# Purification and characterization of DNA from whole blood of children with ALL and normal children

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## Summary:

***Background:*** *The acute lymphocytic leukemias (All) make up about 76% of children leukemias . lymphoid leukemias occur more often than expected in patients with immunodeficiency , chromosomal abnormalities & ataxia _ telangiectasia . A number of chnges occur in preneoplastic & neoplastic cells as the progress towards a greater degree of malignancy . Nuclear DNA may be used as an aid in diagnosis , to predict prognosis & to determent of certain neoplasia.*

***Aim of the work:*** *is to purify DNA from whole blood of predict & normal children & to characterize it by spectral studies.*

***Patients & Methods:*** *Fifteen EDTA-treated blood samples of children with acute lymphoblastic leukemia (ALL) and the same number from normal children were used to isolate DNA from whole blood, then to characterize and compare the DNA of ALL patients with normal individuals. Typical ratio of 260nm/280nm absorbance ( used to assess purity ) for DNA*

***Results:*** *purified were 1.5 and 1.3 for ALL patients and normal children respectively. No real differences between DNA of ALL patients and normal DNA, when characterized by using UV spectrum at PH (7), were found. Infrared spectroscopy in the 4000-200 $cm^{-1}$ region was used to characterize the DNA structure of ALL patients and normal children. A set of IR bands characterize of DNA conformations was proposed.*


***Key words:*** *DNA, Purification, Spectral analysis & ALL.*

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## Introduction

The acute lymphocytic leukemias (ALL) make up about 76% of childhood leukemias, with a peak in incidence around the age of 4 years. ALL occurs slightly more frequently in boys than in girls, several reports of acute leukemia in children have suggested some common environmental factor in etiology, but careful statistical analyses have not support this possibility. Lymphoid leukemias do occur more often than expected in patients with immunodeficiency, chromosomal abnormalities (e.g. Down Syndrome ), and ataxia-telangiectasia. (1) A number of changes occur in preneoplastic and neoplastic cells as they progress towards a greater degree of malignancy (2). These alternations include genetic changes, epigenetic changes, surface alternations and alternation in intercellular interactions. In some instances, these changes are contributing factors to the degree of pathology noted, whilst others are resultant. In many situations, the relationship between these changes and the progression towards neoplasia is not understood(3,4). Nuclear DNA may be used as an aid in diagnosis, to predict prognosis and to determine management of certain neoplasia (5-7). Ultraviolet light absorbance measurements were made from many purposes: to determine the concentration of a substance, to assay certain chemical reactions, to identify material, and to determine the structural parameters of macromolecule (8). In addition to that the ratio between the readings at 260nm and 280nm ($A_{260}$ / $A_{280}$) provides an estimate for the purity of the nucleic acid (9).

The value of IR spectral analysis comes from the fact that the modes of vibration of each group are very sensitive to changes in chemical structure, conformation, and environment. Therefore, there are many applications of infrared spectroscopy, like; identification of the number of hydrogen bonds and the functional groups and measurement of their breakage during denaturation, identification of tautomeric forms, interaction between small molecules, such as riboflavin and adenine, and determination of the ratio of AU to GC pairs in tRNA (8,10,11).

In this paper, DNA was purified from whole blood of children with ALL and normal children by using a simple, rapid method, then characterized by

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spectral studies. A work was under taken to identify the UV spectrum of the purified DNA and IR intensities characteristic of the DNA in the vibration region between 4000 and 200 $cm^{-1}$ .

**Patients and methods:**

Children with ALL were patients of the Clinic of AL-Manssor Pediatric Oncohaematology, Medical city. All children were within the active stage of disease and 1-11 years old. A significant proportion of patients have white blood cell counts between 25000/$mm^3$ and 50000/$mm^3$, and about 20% have counts greater than 50000/$mm^3$. while normal children have counts between 5000/$mm^3$ and 15000/$mm^3$ DNA was purified from whole blood of fifteen healthy children, and fifteen children with ALL. DNA was extracted and purified by the method of Adell and Ogbonna (12). The whole blood was mixed with a lysis buffer (10 m mole/L Tris-HCL, pH 7.5 containing 300 m mole of sucrose and 10 mL of Triton X-100 surfactant per liter) to lyse the cells. A fraction containing nuclear material of the leukocytes, obtained from this mixture by centrifugation, was suspended in a buffer containing strong protein-denaturing agents (100m mole/L Tris-HCL, pH 7.0 containing 10m mole of EDTA, 8mol of urea, and 10 gm of SDS per liter). The dissociated DNA was extracted twice with phenol/chloroform to remove most of the proteins. The sample was rich in DNA at this stage but sill contained some protein. The crude DNA was purified by Sephadex G-25 spin column. The sample was applied to the column and purified DNA was recovered in the first three fractions. DNA concentration was measured by using Burton method (13), standard curve was prepared by dilution the stock solution of Calf-thymus DNA to (10, 20, 40, 80, 100, 200)µg/ml. Purified DNA from children with ALL and normal children were used, with concentration of 20µg/ml, against the blank of TED buffer (10mmole/L Tris-HCL, pH 7.0 containing 1m mole of EDTA per liter) at the wavelength from 200 to 300 nm. Two absorption spectrum of purified DNA were plotted at pH 7. The UV spectra were recorded with a Shimadzu 160 UV- visible recorder spectrophotometer. Bach of purified DNA samples with concentration of ( 25µg/ml) for children with ALL and normal children at pH 7 were lyophilized. Infrared absorption spectra of DNA were obtained by using DNA films. The water content was rigorously controlled, as this parameter is essential to stabilize the structure. The IR spectra were recorded with a Perkin Elmer 180 double-beam ratio recorder spectrophotometer. Statistical analysis was performed by Student's t-test.

