Study serum and saliva samples of leukemic and compare them with Normal Objects by UV-Visible Spectroscopy

Luma N.Joudah[1] Hayder H. Husain[2] Munther B.Hassan[2]

[1]College of health and medical technology Baghdad

[2]Department of Physics Faculty of Science Kufa University.

hyder_ham@yahoo.com

Abstract

A technique of UV-visible spectroscopy was employed to study the spectral differences in the serum and saliva of healthy and leukemic patient. This is based on the differences in the spectral signatures .Took the serum of blood and saliva from the same patient was taken to examine them by UV –visible light spectroscopy techniques.

This technique help in identify the leukemia and its type with high accuracy by easy and fast physical methods .It found the absorbance of patients larger than normal.It is method to measure the leukemia at any type in serum and saliva based on increasing the absorption as supported by usual method in hospitals. The results of this study showed anew efficient method for diagnose or detection the leukemia disease.

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Keywords: UV-Visible spectroscopy, leukemia, spectra of serum human.

دراسة عينات المصل واللعاب من اللوكيميا ومقارنتها مع الكائنات الطبيعية بواسطة مطيافية الأشعة فوق البنفسجية المرئية

لمى ناجي جوده ، [1] حيدر حمزه حسين [2] منذر باقر حسن [2]

[1]قسم الاشعه /كليه التقنيات الصحيه والطبيه/جامعه التقنيه الوسطى /بغداد

[2]قسم الفيزياء /كلية العلوم /جامعة الكوفة

الخلاصة

تقنية مطياف الأشعة فوق البنفسجية - المرئية استعملت لدراسة الفرق في الاطياف في اللعاب والأمصال السليمه والمصابة باللوكيميا . وتعتمد على الاختلاف في زيادة الامتصاصيه في حالة وجود المرض كما مدعم وجود المرض بالطرق المعتاده بالمستشفى مقارنة مع الطبيعي . ناخذ سيرم الدم واللعاب من نفس المريض المصاب باللوكيميا ونفحصه بتقنية مطياف الاشعه فوق البنفسجيه- المرئية هذه التقنيه ساعدتنا في معرفة وجود اللوكيميا ونوعها وبدقه عاليه بواسطة طريقه فيزياويه سريعه وبسيطه والنتائج التي تم الحصول عليها في هذه الدراسه اظهرت لنا اختلاف بالامتصاصيه وشكل وموقع القمه بشكل واضح كما ان النتائج اوضحت زياده بالامتصاص في حالة سيرم ولعاب مريض اللوكيميا مقارنة مع سيرم ولعاب الطبيعي.

الكلمات المفتاحية : تقنية مطياف الاشعه فوق البنفسجيه-الضوء المرئي ، امتصاص ، اللوكيميا ،مصل ، لعاب

Introduction:

Ultraviolet/visible (UV/VIS) absorption spectroscopy has been used in the clinical laboratory for many years. The technique has appeal, as it is almost universal in its application[1]. These analyzers normally utilize a range of analytical techniques such as ion-selective electrodes, turbid metry and UV/VIS absorption in one integrated instrument. Reagent manufacturers are also trying to simplify the analysis even more over so that much simpler instrumentation is required[1]. Cancer is a multi-step process resulting from the accumulation of irreversible and transmittable genetic mutation together with the concurrent presence of epigenetic alterations in susceptible cells[2,3].These alterations contain thousands of mutations [4]. show altered responsiveness to microenvironment [5].

Leukemia is a malignancy (cancer) of blood cells. In leukemia, abnormal blood cells are produced in the bone marrow. Usually, leukemia involves the production of abnormal white blood cells, the cells responsible for fighting infection. However, the abnormal cells in leukemia do not function in the same way as normal white blood cells. The leukemia cells made up mostly of fat cell .found in the inside of a middle of the long bones. White blood cell (lymphocytes)red blood cell and platelets are produced in a red marrow. Red blood cells carry oxygen ,white blood cells fight diseases , platelets are major for blood clotting[8].

The aim of this study was to use the ability of physical instruments as new method based on using the (UV-VIS)spectroscopy technique to diagnose the leukemia by using the samples of serum and saliva .

continue to extend and divide, finally, crowding out the normal blood cells. The end result is that it becomes hard for the body to fight infections, control bleeding, and transport oxygen[6].Leukemia usually begins in the bone marrow, the soft material in the center of most bones where blood cell are formed[7] .There are two types of bone marrow. The first type ;Red marrow made up mainly of myeloid tissue . found in the flat bones such as the breast bone ,skull, vertebrae, shoulder blades , hip bone and ribs. So this can be found at the ends of long bones , such as the humerus and femur. The second type ;yellow marrow ,

MATERIALS AND METHODS

Samples collections

In this study blood and saliva samples were collected from teaching Baghdad hospital (blood disease)and tumor hospital .the samples were collected from (16) patients (males and females),age range between (15to73)years .Healthy group samples were collected from (16) subjects, with the same age range and divided into sex age group (15-25y),(26-35y),(36-45y),(46-55y),(56-65y),(66 -75y) respectively.

*Drawing the blood from the patient in the hospital from brachial vein then divided it ready for the examinations and collect saliva from the same patient .

