Sialic acid concentration changes in acute lymphocytic leukemia and acute myelogenous leukemia patients after chemotherapy treatment

Jian Lateif Hussein¹, Kamaran K. Abdoulrahman¹, Husny M. Hassan², and Hardi Q. Hamad³.

¹Department of Chemistry, College of Science, Salahadeen University, Erbil, Kurdistan Region-Iraq.
²Department of Biochemistry, College of Medicine, Dohok University, Dohok, Kurdistan Region-Iraq.
³Department of Chemistry, Koya Technical Institute, Koya, Kurdistan Region-Iraq.

ARTICLE INFO

ABSTRACT

Sialic acid—rich glycoprotein binds selectively in human and other organisms cancer cells that can metastasize. This helps these late stage cancer cells enter the blood stream. Biological markers can be used to monitor cancer elevation in the serum. Total sialic acid (TSA) concentration was observed in a number of cancer types, the chemotherapy is used as the only cancer treatment. The present study included 51 patients age (4—69 year) with leukemia, they were included 13 patients with acute lymphoblastic leukemia (ALL), 13 with acute myelogenous leukemia (AML) and 25 with chronic lymphocytic leukemia (CLL). This study included also 50 healthy subjects as normal control, all the patients had chemotherapy. The study included measurements of fasting serum total sialic acid (STSA) level, which is significantly increased in post sampling of leukemia patients compared with normal control subjects. A higher significant difference of STSA at the first week during chemotherapy in the three type of the leukemia patients, also there were significant difference of STSA level at the third week during chemotherapy in the ALL and AML patients. On the other hand, there were no significant differences in STSA level after five weeks of chemotherapy treatment for ALL and AML patients. According the duration of chemotherapy dose there were significant differences in STSA level between the first dose week and third dose of chemotherapy. Also, there were significant differences between the first weeks and 5th weeks of chemotherapy treatment for ALL and AML patients.

Keywords:
Sialic acid
Leukemia
Chemotherapy.

*Corresponding Author:
Jian L. Hussein
(jian.hussen@su.edu.krd)

1. INTRODUCTION

Sialic acids (SAs) could be defined as O or N – acetyl derivatives another acid which is Neuraminic, where it is nine carbon sugar that also derived from Mannosamine (Collins, 1987). It plays a big role in human’s biochemical function because its constituents of Glycoproteins. SAs found in some cells that are negative charge (Langana et al.,1995), where it is valuable for cell to cell infarctions, immunological reaction, receptor function and cell recognition (Yoko et al.,1983),(Weigant,1985).

Increased SA concentrations during the process of inflammatory have found by Sillanankee et
al. (Sillanankee and Pannio, 1999), and it may possible resulting when rich sialyted acute-phase glycoproteins is increased, also SA level has found increased level also in diabetes mellitus, chronic renal failure and cancer.

The concentration of serum SA is found as a risk marker for atherosclerosis and cardiovascular disease (Lindbery et al., 1997).

Sialic acid as a tumor marker in hematological malignancy like leukemia, is a cancer of the blood or bone marrow and is characterized by an abnormal production of blood cells, usually white blood cells (leukocytes). Biological markers can be used to monitor cancer elevation in the serum total sialic acid (TSA) concentration has been observed in a number of cancer types, leukemia can be divided clinically in to 2 groups as in below:

1.1. **Acute leukemia**

Produced in the wake of uncontrolled cancer cell division (lymphocytes). Acute leukemia required an immediate treatments due to the fast patterned advanced of the malignant cells. Acute leukemia could be grouped in to ((Saforf et al., 2000):

i. Acute myelogenous leukemia (AML).
ii. Acute lymphoblastic leukemia (ALL).

1.2. **Chronic leukemia**

Chronic leukemia CL is characterize by the extravagant buildup of relatively mature, but still abnormal, white blood cells. It could take months to years for diagnosing, the cells in this type are produced at a much higher rate than normal cells (Colvin and Elfenbein, 2003).

2. **MATERIALS AND METHODS**

2.1. **Experimental Animals**

Fifty one leukemia patients aged (4-69) years were included, at Nanakali Hospital in Hawler City, they were divided into three types of the leukemia: 13 patients (ALL type), AML type included 13 patients and chronic myelogenous leukemia (CML) type included 25 patients. The study also included 50 subjects of normal healthy control.

1.1. **Blood Sampling**

The blood was allowed to coagulate at room temperature and centrifuged at 3000 rpm. for 20 min. The resulting sera were separated. The sera were then stored at –20 °C when it was not used immediately. The stability studies showed that the parameter TSA was constant under these conditions, for up to four months (Gabrlel and Ekeke, 1988).