**Results and discussion:**

The yield and purity of DNA samples, which were isolated from ALL patients whole blood, were higher than these of normal children whole blood (Table 1), and this may be related to the difference in leukocyte counts. As, DNA concentration in human blood varies considerably among individuals, depending on leukocyte counts(12).

Adell and Ogbonna (12) have used a simple spin-column method for purification of DNA from whole blood of myocardial infarction patients. The DNA prepared by this rapid procedure is essentially pure and suitable for numerous applications. The average yield (24.5 µg per milliliter of whole blood) is lower than that obtained by the proteinase K digestion method (14), probably because of the partial recovery of the aqueous phase during the phenol/chloroform extraction step.

Purified DNA samples of children with ALL and normal children were studied by using UV light. Figure (1) show that DNA molecules have the same λmax at (220 and 260) nm at pH 7 for both children with ALL and normal children, except that there was increased for approximately three times in absorbance of peak II (260nm) for DNA sample of normal children than that of children with ALL. This is may be due to several spontaneous hydrolytic reactions, which cause DNA damage. For example, it has been estimated that several thousand purine bases are lost daily from the DNA in each human cell. If they are not immediately replaced, proper base pairing cannot occur, thereby causing a transition type of mutation after the next replication(15).

Most of biological macromolecules absorb ultraviolet (UV) light in a range of wavelengths that is easily measured, as a result of their containing aromatic rings. The absorption spectra of some of the amino acids and of the nucleotide bases in nucleic acids have been well studied and are of great use both in identifying substances and in determining the structure of proteins and nucleic acids. (16)

The absorption spectra of the five bases contained in DNA and RNA are rather similar (though distinguishable), with λmax ranging from 250 to 275nm. All DNA molecules, have the same λmax (259nm) and have nearly indistinguishable spectra, unless, a particular base pair is present in great excess. The most important aspect of nucleic acid absorption spectroscopy is the decrease in the absorbance of the nucleotide bases that occurs when an oligonucleotide forms (8,16).

The IR spectra of purified DNA at pH 7 from whole blood of children with ALL and normal children are presented in figure ( 2:a,b ), between 4000 $cm^{-1}$ and 200 $cm^{-1}$. The wave numbers of the main absorptions as well as their intensities are summarized in (Table:2). From these results some differences between the two spectra were detected :

a. The weak band of DNA purified from whole blood of children with ALL at 1390 $cm^{-1}$ reflects in-plane vibrations of the adenine base

residues involving the N-H and C-H in plane deformation modes(17). This band disappeared in the spectrum of normal DNA.

b. The band (1120-1140) $cm^{-1}$ appeared as a medium band in normal DNA infrared spectrum, while it appeared as a shoulder in the spectrum of DNA isolated from children with ALL. This band reflects symmetric stretching vibration of the phosphate group of thymine residue (17).

c. The band (760-770) cm-1 appeared as a weak band in normal DNA infrared spectrum, and disappeared in the spectrum of DNA isolated from children with ALL. This band reflects out- of- plane ring vibration of cytosine residue(18).

d. The strong band of normal at 1440 $cm^{-1}$ involves the motion of the N7 atom of guanine (18). This band appeared as a weak band in the spectrum of DNA from children with ALL.

In summary, these observations clearly indicate that the DNA prepared by the spin-column procedure is suitably for UV analysis and IR studies. Finally, these results indicate that purified DNA from human whole blood of children with ALL and normal children can be distinguished by IR spectroscopy technique.

**Table (1) : Purity and yield of DNA from whole blood of normal children and children with ALL**

| Sample | Purity ($A_{260}/A_{280}$) Mean ± SD | Yield (µg/ml) Mean ± SD |
|---|---|---|
| Normal children | 1.3 ± 0.3 | 20.0 ± 2.5 |
| Children with ALL | 1.5 ± 0.4 | 30.1 ± 3.5 |

**Table (2) : infrared wave numbers and intensities of DNA bands.**

| Normal DNA | | DNA from children with ALL | |
|---|---|---|---|
| Wave number ($cm^{-1}$) | Intensity | Wave number ($cm^{-1}$) | Intensity |
| 3500-3100 | s | 3400-3200 | m |
| 2900 | m | 2900 | m |
| 1580-1660 | s | 1580-1630 | s |
| 1500 | s | 1500 | m |
| 1440 | s | 1440 | w |
| 1120-1140 | m | 1390 | w |
| 1040 | s | 1120-1140 | sh |
| 760-770 | w | 1040 | s |

