Evaluation of Thyroid Function in Sera of Acute and Chronic Leukemic Patients

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Abstract

Background: This study aims to compare thyroid function between patients with acute and chronic leukemia, through measurement of triiodothyronin, tetraiodothyronin (thyroxin), free triiodothyronin, free tetraiodothyronin, thyroid stimulating hormone, antithyroglobulin, and antithyroperoxidase .

Material and Methods: One hundred twenty subjects collected from the Hematological Center / AL-Mustansiriya University, This subjects divided into two groups depending on the lineage of the original mutated cell: forty patients of chronic lymphocytic leukemia and forty patients of acute lymphocytic leukemia and forty healthy control for comparison with two groups. Complete blood count of each subjects was evaluate by using Hemolyzer 5 instrument, and Hormonal tests which include: triiodothyronin, tetraiodothyronin (thyroxin), free triiodothyronin, free tetraiodothyronin, thyroid stimulating hormone, were measured by using Minividas. antithyroperoxidase and antithyroglobulin were measured by using Enzyme Linked immunosorbent assay.

Result: The result indicated a presence of highly significant increase ($P<0.001$) of red blood cell and platelet among patients with acute lymphocytic leukemia when compared with control groups, while highly significant decreased ($P<0.001$) of red blood cell in chronic lymphocytic leukemia when compared with control groups and highly significant increase ($P<0.001$) of platelets were found in chronic lymphocytic leukemia when compared with control groups. A significant increase ($P<0.05$) of thyroid stimulating hormone, free triiodothyronin, antithyroglobulin, and antithyroperoxidase in sera of both acute lymphocytic leukemia and chronic lymphocytic leukemia patients in comparison to that of the control group. A significant positive correlation ($p<0.05$) was observed between antithyroperoxidase and free tetraiodothyronin in sera of acute lymphocytic leukemia patient while, in contrast with chronic lymphocytic leukemia group there was no significant correlation. A significant negative correlation ($p<0.05$) between antithyroperoxidase and RBC was observed , while a significant positive correlation ($p<0.05$) between antithyroperoxidase with platelet was observed in sera of chronic lymphocytic leukemia patient.

Conclusion: Increase of antithyroglobulin, and antithyroperoxidase with the increase of thyroid stimulating hormone levels of both groups (acute lymphocytic leukemia and chronic lymphocytic leukemia) indicate an autoimmune thyroid disorder, and the free tetraiodothyronin is an important test and it may be used with antithyroperoxidase as a marker for diagnosis of leukemia.

Keyword: Thyroid function test, antithyroglobulin, antithyroperoxidase, acute lymphocytic leukemia and chronic lymphocytic leukemia.

Introduction

The thyroid gland is a butterfly shaped endocrine gland located in the neck. As a response to thyroid stimulating hormone (TSH) [(1)], thyroid hormone is secreted from the thyroid predominantly as the pro hormone thyroxine (T4) which must be activated in tissues to the active form, triiodothyronine (T3), by the action of the 5′-deiodinase enzymes [(2)]. These thyroid hormones are derived from tyrosine and iodine. When released into circulation, they produce a diverse systemic organic and metabolic response [(1)]. Thyroid hormone modulates oxygen consumption, basal metabolic rate (BMR), as well as carbohydrate, lipid, and protein metabolism. It regulates the synthesis and degradation rates of numerous proteins and has fundamental effects on the nervous system [(3)]. The active forms of thyroid hormones are made in in the presence of peroxidase [(4)]. Synthesis of T3 occurs in association with thyroglobulin, which contains iodine trapping mechanisms forming the precursors mono-iodotyrosine (MIT) and di-iodotyrosine (DIT), and it is considered the prohormone to thyroxine [(1)].

Thyroglobulin (Tg) is a protein mostly abundant in the thyroid gland. It is produced by thyroid follicular cells, iodized as active thyroid hormone, and released into circulation through exocytosis [(5)]. It is therefore a precursor of T4 and T3. Tg is glycosylated protein with a molecular mass of 660 kDa and two large subunits. It contains 115 tyrosine residues, each of which is a potential site of iodination [(6)].

