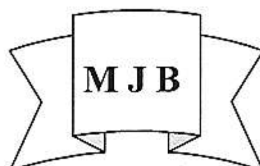
Lipid Associasted α-L Fucose Levels in Sera of Leukaemic Patients

Hamid G. Hassan
Chemistry Department, College of Science, University of Sulaimani, Sulaimania, IRAQ.

E mail:gaforiiq@yahoo.com.uk



Abstract

Lipid associated fucose (LAF) levels were determined in sera of 94 leukaemia patients. These were classified as 50 samples of Acute lymphoblastic leukaemia (ALL), 27 samples chronic lymphocytic leukaemia (CLL), and 17 samples chronic myeloblastic leukaemia (CML). In addition, twenty samples represents normal healthy individuals were used for comparison study.

Results analysis revealed significant ($p<0.05$) increases in the mean + SD of ALF values in sera of leukaemic patients compared with normal subjects.

الخلاصة

تم بحث مستويات الفا-ل-فيوكوز المرتبط باللبيد (فيوكوز-لبيد) فى مصول 94 نموذج من مرضى أبيضاض الدم حيث تضمنت االحالات المدروسة 50 نموذج من نوع أبيضاض الدم الحاد (ALL) و 27 نموذج من نوع أبيضاض الدم المزمن (CLL) و 17 نموذج من نوع أبيضاض الدم المزمن ذات الخلايا الميلوبلاستية (CML). وقد قورنت النتائج مع مثيلاتها فى مصـــول الـــدم الطبيعية .

أكدت النتائج على أرتفاع ملحوظ و نوعى فى مستويات الفيوكوز-لبيد فى الحالات المرضية الثلاثة المدروسة عنها فـــى المصل الطبيعى .

Introduction

Recently, scientific researches were concentrated on the role of Fucose and sialic acid and their related parameters as a tools used for monitoring cell proliferations[1,2,3,4]

A sensitive and specific blood test for early detection and subsequently for the management of cancer patients would be of great clinical value, however, the request for such a test is still going. Increased levels of enzymes, glycoproteins, hormones, glycolipids, oncofoetal proteins and peptides have been considered as potential tumor markers for helping in screening, diagnosis, staging, prognosis and monitoring of cancer treatment.

The cell surface membrane (plasma membrane) plays an important role in the (social) behavior of cells, that is, communication with other cells, cell monement and migration, adherance to other cells or structures, access to nutrients in the microenviroment, and recognition by the body's immune system. Alteration of the plasma membrane in malignant cells may be inferred from variety of properties that

characterizes their growth and behavior, for example the loss of density-dependent inhibition of growth, invasivenes through normal tissue barriers and loss of anchorage dependence[5]. In addition, a number of changes in the biochemical characteristics of malignant cells surface have been observed. These include appearance of new-surface antigen, proteoglycans, glycolipids and mucine, and altered cell-cell and cell-extracellular matrix communication [6].

Tumor associated carbohydrate antigens can be expressed as to be present in much higher concentration on tumor cell, for example, the GM3, ganglioside in melanoma and lewis X (blood group antigens) in gastrointestinal cancer [7].

Tumor-associated carbohydrate antigens can be classified into three groups [8] :

1. Epitopes expressed on both glycolipids and glycoproteins.
2. Epitopes expressed only on glycolipids.
3. Epitopes expressed only o9n glycoproteins.

To be first group belongs the lacto-series structure that is found in most human cancers, such as lung, breast, colorectal, and pancreatic cancers. The common back bone structures for these epitopes is:

Gal a1→3G1NAC a1→3Gal (type 1 blood group)

Gal a1→ 4G1NAC a1→3Gal (type 2 blood group)

The second groups of epitopes expressed exclusively on glycolipids are mostly on the ganglio-series structures. The third groups of epitopes, seen only on glycoproteins are a multianennary branches of N-linked carbohydrates, and the alterations of O-linked carbohydrate chains seen in some mucine[9].

