INTRODUCTION

Genetic factors appear to contribute to virtually every human disease. Cancer is an uncontrolled proliferation of cells or clones depend on its own symptoms and characteristics. Survival of the rapidly renewing tissues of long-lived animals like man requires that they be protected against the natural selection of fitter variant cells is spontaneous appearance of cancer. Cancers are caused by substances that damages DNA and mutagenic in nature – radiation and chemicals that can penetrate to the nucleus and damage DNA.

Leukemia is a clonal disease or cancer of the blood that develops in the bone marrow. In other words it's a proliferative disorder of the leukopoietic cells. The complex origin and nature of this disorder allow its classification into acute and chronic myeloid or lymphoid types based on life expectancy but now are classified according to their cellular maturity. Acute leukemia’s consist of predominantly immature, poorly differentiated cells and chronic leukemia’s have more mature cells.

DIAGNOSTIC ASSAYS BASED ON BLOOD SAMPLE ANALYSIS ARE ATTRACTIVE BECAUSE OF THE SIMPLICITY OF SAMPLE COLLECTION. ACCURATE ANALYSIS OF TUMOR MARKERS IN BLOOD FROM CANCER PATIENTS COULD HAVE SIGNIFICANT IMPACT IN FACILITATING THE SCREENING, DIAGNOSIS, AND MONITORING FOR DISEASE RECURRENCE AFTER INITIAl THERAPY.

ONE OF THE MOST IMPORTANT TOOLS UNDERLYING THE REVOLUTION IN MEDICAL GENETICS IS THE ABILITY TO VISUALIZE SEQUENCE DIFFERENCES DIRECTLY IN DNA AND INFORMATION ABOUT DNA SEQUENCE VARIATION WILL HAVE A WIDE RANGE OF APPLICATIONS FOR ANALYZING DISEASE AND DEVELOPING DIAGNOSTIC, THERAPEUTIC AND PREVENTATIVE STRATEGIES. GENOMIC DNA IS THE OPTIMAL RESOURCE TO ANALYZE QUESTIONS CONCERNING GENETIC CHANGES THAT ARE RELATED TO ONCOGENESIS.

WORKING WITH LEUKEMIA’S, AFTER THE EXTRACTING THE DNA FROM THE PLASMA OR BLOOD, DETECTABLE AMOUNTS WERE FOUND ONLY IN PATIENTS WITH ADVANCED MALIGNANCIES BEARING A LARGE TUMOR CELL BURDEN. IN TWO CASES WITH PROGRESSIVE CANCER WHERE A SECOND DETERMINATION COULD BE PERFORMED IN THE
course of the disease, an increased plasma DNA concentration was measured suggesting a relation between this parameter and tumor evolution. The central purpose of the present study was to compare the quantity of genomic DNA in various leukemia patients such as CML, AML, CLL and ALL to detect the genetic variation accordingly to their disease status.

MATERIALS AND METHODS

Patients

The study is concerned with four Leukemia patients (One from each category - CML, AML, CLL and ALL) selected on the basis of their blast crisis and stage level. All the four leukemia patients visited JNCH&RC for treatment after the diagnosis and received chemotherapy.

Blood Samples and Isolation of DNA

1 ml of Peripheral blood samples were collected in a sterile 10% EDTA centrifuge tubes and thawed the samples to avoid clot formation. DNA was isolated immediately using standard whole blood DNA isolation kit (Bangalore Genei – Cat # KT-23) according to the manufacturer’s protocol. DNA from all samples was analyzed qualitatively by electrophoresis on 0.8% Agarose (SRL, Mumbai) in 1 X TAE buffer in a horizontal apparatus (Bangalore Genei) with a constant power of 150 Volt and quantitatively by spectrophotometry (Shimadzu, Japan) at 260 nm to determine the DNA concentration and purity. Finally, for comparison Low and high molecular weight DNA ladders (Bangalore Genei) were loaded with the test DNA Samples on both the ends of the gel respectively. The gel was visualized on a UV – Transilluminator (Bangalore Genei) and photographed in a Gel documentation system (BD Biosciences).

The results were estimated on the basis of quantification values and simple band sharing ability of leukemic DNA with the markers.

RESULTS AND DISCUSSION

The Present analysis was performed to detect the simple genetic variation in different types of leukemia – CML, AML, CLL and ALL. Patients selected for the study was slightly elevated in their blast crisis and disease stage. For comparison, the DNA pattern of the entire four patients was analyzed with low and high molecular weight markers Fig. 1. DNA concentrations obtained from all the leukemia patients were presented in Table 1. In all the four cases CML patient’s DNA was found to be increased concentration in quantification and in addition a smeared band weighed around 150 bp was observed. It concludes that the elevated amount of DNA in CML. Next, CLL was noted with slight variation with a single DNA fragment and increased in quantification. In the case of AML and ALL there is no variation in DNA concentration and a single band was noticed. Positively, the quantification values also proved as same.

Table 1: DNA Quantification values of leukemia samples – Comparative chart

<table>
<thead>
<tr>
<th>Leukemia samples</th>
<th>UV – Spectrophotometer qualification OD 260-280 nm g/μl</th>
<th>Concentration of DNA μg/μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td>0.985</td>
<td>4.93</td>
</tr>
<tr>
<td>AML</td>
<td>0.968</td>
<td>4.83</td>
</tr>
<tr>
<td>CLL</td>
<td>0.979</td>
<td>4.89</td>
</tr>
<tr>
<td>ALL</td>
<td>0.968</td>
<td>4.83</td>
</tr>
</tbody>
</table>

Fig. 1: Agarose gel electrophoresis shows DNA fragments of leukemia patients. LMW – Low molecular weight marker, Lane 1 – CML, Lane 2 – AML, Lane 3 – CLL, Lane 4 – ALL, HMW – High molecular weight marker.
This correlation study proves that the elevated DNA concentration was found only in the chronic stage of leukemia's as compared to acute leukemia's. Cancer patients tended to have higher levels of circulating DNA than healthy controls. So far the previous work performed in leukemia and its DNA studies showed an increased amount of DNA concentration in plasma and serum samples. Although, we applied two markers with different size and range, the band pattern of the patients was more than the reference range of the ladders i.e. above 10 Kb in size. For further analysis the High molecular weight markers are suggested to obtain a good interpretation.

The present study was performed to test the proposition that the genomic DNA of every human cell contains whatever information is necessary and sufficient for transformation to malignancy.

DNA is present in increased amounts in plasma serum DNA of cancer patients. Cancer patients tended to have higher levels of circulating DNA than healthy controls. Persistently high or increasing DNA levels were associated with a lack of response to treatment. Several studies described an increase in circulating DNA in several malignancies and an association between increased plasma DNA and disease recurrence was suggested. This reveals that accumulation of high DNA will act as a molecular tool in diagnostics.

The advent of molecular biology & genetics has allowed identifying the genetic mechanisms that are involved in the pathogenesis of disease states. The molecular alterations associated with these genetic abnormalities have provided insights in to mechanisms trough which the disease state is generated and have provided a direction in which the genetic therapies of leukemia can be developed. On the basis of these analyses, patient specific molecular markers were established that turned out to be a very good source for monitoring leukemia and minimal residual disease (MRD).

REFERENCES

8. Leon SA, Shaprio B, Sklaroff DM and Yaros MJ. Free DNA in the serum of cancer patients