Thyroid hormone biosynthesis involves a heme-containing peroxidase [(7,8)]. Thyroperoxidase, is a tetrameric protein with a molecular mass of 60 kDa, requires hydrogen peroxide as an oxidizing agent [(6)]. Human thyroperoxidase is a membrane-bound glycoprotein located at the apical membrane of the thyroid follicular cells that catalyzes iodide

oxidation of the TG tyrosine residues, leading to the thyroid hormone synthesis by coupling of iodotyrosine residues [9].

Leukemia is a malignant disease of white blood cells occurs when cells residing in the bone marrow they become cancerous and thier daughter cells crowd normal cells in the bone marrow or are released from the bone marrow and circulate in the blood. Generally, leukemia has been classified as myeloid or lymphoid, depending on the lineage of the original mutated cell [10,11].

In general there are two types of leukemia acute and chronic. Acute leukemia develops rapidly from early immature white blood cells. Chronic types develop more slowly from cells at later stages of development. In addition to acute and chronic forms leukemia are categorized by the types of cells from which they arise. The two major categories are lymphocytic which are chronic lymphocytic leukemia (CLL) and acute lymphocytic leukemia (ALL), and myelogenous which are chronic myelogenous leukemia (CML) and acute myelogenous leukemia (AML) [12, 13].

However thyroid disease has been associated with leukemia and lymphoma, a further studies are needed to explore underlying mechanisms associating thyroid autoimmunity with leukemogenesis [14]. We aims to compare the thyroid function in patients of acute leukemia with that of chronic leukemia through measurement of T3, T4, freeT3, freeT4, TSH, antithyroglobulin, and antithyroperoxidas.

Material and Methods:

The study included 120 subjects collected from the Hematological Center / AL-Mustansiriyah University for period (Dec. 2016-Mar.2017). This patients were categorized into two groups depending on the lineage of the original mutated cell: 40 patients of chronic lymphocytic leukemia ranging in age (23-65 year) and 40 patients of acute lymphocytic leukemia ranging in age (18 -68 year). As a control group 40 apparently healthy with match sex and age were included in this study.

Complete blood count of each patients was evaluate by using Hemolyzer 5 instrument, these include: RBC, HGB, HCT, MCV, MCH. RDWsd, RDWcv, PLT, PCT, MPV, PDWsd, PDWcv, and WBC. Hormonal tests which include: T3, T4, TSH, FT3, and FT4 were measured by using Mini vidas. Thyroperoxidase antibody and antithyroglobulin were measured by using Enzyme Linked immunosorbent assay (ELISA) kits.

The data processing and statistical analysis was done by the computer SPSS (Statistical Package for Social Science –version 20). Data were analyzed in simple statistical measures of mean, standard error and standard deviation. The data were analyzed using one Way ANOVA and person correlation coefficients. Differences were considered significant when $P< 0.05$.

Results:

The results presented in Table 1 indicated a presence of highly significant decrease ($P<0.001$) of MCH, MCHC, RDWcv and a significant decrease ($P< 0.05$) of RDWsd and MPV in all samples compared with control while a highly significant increase ($P<0.001$) of RBC, MCV, PLT and a significant increase ($P< 0.05$) of HCT were found in all samples compared with control group. Furthermore the results shows a presence of highly significant decrease ($P<0.001$) of RBC, MCH, RDWcv in CLL samples compared with control and a highly significant increase ($P<0.001$) of MCV, MCHC, PLT in CLL samples compared with control. The overall results presented in Table 1 indicated an abnormality on a routine blood test of Leuckemia CBC.

Table 2 shows the mean values of thyroid hormones, antithyroid peroxidase, and antithyroglobulin of control, ALL, and CLL groups. There were a significant increase ($P<0.05$) of TSH, and FT3 in sera of both ALL, and CLL patients in comparison to that of the control group. Antithyroid antibody studies are used to evaluate for autoimmune thyroid problems. Although highly significant increase ($P<0.001$) of anti TG was observed in the present study in sera of both CLL and ALL groups in comparison to that level of the control group, it were found a non-significant differences in serum levels of thyroxine T4 and triiodothyronine T3 in all patients compared to control, all group were within the normal rang. In contrast to CLL group, the ALL group showed a highly significant increase of anti TPO, in comparison to that level in the control group.