A variety of chemical changes in tumor cells have been identified that can explain altered glycosylation patterns, for example organizational rearrangement of tumor cell membrane of glycolipids[10]. Similar changes have been noted in the carbohydrate components of glycolipids and of membrane-associated and secreted glycoproteins[11]. Lipid associated fucose (LAF), have been demonstrated in most common human malignant neoplasma's including carcinoma of the colon, ovary, kidney, and breast[12].According to the results appears the LAF could be considered as a biomarker for leukaemia.

Materials and Methods

Chemicals

All common laboratory chemicals or reagents were of analar grade. They were obtained from May and Baker, Riede-DeHaene, Hopkins and William firms. Alpha-L-Fucose was obtained from Sigma firm

Blood Samples Collection and Sera Separation

Twenty sample of blood were taken from normal healthy individuals (5-7ml). Fifty samples of blood were taken from patients with ALL, 27 blood samples with CLL, and 17 blood samples with CML. Leukaemic patients had already been diagnosed by consultant and confirmed with hematological investigations. Blood samples were left for 20 min at room temperature, after coagulation, the serum was separated by centrifugation at 3000rpm for 5 min. Serum specimens were frozen at -20 °C until analysis. The host information of patients and subject are summarized in table-1

Determination of LAF Levels in Leukaemic and Normal Samples

LAF was assayed in serum samples using the method described by Katabodis etal[13].

50 µL serum was diluted with 150µL de-ionized water then vortexed for 5sec and placed in ice bath. 3ml of ice-cold CHCl3/ CH3OH (2:1 v/v) were added and vortexed. 0.5ml cold de-ionized water was added, and centrifuged for 5min at 3000 rpm at R.T. One milliliter of the upper layer was transferred and 50µL of phosphotungstate sol (50%) was added. Vortexed and allowed to stand at R.T for 5min then centrifuged at 3000 rpm. The supernatant was separated and the precipitate was dissolved in 1ml de-ionized water. To 0.1ml ice-cold sol 4.5ml ice-cold sol of H2SO4 (6:1 v/v) was added. The mixture was kept in a water bath at R.T for a few mins. then to a vigorously boiling water bath for 3min then placed at R.T water bath. 0.1ml of 3% cystein HCL was added and absorption was read at 390nm.

A series of concs (5,10,15,20,25,30,35 µg/ml) of fucose were prepared for standard curve determination (fig-1)

Statistical analysis

The results of LAF values was analysed statically by values expressed as mean±SD. The level of significance was determined by using t-test.

Results and Discussion

The standard curve of fucose solutions was used to determined lipid associated fucose (LAF) or glycolipid in serum samples. Serum samples were tested for LAF , or glycolipids, these included 94 patients with various leukemia (table-1). Table-2, shows the mean value±SD of LAF in three major types of leukemia. The results reveal over all elevation in LAF levels in each types of leukemia when compared with normal healthy objects. Figure –2, also show the distribution of the individual values of LAF for leukaemic patients and normal individuals.

The increased levels in LAF in leukaemic sera were statically significant when compared with those obtained from normal objects ($p<0.05$) (table-3). Table-2, also show the percentages of LAF specificity and sensitivity in leukemia patients and normal objects, calculated by the value 5.0 mg/dl as the upper limit of normal. In general high sensitivity was observed especially for ALL and CML[14].

Serum LAF levels have been suggested to be a useful tumor marker, more satisfactory than other fucose related compounds[15]. Dwivedi and Hard[16] measured the LAF levels in eight different cancers, the results were compared with normal healthy objects, and shows that LAF is a useful marker as a prognostic determination in a variety of neoplastic condition.

Some authors have observed an increased level of fucose containing glycoconjugates in sera of malignancy patients[17]. These investigators reported significantly elevated serum concentrations of total fucose and its lipid associates. In another study, LAF levels have been reported to be useful indicator in monitoring patients with melanoma. Some others used TF, TF/TP, and LAF in diagnosis of children with various malignancies, a significantly elevated TF,TF/TP, and LAF were observed in different tumors, a decreasing in TF,TF/TP and LAF was noted in neuroblastoma and yolk sac tumors after a successful treatment of the malignancy[18].

