormalities or somatic mutations. But after these therapies patients may remain at risk of early mortality due to the infectious and hemorrhagic consequences of severe bone marrow failure. Elderly patients have decreased bone marrow reserve and have an antecedent bone marrow stem cell disorder such as myelodysplastic syndrome (either recognized or not). At this time activity of other pathological agent become fatal for AML patients unless the tested agent gains the ability to repair the underlying bone marrow failure syndrome. In some cases advances in supportive care may allow older patients to tolerate severe myelosuppression. On the other hand, morphologic clearance of AML blasts without reconstitution of normal hematopoiesis has not been shown to improve overall survival of AML patients. It is possible that morphologic clearance of AML may facilitate allogeneic hematopoietic stem cell transplantation (HSCT) as a post remission therapy, i.e., a bridge to transplant (Cassileth et al; 1998, Matthews et al; 2001). In AML; a good cytogenetic abnormality also trumps other bad prognostic factors such as secondary leukemia...
and an associated myelodysplastic disorder. The presence of bad cytogenetics alone is a poor prognostic factor, especially in the clinical setting of secondary leukemia (Frohling et al; 2005, Mrozek et al; 2007).

The other prognostic factors of acute myeloid leukemia include the patient's age, white blood cell count, environmental risk factors, AML subtype and response to chemotherapy. Younger patients tend to have a better prognosis than older patients. Treatment outcome is worse in older patients than in younger patients in term of complete remission, overall survival and remission duration (Kantarjian et al; 2000, Munoz et al; 2003, Medeiros et al; 2010). Typically, the higher the white blood cell count, the worse the prognosis. Patients who achieve a complete remission within 4 to 5 weeks of starting treatment tend to have a better prognosis than those in whom this takes longer. Patients who don't achieve a complete remission at all have a poorer outcome.

Comprehensive epidemiological information on acute myeloid leukemia (AML) in India is not available. Most of the data are institution based, from large medical institutes or cancer centers with referral facilities. However there had been no attempt to systematically study the burden of acute myeloid leukemia in India and there is insufficient information to understand how the occurrence and outcome of the disease varies across the country. Keeping in view of the above information the present study of pathology, immunophenotyping and cytogenetic analysis of acute myeloid leukemia patients has been conducted with following objectives:

**Study objectives:**

1. To identify various hematological malignancies in relation to morphology, age and gender in Haryana population.
2. To study the pathological characteristics of acute myeloid leukemia.
3. Immunophenotyping of acute myeloid leukemia patients.
4. Cytogenetic analysis to find out karyotypic abnormalities of acute myeloid leukemia patients.
5. Prognostic formulation from cytogenetic, immunophenotyping and pathology of acute myeloid leukemia.