A significant positive correlation ($p<0.05$) was observed between anti TPO and FT4 in sera of ALL patients group (Figure 1) .In contrast with CLL group a non-significant correlation ($p>0.05$) between anti TPO and FT4 was observed(Figure 2). Meanwhile in sera of CLL group a significant negative correlation ($p<0.05$) between anti TPO and RBC was observed (Figure 3) while a significant positive correlation ($p<0.05$) between anti TPO with PCT and PLT were observed (Figure 4).

Table 1: The distribution of the studied groups according to complete blood picture

Parameters	Group	Mean	±SD	P value
WBC	ALL	13722	5.79739	0.096
	CLL	9.8205	34.454	0.587
	Control	6.3100	1.11067	-
RBC	ALL	5.8057	1.3634	0.00
	CLL	4.470	0.5648	0.00
	Control	4.5280	0.7140	-
HG	ALL	11.1350	2.2497	0.43
	CLL	11.745	1.046	0.49
	Control	11.7550	1.14638	-
HCT	ALL	39.2800	7.3952	0.02
	CLL	33.086	8.464	0.82
	Control	31.3470	11.40662	-
MCV	ALL	68.9950	7.4192	0.00
	CLL	78.270	6.469	0.00
	Control	31.3470	11.40662	-
MCH	ALL	20.7300	6.2605	0.00
	CLL	26.5200	2.8949	0.00
	Control	31.3470	11.40662	-
MCHC	ALL	29.6050	4.9125	0.00
	CLL	33.8300	1.7345	0.00
	Control	31.3470	11.40662	-
RDWsd	ALL	24.3650	4.16139	0.03
	CLL	32.5600	8.12627	0.89
	Control	31.3470	11.40662	-
RDWcv	ALL	13.0000	1.27939	0.00
	CLL	13.8750	5.19158	0.00
	Control	31.3470	11.40662	-
PLT	ALL	392.300	134.482	0.00
	CLL	236.650	100.001	0.00
	Control	31.3470	11.40662	-
PCT	ALL	0.1470	0.06071	0.34
	CLL	0.8870	3.55768	0.74
	Control	1.7050	4.8892	-
MPV	ALL	3.8900	0.2359	0.03
	CLL	4.5300	0.8980	1.0
	Control	4.5300	0.96141	-
PDWsd	ALL	11.9150	2.9322	0.99
	CLL	11.8300	1.7060	0.98
	Control	11.9550	1.67001	-
PDWcv	ALL	31.0000	0.0000	1.0
	CLL	31.0000	0.0000	1.0
	Control	31.0000	0.0000	-

Table 2: Mean values of Thyroid hormones, antithyroid peroxidase, & antithyroglobulin of 3 groups

Parameters	Group	Mean	±SD	P value
T3 nmol/L	ALL	1.7950	1.16956	0.991
	CLL	1.5100	0.4933	0.537
	Control	1.7650	0.30310	-
T4 nmol/L	ALL	88.0500	46.73495	0.863
	CLL	80.000	38.44066	0.452
	Control	94.0500	19.55148	-
TSH nmol/L	ALL	15.6515	25.96425	0.025
	CLL	22.4000	10.95157	0.001
	Control	1.8500	0.33950	-
FT3 nmol/L	ALL	6.7650	2.36048	0.006
	CLL	6.625	2.45547	0.010
	Control	4.7400	0.32347	-
FT4 nmol/L	ALL	16.5300	3.13773	0.909
	CLL	16.1750	3.33496	0.694
	Control	16.9000	1.61897	-
Anti TPO pg/ml	ALL	164.5000	38.505052	0.00
	CLL	58.6250	50.97303	0.07
	Control	86.9100	20.02270	-
Anti TG pg/ml	ALL	159.1000	25.1373	0.000
	CLL	166.5500	39.2381	0.000
	Control	52.4000	5.32521	-