In this study, the author findings, support that the fucosylation of the LAF may play an important role in discrimination of normal and leukaemic patients. Since LAF exhibit a high sensitivity, the combined use of LAF

with others provide high degree of marker positivity.

Conclusion

The results which obtained in this study reflects an increase in the levels of LAF which may be useful as an additional follow-up tool in those who develop cancers, and it may be applicable in following those patients who have had leukemia. Generally, estimation of LAF, is an additional piece of information to be weighed along with physical diagnosis and with other tests.

References

1. Toshihiko Maryam etal, Int.J.Cancer, 1997 ,70,575.

2. Jay etal, AM.J.Pathol, 1998, 110,425.

3. Abbas LB, PhD Thesis,Biochemistry Dept, College of medicine, University of Salahuddin, Erbil-IRAQ. Supervised by Hamid G Hassan.

4. Hassan HG "Serum L-Fucose level and some correlated biomarkers in Type-II Diabetes mellitus in Sulaimania city" In press. Accepted in Ibn Al-Haitham J , 2004.

5. Wareen L, Fuhrer JP, and Buck CA "Surface glycolipids of normal and transformed cells", Acad.Sce.USA 1972.

6. Hakomor SI "Biochemical basis of tumor-associated carbohydrate antigen"Immunol Allergy Clin., 1990 ,10,781.

7. Aoyagi Y etal, Lancet, 1985, 11,1353.

8. Johnson VG etal, Cancer Res., 1986, 46,850.

9. Mizuochi T etal, J. Biol. Chem, 1983 258, 14126.

10. Yashiko T etal, Am. J. Nephrol , 1988, 2, 21.

11. Oser BL "Hawk'sphysiological chemistry" 14th ed. PP:179-181, McGraw-Hill, New York ,1965.

12. Jussawalia DJ and Yeolo BB. Epidemiology of breast cancer in India, Current rospectives in Breast Cancer. Co. Ltd New Delhi ,1994.

13. Katapodis N etal, Cancer Res. , 1982, 42, 5270.

14. Takul IA etal, Leuk-lymphoma, 2002, 43, 11, 2241.

15. Iozzo RV etal J.Biochem, 1985, 260, 764.

16. Dwivedi GM and Hard RF, Experienta, 1990, 40, 91.

17. Reinigein DS etal, An. Plast. Surg., 1992, 28, 55.

18. Rompannen J etal, Anti-Cancer res., 1998, 18, 413, 2793.

Table 1 The host information of leukemic patients and normal subjects studied

Cases	No. of cases	Female	Male	Age (years)
Leukemia	94	34	60	2 – 80 yr.
ALL	50	20	30	2 – 58 yr.
CLL	27	10	17	42 – 80 yr.
CML	17	10	7	25 – 68 yr.
Normal	20	10	10	25 – 60 yr.