*After centrifuging the blood and taking the serum labeling the samples then they are kept to prepare for examination by UV-Visible light spectroscopy

Samples preparation

Blood samples collected5mlor 4mlof blood withdrawn from brachial vein from donors using tunica and syringe. Empty the withdrawn blood from syringe into tube with white cover (without anti-coagulant factor) slow and on the wall of the tube to avoid broken of blood cell (Hemolysis). Serum was prepared by placing the test tubes of blood at centrifuge instrument.

Serum was isolated by micropipette volume (1ml) and divided into two part by put it in another two test tubes with white cover .The test tubes were frozen to keep for examination it by (UV-VIS) after labeling the tubes. Collect the amount of saliva directly from the mouth of patient and put it in test tubes with white cover then freezing it to keep for examination by (UV-VIS) spectroscopy after labeling it.

Ultra-violet visible spectroscopy measurement for serum and saliva

Take (0.025ml)of kept serum by micropipette then Put (3ml)of deionizer water in the tube and removed from the water (0.025ml)and added the measured serum to the test tube .

The tube shakes circularly to mix well. The instrument was blank by put deionizer water (diluting solution)at the two cell which accessed with the instrument The (deionizer water and serum) which wanted for measurement put on one the two quartz cell and the other remained. Then the spectrum was recorded to detect the leukemia. The same technique used for saliva with observed that the labeling put on each sample.

Results and Discussions

The UV-Visible spectroscopic study of blood and saliva in health and diseased people has already been reported[9,10]. Various plasma and cellular constituents reflect physiological and pathological changes that take place in the tissues. This variation was explored for understanding the spectral UV-Visible spectral differences between normal healthy sera and that affected with certain diseases.

UV-Visible spectral analysis

Table (1) showing the absorption of serum samples for normal and patient with leukemia at each age group and table (2) showing the absorption of

saliva samples for normal and patient with leukemia at each age group

Table 1:The absorption of serum samples for normal and patient with leukemia at each age group.

Age group	278 nm		345 nm		411nm		541nm		576nm	
	L	N	L	N	L	N	L	N	L	N
15-25y	2.898 2.885 2.760 2.859	1.40 1.289 2.212 1.238	- - - -	- -	0.163 0.087 - -	0.063 0.084 0.071 -	- -		0.050 0.023 - -	0.013 - 0.012 -
-35y	*0.775 2.536 2.778 *2.136 2.898 *1.763	1.321 1.44 1.045 1.643 2.276 1.437	- - - -		0.165 0.186 - 0.358 - 0.039		- - - 0.059 - -		0.031 - - 0.056 - 0.040	
-45y	2.112 2.371 2.627	1.289 2.178 1.238	- - -	0.045	0.132 0.105 0.089	0.077	- - -		- - -	0.006 - -
-55y	2.246 2.547	1.437 2.083	- 0.942		0.271 3.836	0.124 0.211	0.038 0.472		0.046 0.498	
-65y										
-75y	2.254	2.276	-		-	0.099	-		-	0.019

The overlay UV-Visible spectrums of normal and leukemia sera are presented in figure 1,2,3,4,5,6,7,8,9 and 10.

a. Serum samples

Leukemia disease sera in each group of age are analyzed by the characteristic absorption in UV-Visible spectral region.

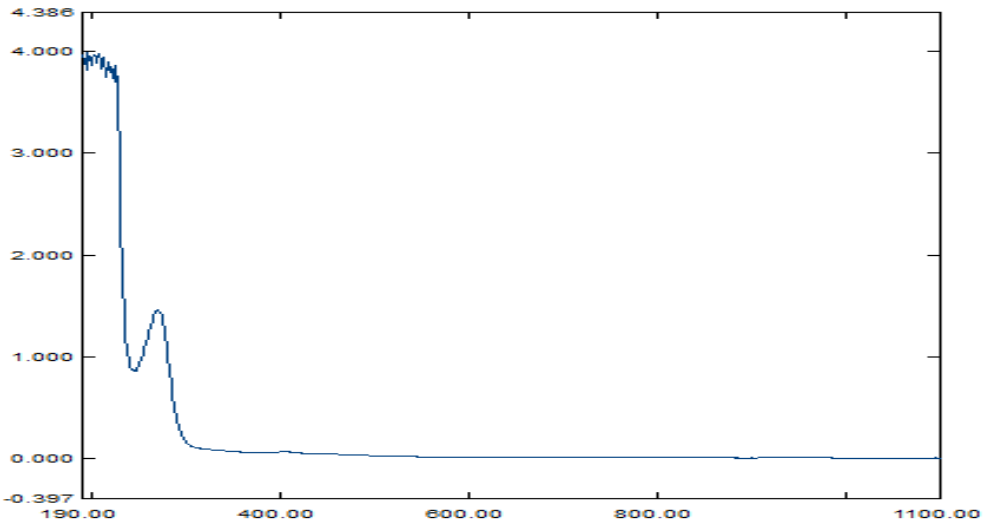


Figure (1) :UV- visible spectrum for normal serum sample at first age group.