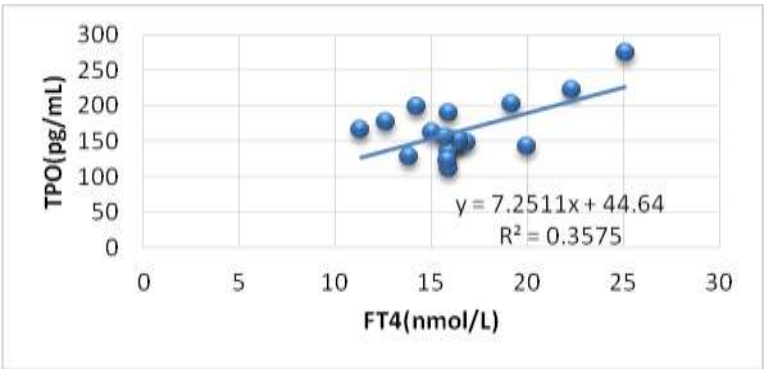

Figure 1: Relationship of FT4 with TPO in sera of ALL patients

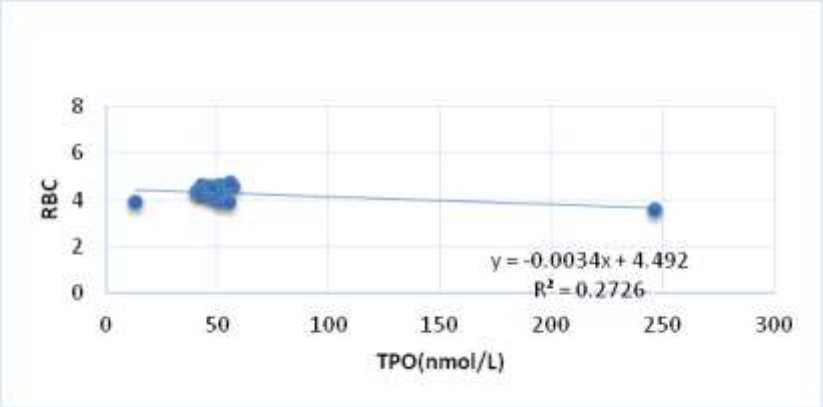

Figure 2: Relationship of RBC with TPO in sera of CLL patients

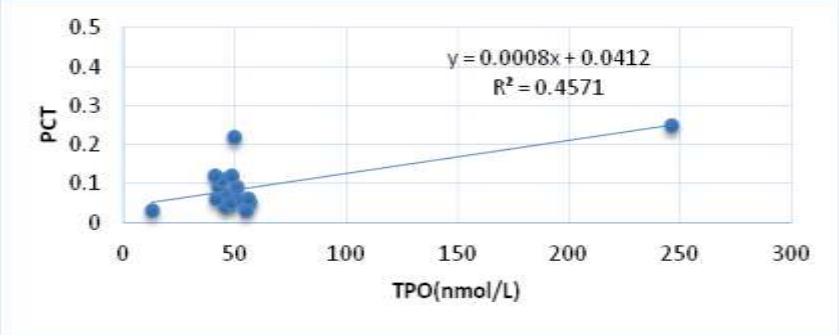

Figure 3: Relationship of PCT with TPO in sera of CLL patients

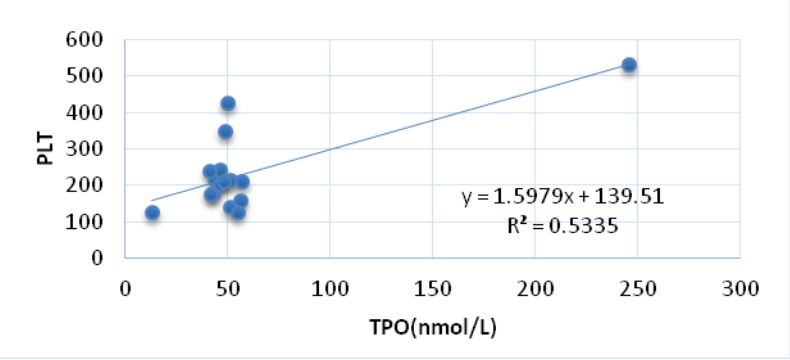

Figure 4: Relationship of PLT with TPO in sera of CLL patients

Discussion

The complete blood count (CBC) test shows how many red blood cells and platelets are present. It also shows the number and type of white blood cells and if they are normal in size and appearance and how much oxygen-carrying hemoglobin the blood has. Table 1 represents CBC for studied groups which indicated an abnormality on a routine blood test of Leuckemia CBC. Those results were agreement with the fact which blood tests of patients with leukemia often show a lower than expected number of red blood cells and platelets. In chronic leukemia, the number of white blood cells is always high. With acute leukemia, white blood cell numbers may be very high, may be lower than normal,or the cells may be immature and unable to function normally[15].

