ABSTRACT

Acute leukemias are the most common type of childhood malignant illnesses. For this reason, their diagnosis is very important in clinics. There are many candidate molecular markers for diagnosis of acute leukemias today. Polyamines (PA) are ubiquitous cationic molecules, which provide cell homeostasis. They especially increase in rapid dividing cells. In many studies, PAs are shown as good prognostic and diagnostic markers in malignancies. Their usage in clinics is easy applicable and there are many methods for estimation of polyamines from samples. [Turk J Cancer 2004;34(1):5-10]

KEY WORDS:
Polyamines, ALL, childhood, putrescine, spermidine, spermine, tumor marker

INTRODUCTION

The acute leukemias are characterized by aberrant differentiation and proliferation of malignantly transformed hematopoietic progenitor cells. These cells accumulate within the bone marrow and, once a substantial burden of leukemia cells is present, lead to suppression of the growth and differentiation of normal hematopoietic cells. Although virtually any organ system may become involved, once leukemia cells enter the peripheral blood, the lymph nodes, liver, spleen, central nervous system, and skin are the most common sites detected clinically (1-4).

The first recognition of leukemia as a distinctive entity is usually accorded independently to Virchow in Berlin and Bennett in Scotland in 1845. In 1847, Virchow coined the term "leukemia" (from Greek leuk, white cells, and emia, in the blood) to replace "weisshame," a German term. Leukemic disorders are currently classified by their presumed cell of origin. Acute myeloid leukemia (AML) results from the malignant transformation of a bone marrow (myeloid) progenitor cell or stem cell, which is the normal precursor for granulocytes, erythrocytes, and megakaryocytes (2). Acute lymphoblastic leukemia (ALL) is a malignant disease of early precursor cells of the B cell and T cell lymphocytic lineages. ALL is known as the most common cancer type in childhood. Each year about 1200 patients who are under 15 years old were diagnosed with ALL in Turkey and mortality rate is 25% in the diagnosed population. ALL in childhood peaks at approximately 4
years of age and accounts for 80% of all acute leukemia. In contrast, adult-onset ALL occurs predominantly in an elderly (older than 65 years old) population and accounts for only 20% of cases of acute leukemia, whereas AML accounts for the majority of cases in adults (5).

The cause of ALL and also AML is unknown however; epidemiological studies suggest some factors associated with an increased incidence of the disease. These include the presence of Down’s syndrome, Fanconi’s anemia, neurofibromatosis, Bloom’s syndrome and ataxia-telangiectasia. In addition to congenital predisposition, environmental toxins and infectious agents including ionizing radiation, benzene, cytostatic agents, and viruses have been implicated in the pathogenesis of this disease (6).

Generally, patients frequently have severe anemia, thrombocytopenia, and granulocytopenia with resulting pallor, fatigue, spontaneous bruising, bleeding, fever and infection. Extramedullary disease, manifesting mainly as asymptomatic enlargement of the liver, lymph nodes, spleen or all of these is present in the majority of patients. A definitive diagnosis of ALL or AML requires examination of bone marrow and peripheral blood analysis (6-7). Immunological, cytogenetic and molecular analysis have provided good diagnosis for acute leukemias (8-9). However, new type of markers to understand cancer grade and prognosis are needed.

The natural polyamines (PAs); putrescine (Put), spermidine (Spd) and spermine (Spm) are ubiquitous low-molecular aliphatic amines that play multifunctional roles in cell growth and differentiation have important functions in cellular processes and they are widely spread in the human body (Figure 1). They are cationic small molecules, which are attached to negative charged part of cells. As a result of this activity they provide stability in DNA, RNA and cell membrane. They provide homeostasis inside the cell. When their concentration increases, cells are divided rapidly and loose the self-control. In contrary, if their concentrations decrease, PA depletion occurs and cells cannot survive (10-15).

About the usage of PA as a marker in clinics there are two different ways. One of them is estimation of extracellular PA levels circulating in body; other is intracellular PA level in problematic tissues. However, the rise of PAs in tissues and biological fluids is not only specific for malignant states but is also found in proliferating tissues in diseases such as pernicious anemia, hemolytic anemia, polymyositis, pulmonary tuberculosis and psoriasis (16).

