Comparative Study of Thymidine Kinase -1 and Total Antioxidant Capacity in Iraqi Children with Platelet Count Disorder

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Abstract

This study aimed to find relationship between thymidine kinase-1 (TK-1) as tumor marker and total antioxidant capacity (TAC) in Iraqi children patients with thrombocytopenia and with thrombocytosis. The present study conducted 60 children patients (30 patients with idiopathic thrombocytopenia purpura (ITP) and 30 patients with thrombocytosis caused by leukemia) attending the Children Fever Hospital in the Medical City / Baghdad, and 30 healthy children as a control group. All study groups were with range ages (1-15) years, and they were diagnosed by assay of platelet count, Prothrombin Time (PT), and partial Thromboplastin Time (PTT). The results shown elevation in plasma TK-1 and TAC values in children patients with thrombocytopenia and with thrombocytosis when compared with control group, and there was no significant different in TK-1 level and a significant different in TAC level in two patient's groups. There was a highly significant positive correlation between TK-1 and TAC levels in both Iraqi children patients with thrombocytopenia and with thrombocytosis. The current study concluded that TK-1 may be a novel biomarker for platelet count disorder disease and there was a probability of expose these patients for tumor diseases.

Keywords: thrombocytopenia, thrombocytosis, thymidine kinase-1, total antioxidant capacity.

1. Introduction

Platelets assume a basic part in hemostasis and thrombosis, additionally are critical in the improvement of obsessive procedures including atherosclerosis and blood vessel thrombosis [1]. Platelets are created in the bone marrow by megakaryocytes in a procedure that is managed principally by thrombopoietin (TPO). TPO is created mostly by the liver [2]. Both strangely low (thrombocytopenia) and lifted (thrombocytosis) platelet include are regular discoveries a few sicknesses including liver maladies, contaminations, immune system issue and malignancies [3]. Idiopathic thrombocytopenic purpura (ITP) is an immune system infection in which the resistant framework botches the platelets as being remote and crushes them. It can take after an infection, immunization or certain medicines, however for the vast majority the reason is obscure. ITP is now and again called insusceptible thrombocytopenic purpura or just, safe thrombocytopenia. Childhood resistant thrombocytopenia (ITP) is a gained immune system issue described by confined thrombocytopenia (fringe blood platelet check < 100 x 109/L). ITP is a standout amongst the most widely recognized draining issue in youngsters, with a rate of roughly four for every 100.000 every year [4,5]. Thrombocytosis (TS) or height in the fringe blood platelet tally to values >400,000/μ L is basic in outset and

youth, happening in 3 to 13% of youngsters. In youngsters in relatively every case the raised platelet check is because of another therapeutic condition, for example, intense contamination, interminable aggravation, collagen vascular and renal ailments, Langerhans cell histiocytosis, press insufficiency, hemolytic sickliness, and Kawasaki malady (KD) [6].

Thymidine kinases have a key capacity in the combination of DNA and along these lines in cell division, as they are a piece of the special response tie to bring thymidine into the DNA. Thymidine is available in the body liquids because of debasement of DNA from nourishment and from dead cells. Thymidine kinase has been having a developing effect in the disease investigate network. It has been discovered that hoisted blood serum levels of TK-1 relates with metastatic abilities of the growth and in this way can be utilized to recognize threatening kinds of tumor, moreover TK-1 has been found to appear in blood serum even before clinical side effects even begin to demonstrate [7]. In clinical science TK is utilized as an expansion marker in the analysis, control of treatment and follow-up of dangerous malady, chiefly of hematological malignancies [8]. Well evolved creatures have two isoenzymes that are artificially altogether different, TK1 and TK2. The previous was first found in fetal tissue, the second was observed to be more inexhaustible in grown-up tissue, and at first they were named fetal and grown-up thymidine kinase. Before long it was demonstrated that TK1 is available in the cytoplasm just fully expecting cell division (cell cycle-subordinate) [9].

Total antioxidant capacity is the term used to portray the capacity of cancer prevention agents in various sustenances to clean unsafe free radicals in the blood and cells. Restricted examinations in which plasma add up to cell reinforcement status (TAS) was explored show that platelet work changes initiated by intense and interminable exercise might be identified with modifications in cancer prevention agent limit [10,11]. Add up to cancer prevention agent limit (TAC) test use as a biomarker of sickness in organic chemistry, prescription, sustenance and nutritious sciences. In a wide range of pathophysiological conditions (heart and vascular illnesses, diabetes mellitus, neurological and mental clutters, renal disarranges and lung sicknesses), TAC could be a solid biomarker of diagnostics and prognostics. TAC could be helpful to assess nutritious mediations with TAC-rich sustenances on ailment hazard and avoidance, including hostile to maturing techniques [12].

This study aimed to find relationship between thymidine kinase-1 as tumor marker and total antioxidant capacity in Iraqi children patients with thrombocytopenia and with thrombocytosis.

2. Materials and Methods

2.1 Subjects

During the period from November 2017 to April 2018, blood samples were collected from (60) children patients were divided into (30) patients with idiopathic thrombocytopenia purpura (ITP) and (30) patients with thrombocytosis caused by leukemia, in addition to (30) healthy children as control in Children Fever Hospital in the Medical City / Baghdad. All study groups were with range ages (1-15) years, and they were diagnosed by assay of platelet count, Prothrombin Time (PT), and partial Thromboplastin Time (PTT).