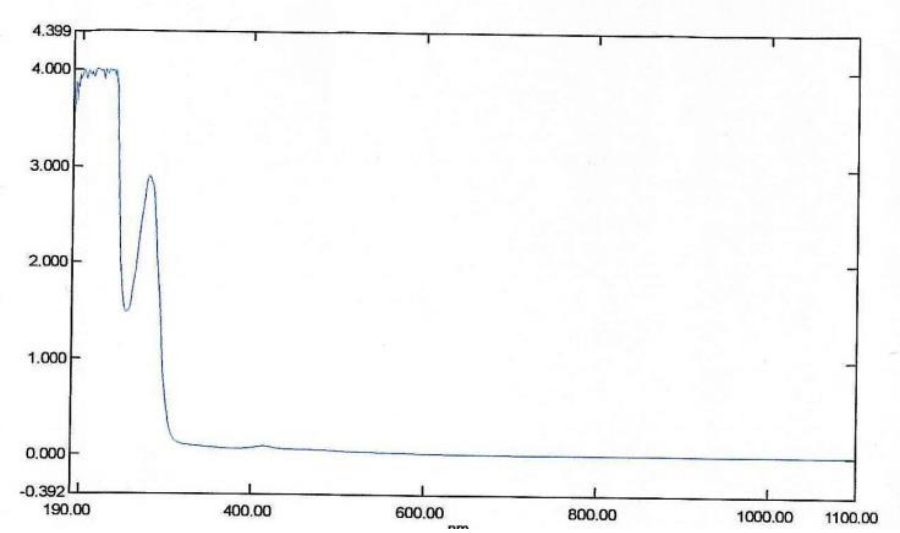


Figure (2) :UV- visible spectrum for patient with leukemia serum sample at first age group.

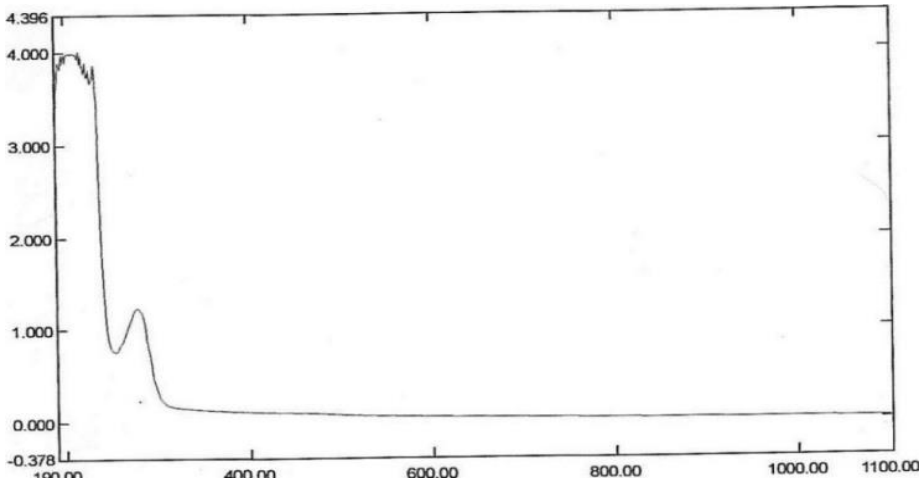


Figure (3): UV- visible spectrum for normal serum sample at second age group

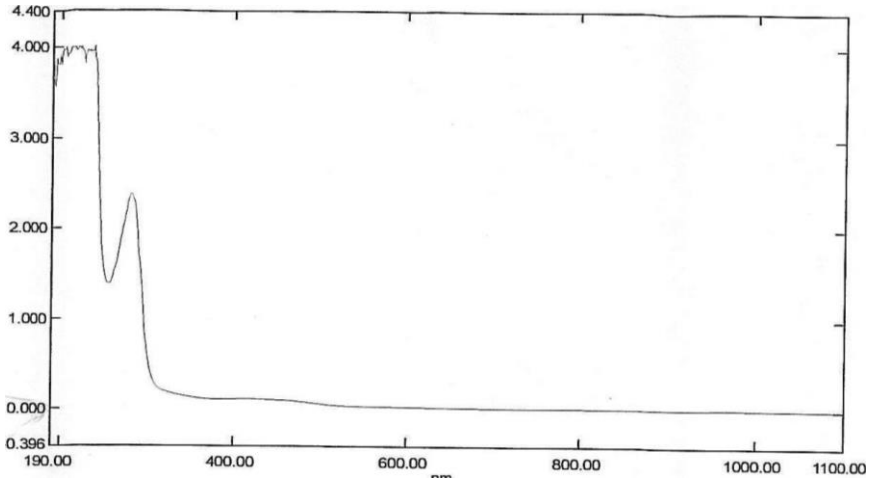


Figure (4) :UV- visible spectrum for patient with leukemia serum sample at second age group.