**Extracellular PA levels circulating in body**

During past years the researchers have tried to gather information on extracellular PA, blood PA in particular, and they have tried to answer the following two questions: 1.) "What is the diagnostic significance of blood PA?" and 2.) "Is it possible to use the information obtained from blood PA determination in antiproliferative cancer therapy?" In fact, extracellular PAs are not only blood PAs. Cerebrospinal fluid and urine are the other main centers for estimation of circulating PAs (17).

PA concentrations and their acetyl conjugates increase significantly in the biological fluids and the affected tissues of cancer patients. The first known study about estimation of circulating extracellular PAs in body fluid was done by Russell in 1971 (18). Also Lee et al. (19) have revealed the importance of PA levels in leukemia prognosis. Abdel-
Monem and Ohno (20) suggested that the amount of N-acetylputrescine and N1-acetylspermidine were increased in cancer patients. Also elevated ratio of N1-N8 acetylspermidine was reported in patients with non-Hodgkin’s lymphoma. Moreover, diacetylpolyamines were found in healthy human urine as regular constituents and increased much more frequently than any other PAs in human malignancies. Lee et al. (19) revealed elevated PA concentrations in urine of leukemic patients before the onset of chemotherapy. Also Russell (18) examined the PA excretion especially Spd, in hydrolyzed urine of adult patients with leukemia. It was reported that higher amounts of N1, N8-diacSpd and N1, N12-diacSpm were excreted in the urine of patients with malignant disease. In the same study Lee showed significant N1, N12-diacSpm in the urine of patients with leukemia than any other PAs (18-19).

Marton et al. (21) showed that cerebrospinal fluid PA levels had better followed-up after operation medulloblastoma. By the end of 70’s the unclear biochemical view of the potential roles of PAs as markers for cancer led to an almost interesting pathway for their diagnostic usage (Figure 2). There are many thoughts about circulating PAs; one of them is how they can be carried in blood? After many investigations two principal of transporter of PAs in blood were identified. Peripheral blood cells especially red blood cells (RBCs) and specific proteins were consisted essentially of transporter groups in blood. The major protein groups as a transporter are antipolyamine antibodies (22,23). They consisted of very lower percentage of human serum as well as in rabbit serum. In lung cancer patients their immunohistochemical identification were done, but the data were not significant for malignancy.

Fig 2. Polyamine metabolism. Biosynthesis pathways and catabolism enzymes
Yet, free PAs in blood were not immunogenic because of their small size. In another study lipoproteins are another carrier in blood for free PAs. Especially, lipospermine complexes have been found in some cases for cancer patients. Also distribution of noncovalently bound PA to plasma lipoproteins might be an explanation for fossell index of cancer (24).

In blood, the proportion of PAs which is bounded to a transporter protein does not exceed 2% of total blood polyamine pool (25). Therefore, transporters have minor importance. Most of the circulating Spd and Spm is transported by RBCs. Also 98% of Spd and Spm exist in a free form in the cytosol of an RBC. Using multivariate analysis some researchers have established in childhood acute leukemias, at diagnosis, clinical usefulness of RBC PA levels if they are used together with other clinical criteria (26).

In leukemic diseases the main diagnostic factors are WBC count, immunohistochemical identification of some antigens, nowadays cytogenetics methods and also some biochemical markers. However, sometimes for diagnosis and to get the responses of therapy these laboratory data may not be sufficient. Bergeron et al. (29) explored whether RBC PA level could be a biological parameter for the measurement of the leukemic risk in ALL and could be of help to discriminate between good and poor prognosis particularly in ALL with intermediate WBC counts. In their study Spd and Spm levels are correlated, but the power of discrimination of Spm is always more important than Spd. On the other hand, they could not find any correlation between RBC PA level and WBC count in ALL cases (27-29).