**Table.1: Age data of hosts used in the study.**

| Groups  | Number of subjects | Mean age (years) | Age-range (years) |
|---------|--------------------|------------------|-------------------|
| control | 50                 | 25               | 10-69             |
| AML     | 13                 | 20               | 10-69             |
| ALL     | 13                 | 25               | 4-60              |
| CML     | 25                 | 25               | 10-66             |

1.2. **Biochemical Tests**

The biochemical tests include serum fasting TSA determination

1.3. **Methods**

Determination of serum total sialic acid (STSA). The principle of this method depends on the formation of chromogen in addition of
resorcinol reagent into the test tube. The chromogen formed was extract by butyl acetate methanol reagent and measured at 580 nm (svennerholm,1975).

1.4. Statistical Analysis
Statistical analysis was done by using student t-test. All the data were presented as mean ± standard deviation. P values < 0.05 were considered significant.

3. RESULTS AND DISCUSSION
Table (1) shows the mean ±SD of TSA level expressed in mg/dl of normal control and leukemia patients and biostatistical calculation and student’s t-test. There is a significant increase in the serum TSA level in leukemia patients post dose of the chemotherapy injected as compared to the normal control group. In case of ALL patients, table (2) shows the mean ±SD of TSA level expressed in mg/dl of normal control subjects and type ALL patients post the chemotherapy injected for three stages, there is a significant differences in the serum TSA level in the ALL patients at the first and second dose, while there is no significant differences in the serum TSA level at the third dose (day 28th) compared to the normal control. Within the groups there were significant differences in the TSA levels between the first and second dose, also between the first and third doses (Figure 3), it is well established that serum TSA is elevated in patients with leukemia , the serum concentration of TSA may be increased through changes in the biosynthesis of the acute phase glycoproteins in the liver (Van Dijk et al,1991).The results in this study indicated highly significant differences in serum TSA level in leukemia patients after chemotherapy at the first week compared to normal control (p<0.0001) Table (1). This is in agreement with previous findings of Warren L.1959,( Warren ,1959). In case of the ALL patients there were significant differences in the level of the TSA post chemotherapy (after the first and third dose) (p<0.0001). Table .3 shows the mean ±SD of TSA level expressed in mg/dl of both normal control subjects and AML patients post three stages of chemotherapy. Biostatistical calculations and student’s t-test, there showed a significant differences in the serum TSA level in the AML patients at the first and second dose, while there is no significant differences in the serum TSA level at the third dose (2 weeks) as compared with normal control subjects. There was a significant increase in TSA levels between the first and second doses between the groups. There were also significant differences between the first dose and third dose (Figure 4). Figures ( 1 and 2 ) show the concentration of TSA of the ALL and AML patients (weeks 1th,3th, 5th) during chemotherapy treatment.

While there is no significant difference between the patients of post chemotherapy (after 5th weeks) and normal control (Table2), increase in sialic acid level in lymphoma cells may be due to enhanced activity of enzymes involved in sialic acid synthesis and /or transfer (Onodera et al.,1976),elevated sialic acid level in malignant cells have also been observed (Prasad and Giri,1994).On the other hand, the TSA level decreases with post chemotherapy after (5th week) treatment this may induce specific changes in tumor cells which could be associated with tumor regression (Cors et al.,2004). (Figure 3). A higher significant difference in TSA level in AML patients as compared with normal control after the AML patients treatments at the first and third week (p<0.0001), while there was no significant difference between the 3rd week and normal healthy control (Table 2) after chemotherapy. Chemotherapy for leukemia is effective at inducing complete reduction for most patients, the development of novel approaches to
leukemia therapy is essential due to high rates of relapse and outgrowth of drug resistant tumors (Cors et al., 2004). The present study was undertaken to elucidate quantitative changes in sialic acid concentration after treatment with chemotherapy during (1st-5th weeks) to illustrate the effects of these drugs on the sialic acid content of various host tissues. Increased sialic acid concentration may be due to at least in part to defective de novo synthesis transport, excretion and/or metabolic regulation of the sialic acid in the cell (Thomas et al., 1985). The observation of increased sialic acid content in the tissue of tumor could be helpful for lymphoma cells since sialic acid is known as an important compound in the transport of protein, amino acids and ions to the cancer cells (Donagh and Nathan, 1990).