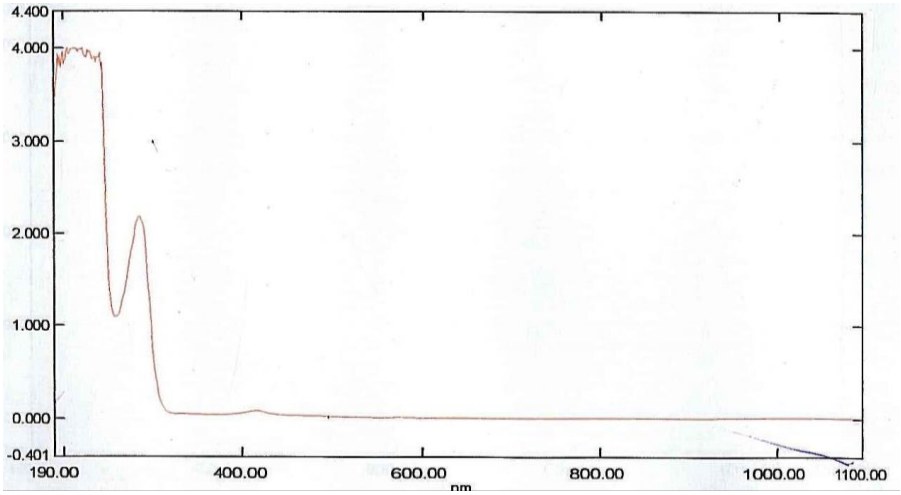


Figure (5): UV- visible spectrum for normal serum sample at third age group.

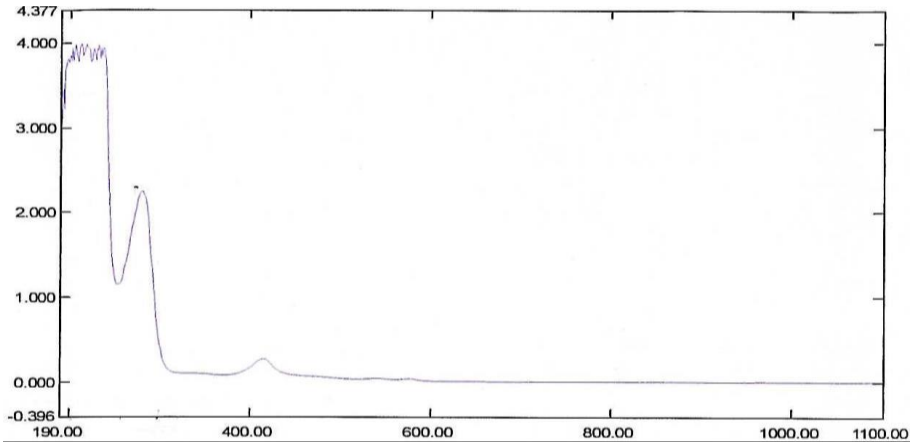


Figure (6) UV- visible spectrum for patient with leukemia serum sample at third age group.

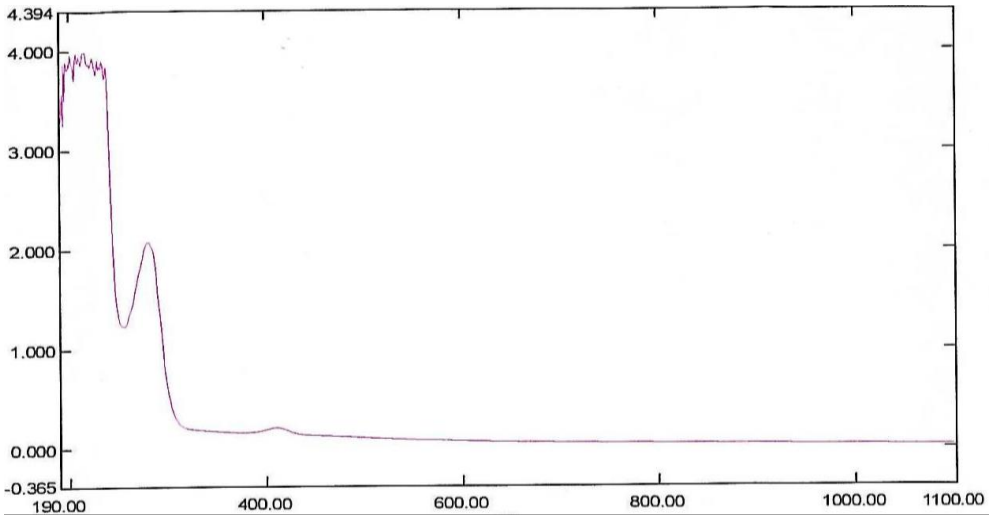


Figure (7): UV- visible spectrum for normal serum sample at fourth age group.