On the other hand, RBC PA levels can be detected for therapeutic follow up marker. For instance in 100% of cases after bone marrow transplantation, the first signs of bone marrow regeneration were preceded one to three weeks by an increase in RBC PA levels denoting the early appreciation of bone marrow graft survival (28).

Central nervous system (CNS) leukemia remains a significant clinical problem in the management of childhood leukemia. Its diagnosis is possible only after morphologically immature cells are identified in the cerebrospinal fluid. Since early diagnosis of the disease is desirable, the study of sensitive markers for CNS leukemia was undertaken. Cerebrospinal fluid PAs have been shown to be of value in monitoring patient status and response to therapy of CNS tumors (30). Also Rennert et al. (31) investigated the use of cerebrospinal fluid PAs as indicators of CNS leukemia in children with acute lymphoblastic leukemia. Many studies showed that CNS leukemia patients PA levels in cerebrospinal fluid might provide a reliable index for leukemic disorder. In another study Smith et al. (26) measured cerebrospinal fluid PAs in non-Hodgkin’s lymphoma and ALL, ranging from complete remission to active CNS and/or bone marrow involvement. Heby and Anderson (32) found that PA elevations correlated with tumour cell death rather than tumor cell proliferation and suggested that tumour cell death was the most likely cause of increased PAs in the physiological fluids of cancer patients.

Based on data from animal and patient studies it was proposed that spontaneous cellular death and lysis of tumor resulted in high levels of PAs in urine and serum of untreated patients. Also, cell killing due to chemotherapy increased the release of PAs from tumour cells (33). Garnica et al. (16) showed that amonafide receiving AML patients have different PA levels in plasma after treatment. They also established pharmacokinetics of amonafide due to PA levels in plasma.

Also pretreatment of PA values could be used to evaluate a number of tumor characteristics including tumor size, growth rate and prognosis. For example, according to Lee et al. (19) urine samples from leukemia patients were good markers for identification of disease grade. They also found that N\(^1\)-acSpd/N\(^8\)-acSpd was a good diagnostic marker for leukemia patients.

**Intracellular PA levels**

Intracellular polyamines have an important role in the proliferation of normal and malignant cells. The recognition of their critical role in cell growth and differentiation has led to the development of several inhibitors of polyamine biosynthesis (34). Therefore, intracellular PA metabolism is closely associated with the cell cycle and ornithine decarboxylase is a key enzyme of PA biosynthesis. *In vivo*
studies have shown that RBC PA levels can be used as an index of neoplastic and normal cell proliferation (35-36).

Rennert et al. (31) have established that naturally occurring PAs have a great potential in clinics and they might be good markers for malignancy. They estimated PA levels in bone marrow of several non-leukemic diseases as control group and compared to bone marrow aspirates of children with leukemia. They found marked elevation of Put in all leukemic samples. Also they determined similar increment in Spd and Spm ratios.

Miale et al. (37) showed significant increment in Put levels, which coincided with an increase in the abnormal lymphoblastic compartment of the bone marrow after relapse. Also they established that high bone marrow cellularity was similar with high PA levels. Buyuktuncer et al. (38) suggested intracellular PA measurement in leukemia diagnosis as a biological marker. In their study PA levels was found high in leukemic patients when compared to control subjects. Especially Spm level in leukocytes which are the abnormal cells in peripheral blood was found 40 fold increased. They also noticed that PA measurement in acute leukemias as a clinical data is cheap and usable in laboratory.

CONCLUSION

Tumor markers in future shall be cheap and easy, applicable in clinics. On the other hand they shall be more specific and sensitive. Therefore diagnostic and also therapeutic biological markers are still the main topic of researchers. PA metabolism is a good point for cancer studies. Production of antineoplastic agents using PA pathway in the cell are still studied in phase III trials. Also estimation of PAs in different body parts are expected to be future diagnostic tools. Also circulating PAs are accepted markers of cellular proliferation. As demonstrated PA depletion of the tumor bearing animals, extracellular PAs participate in supporting the state of malignant proliferation. Therefore, determination of circulating PAs which have similar characteristics to intracellular PAs in proliferating cells will be important tumor markers in the future.

References