The mean values of thyroid hormones, antithyroid peroxidase, and antithyroglobulin of control, ALL, and CLL groups presented in Table 2 were disagreement partially with previous study of Delvecchio et al in TSH and agreement in FT4 level where they reported that TSH mean and FT4 level did not statistically differ between controls and ALL patients [16]. According to Stephen M. Shalet *et. al.* who's reported an elevated basal thyroid stimulating hormone levels, radiation-induced damage to the hypothalamic-pituitary region is thought to be the cause of these abnormalities in secretion of TSH and other hormone [17]. The thyroid gland seems more prone to be damaged by chemotherapy at a younger age [16].

Although highly significant increase (P=0.00) of anti TG was observed in the present study in sera of both CLL and ALL groups in comparison to that level of the control group, it were found a non-significant differences in serum levels of thyroxine T4 and triiodothyronine T3 in all patients compared to control, all group were within the normal rang, a result similar to that indicated by Mohn A *et. al*. [18]. According to Moskowitz C *et. al.*, there is an increased association of autoimmune thyroid disease and acute leukemia. Since thyroid hormones are important regulators of hematopoiesis and utilize receptors similar to those of differentiating factors such as retinoids [12].

The risk of thyroid dysfunction and thyroid cancer was increased among childhood ALL survivors treated with craniospinal radiotherapy [19]. Drugs with antitumor and anti angiogenic effects such as and TKIs are currently used by most oncologists have been largely used as first- or second-line therapy of various solid tumors or haemopathies, among their side effects, thyroid dysfunctions which include hypothyroidism and hyperthyroidism [20]. The mechanism of the antithyroid effect of tyrosine kinase inhibitors appears to be inhibition of peroxidase activity [21].

Conclusion:

The overall increase of anti TPO and anti TG with the increase of TSH levels of both groups ALL and CLL indicate an autoimmune thyroid disorder, and the FT4 is an important test and it may be used with anti TPO as a marker for diagnosis of leukemia. More studies are required to improve if the anti TPO increase are a risk factor of Leukemia or as a results of , dependent on its correlation with RBC and platelet.

References:

1-Anthony W. Norman,Helen L. Henry, "Hormones," in *Thyroid Hormones*, 2015, p. 89–107.

2 -Wang Z, Zhang H, Zhang X, Sun J, Han C, Li C, Li Y, Teng X, Fan C, Liu A, Shan Z, Liu C, Weng J, Teng W., "Serum thyroglobulin reference intervals in regions with adequate and more than adequate iodine intake Medicine (Baltimore).," *Medicine,* vol. 95, no. 48, p. e5273., 2016.

3

Fiorella S. Belfortea, Alexandra M. Targovnikc, Rodolfo M. González-Lebrerod, Carolina Osorio, "Kinetic characterization of human thyroperoxidase. Normal and pathological enzyme expression in Baculovirus System: A molecular model of functional expression.," *Molecular and Cellular Endocrinology,* vol. 404, no. 15, pp. 9-15, 2015.

4

D. E. Harmon, "Cancer and modern science Leukemia: Current and Emerging Trends in Detection and Treatment," in *types of leukemia*, Rosen Publishing Group , 2012 , pp. 23-31.

5

C. Goldsmith, Leukemia, Twenty-First Century Books, 2011.

6

Delvecchio M, Cecinati V, Brescia LP, Faienza MF, De Mattia D, Cavallo L, Santoro N., "Thyroid function and thyroid autoimmunity in childhood acute lymphoblastic leukemia off-therapy patients treated only with chemotherapy.," *J Endocrinol Invest*, vol. 33, no. 3, pp. 135-139, 2010.