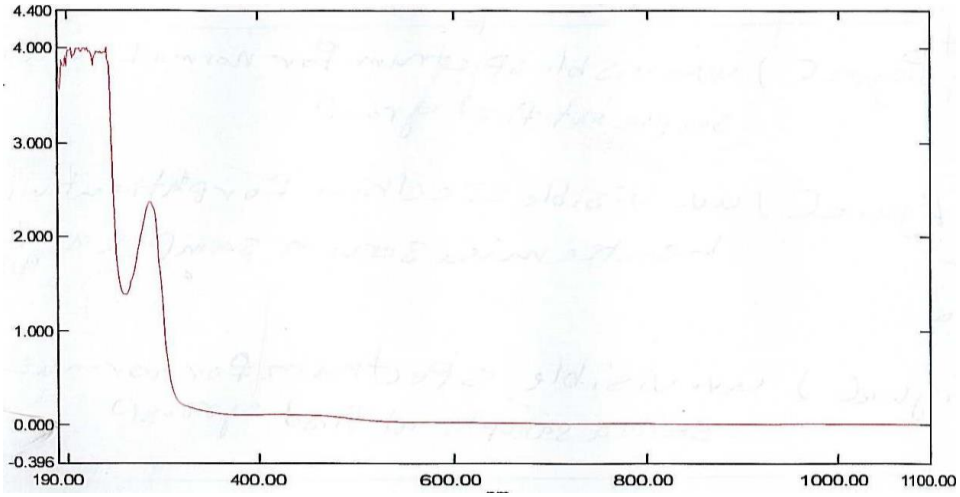


Figure (8) :UV- visible spectrum for patient with leukemia serum sample at fourth age group.

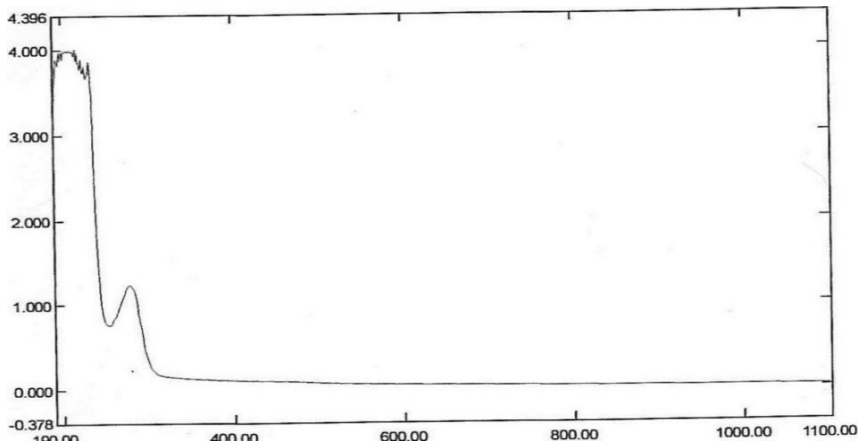


Figure (9) :UV- visible spectrum for normal serum sample at fifth age group

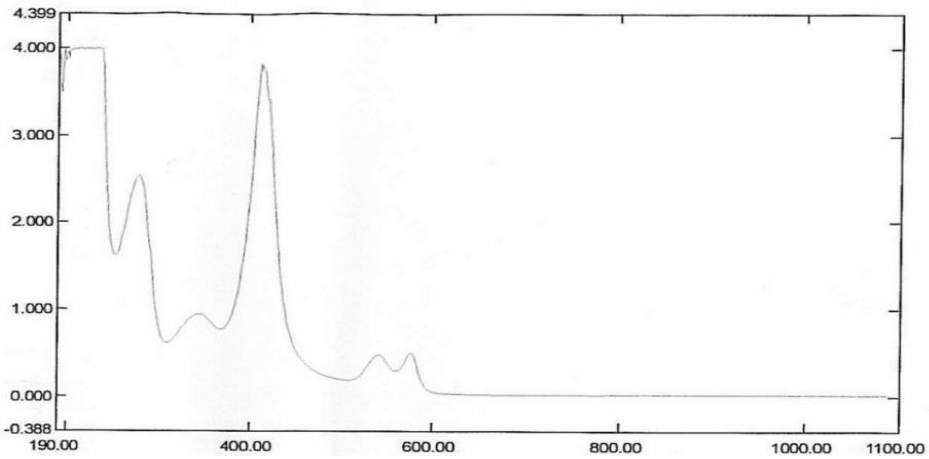


Figure (10) :UV- visible spectrum for patient with leukemia serum sample at fifth age group.

Many peaks were observed due to constituent of serum but one peak is clearly observed at 278nm because of the strongly absorption peak at 280nm due to the amino acids like tyrosine and tryptophan [11]. The increasing absorption in protein peak in leukemic sera compared with normal sera but with vary intensity because increasing ratio of albumin to globulin, the type of protein in serum.

Albumin to globulin ratio=albumin level in serum /(protein level in serum –albumin level in serum). The normal ratio 1/2 or 1/1.and increasing this ratio in case have increasing albumin level or in case have decreasing in globulin level or two cases .in leukemia disease the globulin decreasing lead to increasing in ratio and increasing in absorbent.

In 345nm it was found two samples one normal serum and another of patient may be related to the melanin because the attenuation of UV-Visible spectroscopy in (320-400)nm is primarily via melanin.

In peak 411nm it was found because the scattered in this area more efficiently than other wave length and may be because found some sera hemolysis especially L42 the absorption (3.836) increasing in absorption.