Table 1: Biostatistical calculations and student’s t-test for TSA level mg/dl in serum of leukemia patients post the first dose.

| Groups | Control | First dose | Second dose | Third dose |
|--------|---------|------------|-------------|------------|
| Biostatistical group | (1st week) | (3rd week) | (5th week) |
| Number of patients + control | 50 | 13 | 13 |
| Mean | 53.0276 | 73.9700 | 62.3623 | 53.3185 |
| SD | 4.64617 | 9.84832 | 4.69813 | 0.57849 |
| SE | 0.65059 | 2.73143 | 1.30303 | 0.16044 |
| T-test with control | \(P < 0.0001\) | \(P < 0.0001\) | \(NS\) |

\(P\) = probability of significant

\(NS\) = non significant
Table 3: Biostatistical calculation and student’s t-test for TSA level mg/dl in serum of leukemia patients post the first dose, second dose and third dose of the AML patients

| Groups                | Control group | Leukemia patients post the first dose |
|-----------------------|---------------|--------------------------------------|
| Biostatistical Calculations |               |                                      |
| Number of patients     | 50            | 51                                   |
| + control              |               |                                       |
| Mean TSA levels mg/dl  | 53.02         | 71.97                                |
| SD                     | 4.6           | 13.43                                |
| SE                     | 0.65          | 1.88                                 |

$T$-test with normal control $P < 0.0001$

$P$=probability of significant

Figure (1): TSA concentrations mg/dl for ALL patients during chemotherapy treatment.

Figure (2): TSA concentration mg/dl in AML patients during chemotherapy treatment.

Figure (3): The effect of chemotherapy on TSA level mg/dl in ALL patients.

$NS$= non significant
2. CONCLUSION

During chemotherapy treatment for ALL and AML patients the TSA level is significantly different in the 1st and 3rd dose, this make the test useful for monitoring disease status and important new clinical tool for the diagnosis of cancer. TSA level is decreased in the 5th week of the post chemotherapy by both lympho ALL and AML patients. In the first week of chemotherapy treatment the TSA level is higher in the AML patients, than the ALL patients.

REFERENCES

Collins, P., M. 1987. Carbohydrates Metabolism. Chapman & Hall, 156.

Colvin, G.A., Elfenbein, G. 2003. "The latest treatment advances for acute myelogenous leukemia". Med Health J. 86 (8): 243-63.

Cros, E., Jordheim, J., Dumon, C., et al. 2004. Problems related to resistance to cytarabine in acute myeloid leukemia. Leuk. Lymphoma; 45: 1123-132

Gabriel, L. Ekeke, O. 1988. Analysis of glycoprotein J.Clin. Chem., 347: 1443-1446.

Langana, A., M. Pardo, B., Marinp, A. 1995. Determination of total sialic acid in genitourinary malignancy by fluorimetric high performance liquid chromatography. Clin. Chem. Acta.; 29: 243(2), 165-179.

Lindberg, G., H. Oiso, H., Rastam, L. 1997. Serum sialic acid and its correlates in community samples from Akita, Japan and Minneapolis, USA. International Journal of Epidemiology. 26(1): 58-63.

McDonagh, J.C., Nathan, R., D. 1990. Sialic acid and the surface charge of delayed rectifier potassium channels. Journal of Molecular and Cellular Cardiology, 22: 1305-1316.

Onodera, K., Yamaguchi, N., Kuchino, T. 1976. Alterations in surface glycoproteins and level of sialytransferase of cells transformed by a temperature-sensitive mutant of SV40. Proceedings of the National Academy of Sciences, USA. 731, 4090-4094.

Prasad, S.B., Giri, A. 1994. Antitumour effect of cisplatin against murine ascites Dalton's lymphoma. Indian Journal of Experimental Biology., 32: 155-162.

Sanford, A., Harold, R., William, R. 2000. Handbook Of hematologic Pathology. New York, N.Y: Marcel Dekker, 193-194. ISBN 0-8247-0170-4.

Sillanaukee, P., Pannio, M. 1999. Occurrence of sialic acid in healthy humans and different disorder. Eur. J. Clin- Invest., 29 (5): 413-25.

Svennerholm, L. 1975. Determination of sialic acid. Biochem- Biophys. Acta., 234: 1971.

Thomas, G.H., Scocca, J., Miller, C.S., & Reynolds, L.W. 1985. Accumulation of Nacetylneuraminic acid (sialic acid) in human fibroblasts cultured in the presence of N-acetylmannosamine. Biochimica et Biophysica Acta. 846: 37-43.

Van Dijk, W., Pos, O., Van der, M.E., Eap, C.B. 1991. Inflammation-induced changes in expression and glycosylation of genetic Variants of _1acid
Blvycoprotein. Studies with human sera, primary cultures of human hepatocytes and transgenic mice. *Biochem j*; 276: 343-347.

Warren, L. 1959. The thiobarbituric acid assay of sialic acids. *J Biol Chem*; 234: 50, 1971-1975.

Wiegant, H. 1985. Glycolipids. New Comprehensive Biochemistry. Amsterdam. Elsevier.10.

Yoko, S. Mosao, P. Jaceews, I. 1983. Determination of NANA by gas chromatography – mass spectrometry with a stable isotope as internal standard. Analytical Biochemistry. J. 132:147-151.