**Abbreviations: s: strong, m: medium, w: weak, sh: shoulder**

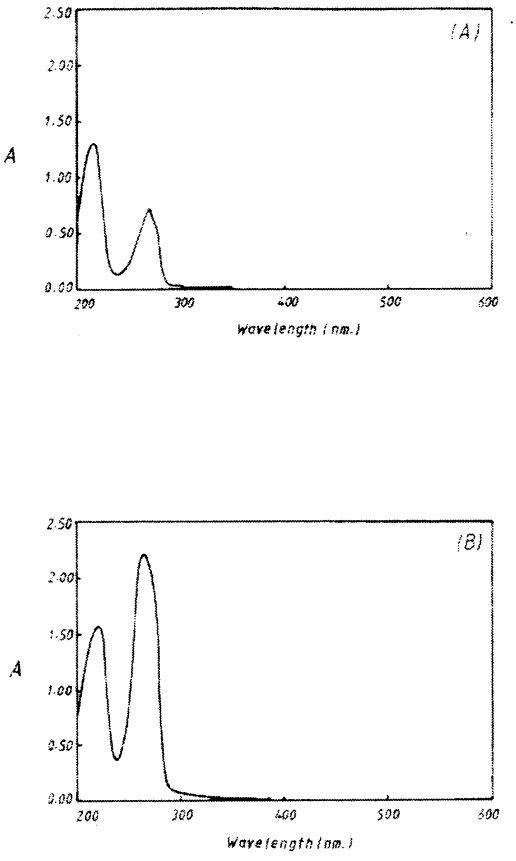


Fig.(1): Ultraviolet absorption spectra of purified DNA at pH(7), (A) DNA from whole blood of children with ALL, (B) DNA from whole blood of normal children.

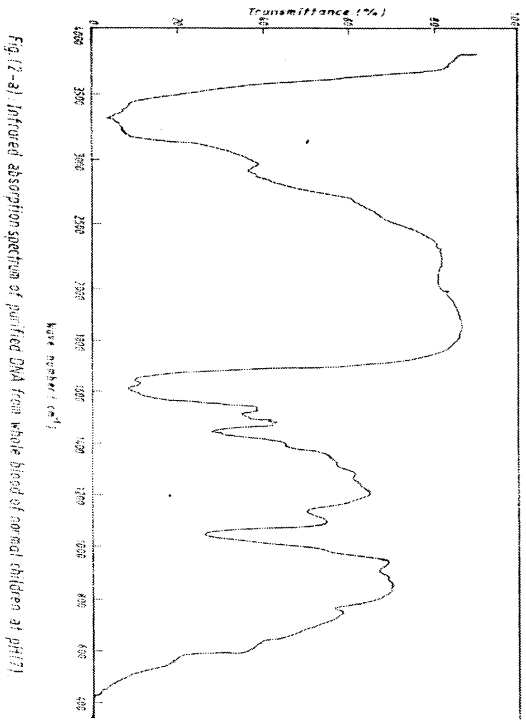

Fig (2-a): Infrared absorption spectrum of purified DNA from whole blood of normal children at pH (7)

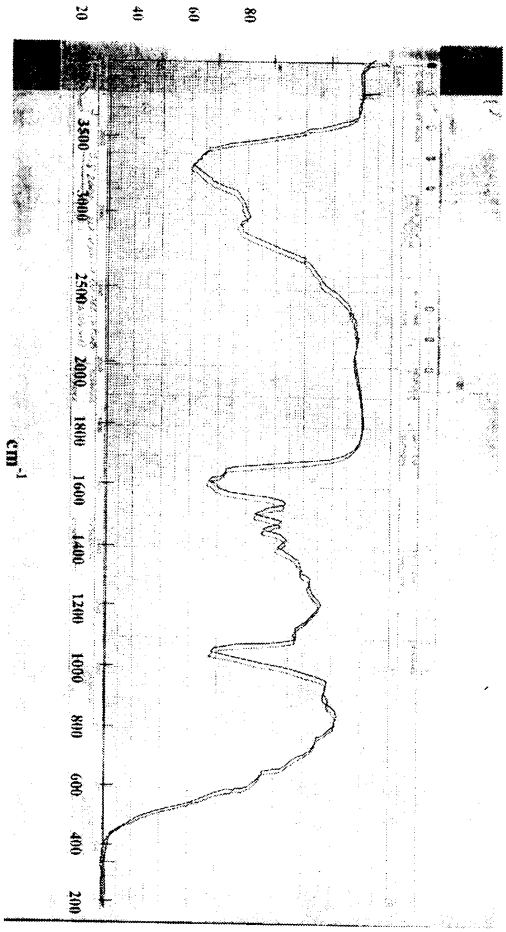


Fig. (2-b): Infrared absorption spectra of purified DNA from whole blood of children with ALL at pH (7).


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