And (541,576) nm peaks appeared may be related to take drug and in some patient treatment with chemotherapy or serum hemolysis.

b. Saliva samples

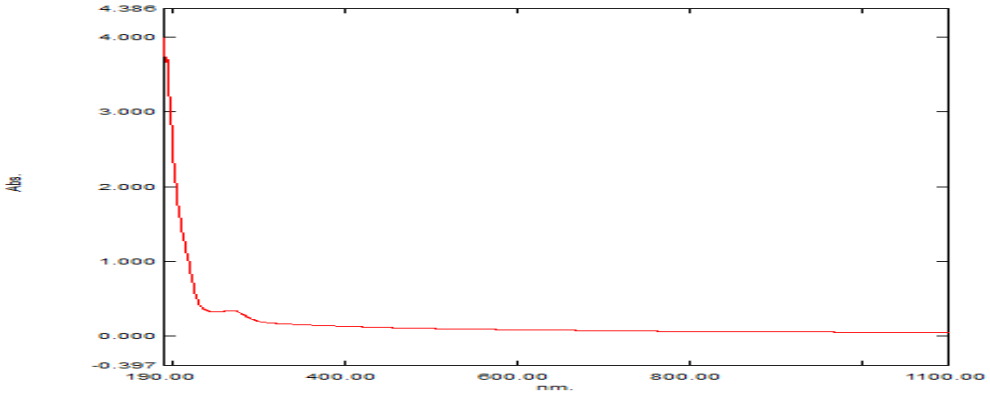
Saliva like serum contain abundance of protein and nucleic acid molecule that reflected physiological status ,salivary diagnostic becomes a key player in routine health monitoring of the early detection of some disease using effective assay [12].

Table 2 : The absorption of saliva samples for normal and patient with leukemia at each age group.

Group of age	**278 nm**		**345 nm**		**411nm**	
	L	**N**	**L**	**N**	**L**	**N**
15-25y	0.197	0.337	-	-	-	-
	0.298	0.262		-		-
	0.213	0.121		-		-
	0.204	0.095				
-35y	0.131	0.164	-	-	-	-
	0.134	0.124	-		-	
	0.148	0.112	-		-	
	0.281	0.173	-		-	
	0.256	0.190				

	0.575	0.132				
-45y	0.170 0.382 0.344	0.172 0.112 o.165	-		-	- -
-55y	0.205 0.281	0.193 0.713	- -	- -	0.088 0.121	
-65y			-			
-75y	0.567	0.213			0.055	

The UV-Visible spectrums of normal and patient with leukemia saliva are presented in figure (1-11),(1-12),(1-13),(1-14),(1-15) (1-16),(1-17),(1-18),(1-19),(1-20).



Figure(11): UV- visible spectrum for normal saliva sample at first age group.

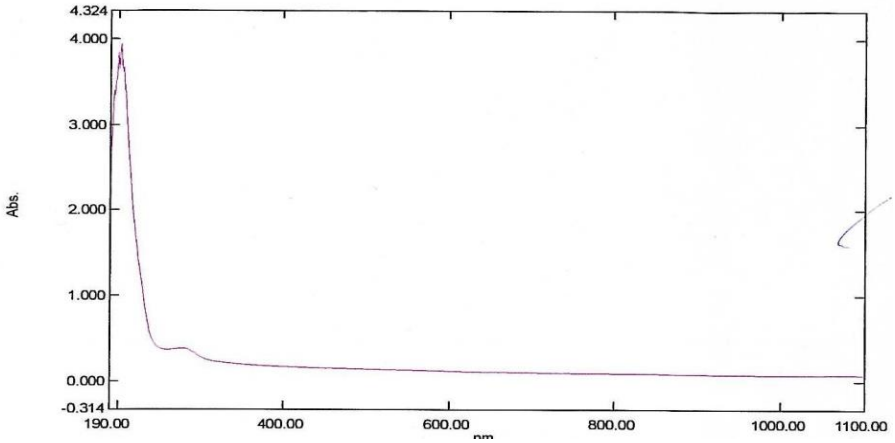


Figure (12): UV- visible spectrum for patient with leukemia saliva sample at first age group.

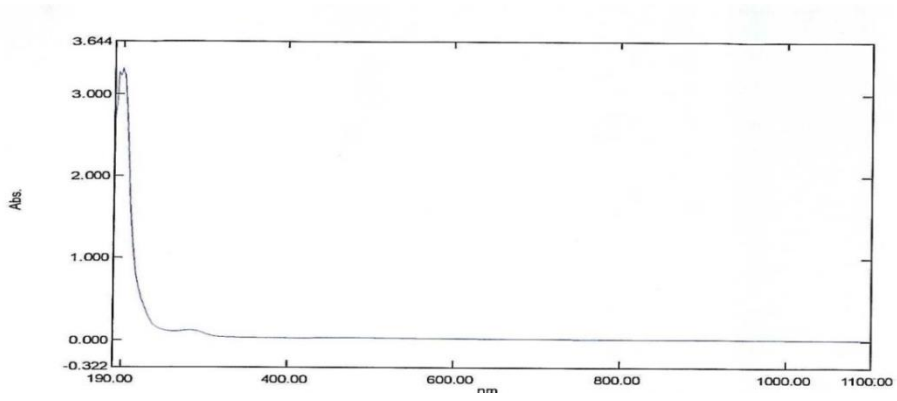


Figure (13) :UV- visible spectrum for normal saliva sample at second age group.