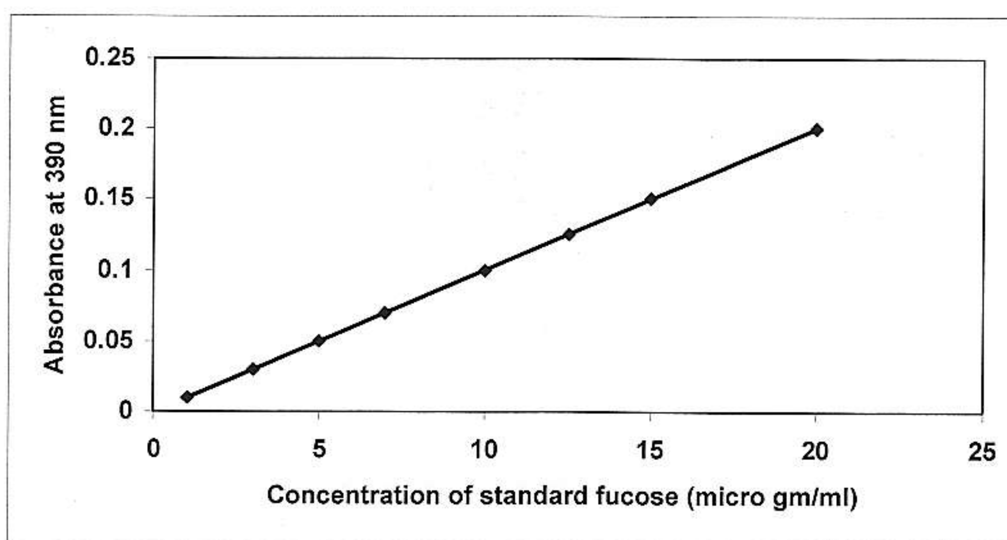


Figure 1 The standard curve for determination of lipid associated fucose (LAF) concentration

Table 2 The mean value , sensitivity and specificity of (LAF) measurments

Group	Sample No.	Glycolipid (mg/dL) ±SD	Sensitivity*	Specificity**
Normal	20	4.0	10 %	90 %
CLL	27	8.5 ± 4.2	55.6 %	44.4 %
CML	17	12.0 ± 3.5	85 %	15 %
ALL	50	10.0 ± 4.5	88 %	32 %

(*) The numbers of cases have (LAF) values > 5.0 mg/dL divided by the total number of cases multiplied by 100.

(**) The numbers of cases have (LAF) values < 5.0 mg/dL divided by the total number of cases multiplied by 100.

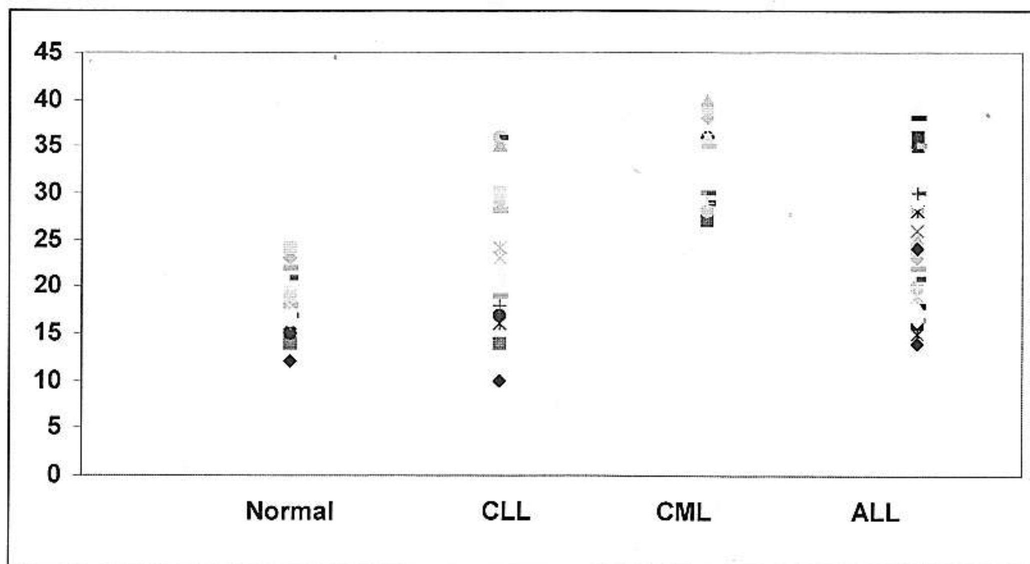


Figure 2 Distribution of individual values of glycolipid in sera of normal healthy subjects and luekemic patients

Table 3 Biostatistical calculations, and student t – test for (LAF) level in sera of normal healthy subjects and luekemic patients

Serum glycolipid (mg/dL)	Normal	ALL	CLL	CML
Sample size	N = 20	N = 50	N = 27	N = 17
Mean	4.0	10.0	8.5	12.0
Standard deviation	2.078	5.851	6.814	4.935
Standard error of the mean	0.464	0.827	1.362	1.197
Confidence interval of the mean	4.0 ± 0.27	10.0 ±1.66	8.5 ± 2.8	12.0 ± 2.53
T – test		7.56	3.72	12.49
Probability		0.01	0.0005	0.005