7

Stephen M. Shalet.Colin G. Beardwell. James A. Twomey. Patricia H. Morris Jones. Dorothy

Pearson., "Endocrine function following the treatment of acute leukemia in childhood," *The Journal of Pediatrics*, vol. 90, no. 6, pp. 920-923, 1977.
8
Mohn A, Chiarelli F, Di Marzio A, Impicciatore P, Marsico S, Angrilli F., "Thyroid function in children treated for acute lymphoblastic leukemia," *J Endocrinol Invest*, vol. 20, no. 4, pp. 215-219, 1997 .
9
Eric J. Chow, Debra L. Friedman, Marilyn Stovall,Yutaka Yasui, John A. Whitton, Leslie L. Robiso, "Risk of thyroid dysfunction and subsequent thyroid cancer among survivors of acute lymphoblastic leukemia A report from the Childhood Cancer Survivor Study.," *Pediatric Blood & Cancer* , vol. 53, no. 3, p. 432–437 , 2009.
10
V. B. D. B. K. M. K. P. J. &. W. P. A. Rodwell, "Harper's Illustrated Biochemistry," in *The Diversity of the Endocrine System*, McGraw Hill Professional., 2015, pp. 436-438.
11
Illouz, F., Braun, D., Briet, C., Schweizer, U. and Rodien, P, "Endocrine side-effects of anti-cancer drugs: thyroid effects of tyrosine kinase inhibitors," *European Journal of Endocrinology*, vol. 171, no. 3, pp. R91-R97, 2014.
12
Wong, E., Rosen, L.S., Mulay, M., VanVugt, A., Dinolfo, M., Tomoda, C., Sugawara, M. and Hershman, J.M., "Sunitinib Induces Hypothyroidism in Advanced Cancer Patients and May Inhibit Thyroid Peroxidase Activity," *Thyroid*, vol. 17, no. 4, pp. 351-355, 2007.
13
Dalamaga, M., Lekka, A., Karmaniolas, K., Stathopoulou, E. and Dionyssiou-Asteriou, A., "Is thyroid autoimmunity a risk factor for developing primary myelodysplastic syndrome?," *Cancer Causes & Control.*, vol. 19, no. 4, pp. 371-378, 2008.
14
Moskowitz, C., Dutcher, J.P. and Wiernik, P.H.., "Association of thyroid disease with acute leukemia," *American Journal of Hematology*, vol. 39, no. 2, pp. 102-107, 1992.
15
TOLLERUD, D., AMINOFF, M., GOODMAN, S., HERTZ-PICCIOTTO, I.R.V.A., HOEL, D., OLSHAN, A., HOWARD OZER, M.D., RAMOS, K., NOEL ROSE, M.D., BUTLER, D.A. and BALIGA, S.S , "Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides (Ninth Biennial Update); Board on the Health of Select Population," Veterans and Agent Orange., Washington, 2012.
16
Arneson, W.L. and Brickell, J.M. , "Clinical Chemistry A Laboratory Perspective," in *Endocrine Disorders and Function*, F. A. Davis Company, 2007, pp. 371-425.
17
J. e. Collins, "Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals," in *Iodine and Thyroid Hormone Synthesis, Metabolism, and Action*, Academic Press., 2017, pp. 143-150.
18
Ruf J, Carayon P, "Structural and functional aspects of thyroid peroxidase," *Archives of Biochemistry and Biophysics.* , vol. 445, no. 2, pp. 269-277, 2006.
19
Manna D, Roy G, Mugesh G., "Antithyroid drugs and their analogues: synthesis, structure, and mechanism of action," *Acc Chem Res*, vol. 46, no. 11, pp. 2706-15, 2013.
20
Carvalho DP, Dupuy C, "Thyroid hormone biosynthesis and release.," *Mol Cell Endocrinol*, Vols. pii: S0303-7207, no. 17, pp. 30051-5., 2017
21
Willems E, Valdes-Socin H, Betea D, Beckers A, Beguin Y., "Association of acute leukemia and autoimmune polyendocrine syndrome in two kindreds.," *Leukemia.* , vol. 17, no. 9, pp. 1912-4., 2003.

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