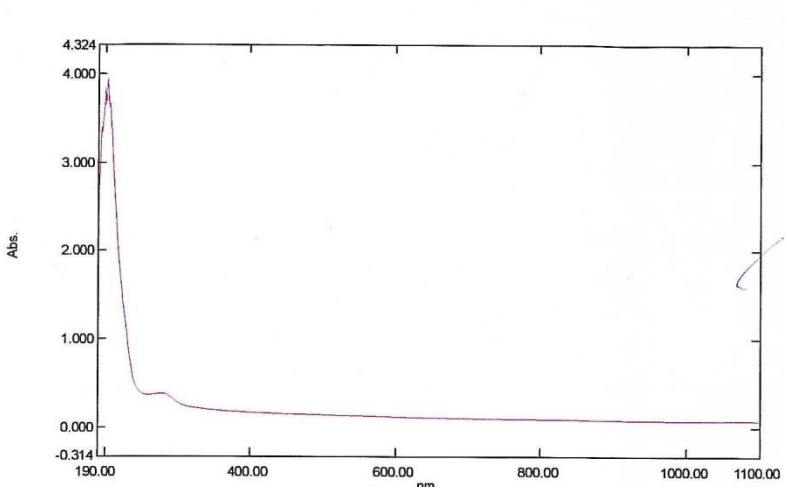


Figure (14) :UV- visible spectrum for patient with leukemia saliva sample at second age group.

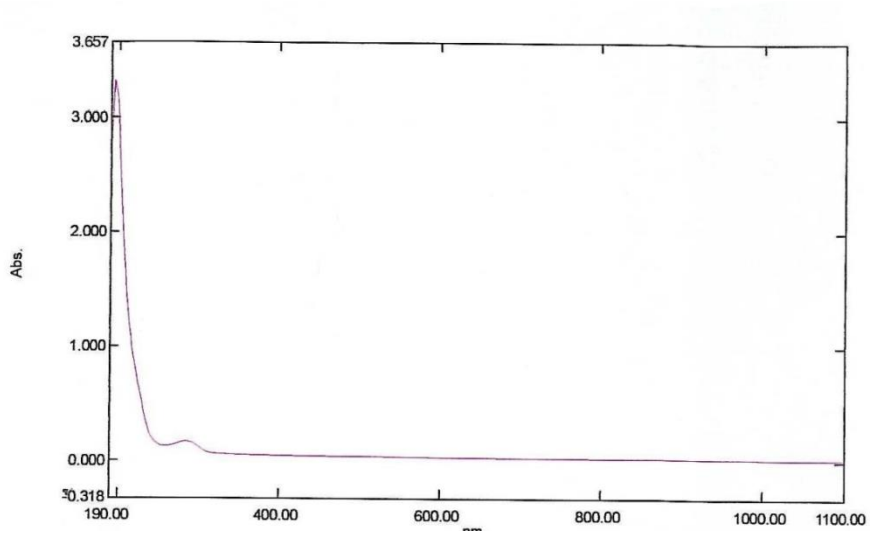


Figure (15) :UV- visible spectrum for normal saliva sample at third group.

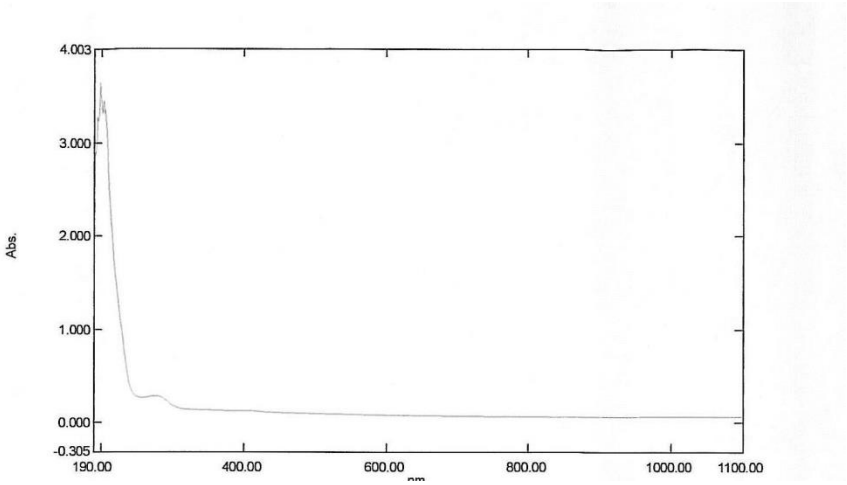


Figure (16): UV- visible spectrum for patient with leukemia saliva sample at third age group.

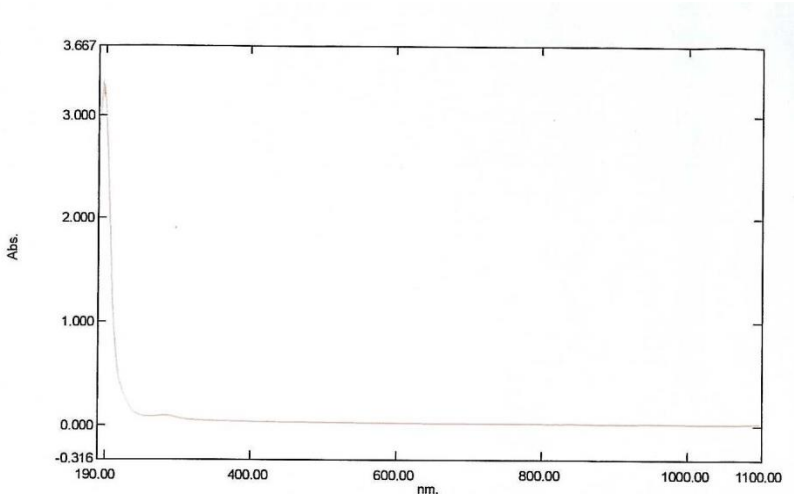


Figure (17) :UV- visible spectrum for normal saliva sample at fourth age group.

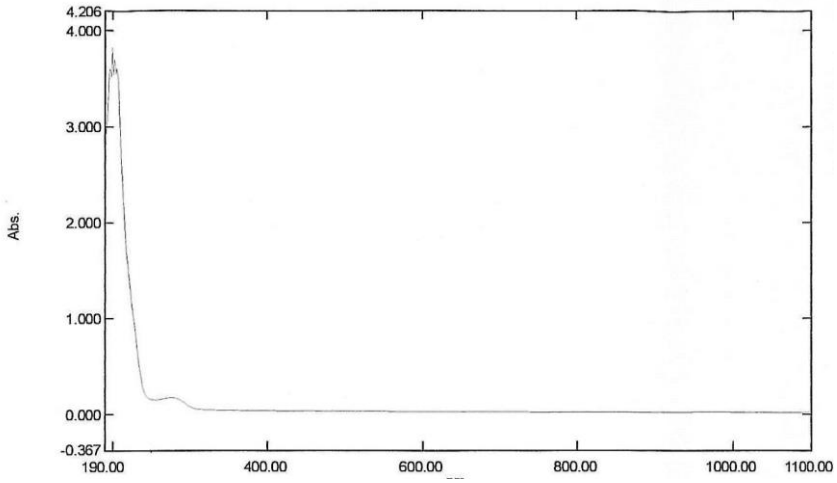


Figure (18) :UV- visible spectrum for patient with leukemia saliva sample at fourth age group.

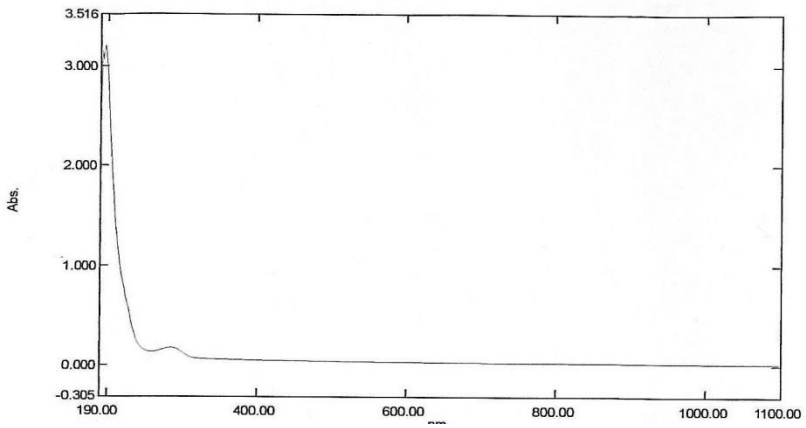


Figure (19): UV- visible spectrum for normal saliva sample at fifth age group.

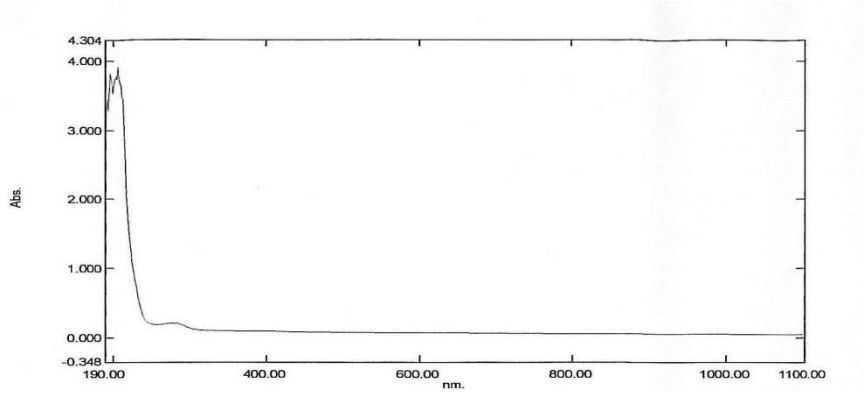


Figure (20) :UV- visible spectrum for patient with leukemia saliva sample at fifth age group.

Two peaks are observed but one peak is clearly observed at 278nm that area of total protein. It was found that there was difference between normal and leukemia saliva in patient with leukemia increasing in absorption and the difference is vary and did not observed as in serum, and in three case of leukemia saliva peak 411nm appear may be the scattered in this area more efficiently than other wave length.

Conclusion:

UV-Visible spectra of serum and saliva samples may be used to differentiate between the normal and leukemic diseases at each group of age.

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