2.2 Estimation of Platelet Count in Blood

Evaluation of platelets in venous blood is a typical research facility estimation, performed via mechanized hematology analyzers which use a reference extend for an ordinary platelet check between 150,000 to 450,000 platelets/μL of blood.

2.3 Estimation of Plasma Thymidine Kinase -1 (TK-1) (ng/ ml)

Human thymidine kinase -1 (TK-1) was determined by enzyme –linked immune sorbent assay (ELISA) kit that supplied by Shanghal Yehuda Biological Technology-China, which that based on biotin double antibody sandwich technology.
Assay range: 0.2ng /ml- 20 ng/ml

2.4 Estimation of Plasma Total Antioxidant Capacity (TAC) (U/ml)

Total antioxidant capacity uses enzyme –linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Total antioxidant capacity, soluble (T-AOC). Add Total antioxidant capacity to wells that are pre-coated with Total antioxidant capacity monoclonal antibody and then incubate. After incubation, add anti T-AOC antibodies labeled with biotin to unite with streptavidin –HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of Human Total antioxidant capacity are positively correlated.
Assay range 0.3U|ml-90U|ml

2.5 Estimation of Prothrombin Time (PT) (Sec)

Prothrombin time (PT) was examined by pack from STAGO-France. The guideline of the test comprises of the utilization of calcium thromboplastin to gauge the coagulating time of the patient s plasma and to contrast it and that of a typical standard. The test measures, all in all, the action of the coagulation factor ll (prothrombin), factor V (proaccelerin), factor Vll (proconvertin), factor X (stuart factor) and factor l (fibrinogen) [13]. Normal Value: 12-15 Sec.

2.6 Estimation of Activated Partial Thromboplastin Time (APTT) (Sec)

The Kaolin – initiated halfway thromboplastin time (APTT) was controlled by pack from STAGO-France. The test includes the recalcification of plasma within the sight of an institutionalized measure of cephalin (platelet substitute), and the factor Xll activator (Kaolin). The APTT investigates the characteristic coagulation pathway (factors Xll, Xl, lX, Vlll,X, V, ll and l) aside from the platelets[14].
Normal Value: 28-40 sec

2.7 Statistical Analysis

Information were communicated as mean ± SEM. The correlation amongst patients and control bunches was communicated by t-test. P-estimation of < 0.005 and < 0. 05 were considered profoundly huge and huge, individually. Pearson's connection coefficient (r) is utilized for portraying the relationship between the distinctive investigation parameters.

3. Results and Discussion

The levels of diagnostic parameters in children patients and control groups are summarizes in **Table 1**. The results which expressed as (mean± SEM), showed a highly significant decrease (p < 0.005), and a highly significant increase (p < 0.005) in platelet number in both

idiopathic thrombocytopenic purpura patients (G1) and thrombocytosis caused by leukemia patients (G2), respectively, when comparing with control group,while there was a highly significant different (p < 0.005)   in platelet number between two groups.

Thrombocytopenia is defined as a platelet count of less than 150×10^3 per μL. Normally, the platelet count in the peripheral blood is controlled by complex in teractions regulating platelet production in the bone marrow, platelet pooling in the liver and spleen, and their elimination in the reticuloendothelial system, which then feeds back into thrombopoietin regulation [15,16]. The platelet count is rather constant in an individual person. In critically ill patients, however, these mechanisms can fail, which results in a disturbance of the balance among platelet production, platelet pooling, and platelet consumption. Thrombocytopenia should be seen as a sensitive marker for considerable alteration of normal physiology [17]. In kids in relatively every case the hoisted platelet check is because of another medicinal condition, for example, intense contamination, incessant aggravation, collagen vascular and renal maladies, Langerhan's cell histiocytosis, press lack, hemolytic pallor, and Kawasaki disease [18]. In endless myelogenous leukemia, there are diminished levels of serum erythropoietin in polycythemia vera and diminished levels of granulocyte province fortifying element. In myeloproliferative scatters, there is diminished official of thrombopoietin to megakaryocytes due to the diminished number and capacity of thrombopoietin receptors, yet in fundamental thrombocythemia, these ancestors are additionally uniquely touchy to the activity of the hormone. This prompts the expanded megakaryocyte multiplication and platelet generation found in fundamental thrombocythemia [19]. Despite the fact that thrombocytopenia is a sign of intense lymphoblastic leukemia (ALL), in a few arrangement upwards of 25% of youngsters have been found to have platelet tallies surpassing 100,000/mm3 at conclusion. In any case, thrombocytosis isn't normally perceived as an introducing highlight of ALL. We as of late observed a patient with an underlying platelet check of more than 500,000/mm3, a discovering which had postponed doubt of hematologic danger. In this way, it appears to be likely that the platelet include heights were some path due to the leukemic procedure [20].

The present study showed a significant elevation (p < 0.05) in PT levels in G1 and G2 patients when compared with control, and there was no significant different in PT levels between two groups. Also there was a highly significant increase in PTT level (p < 0.005) in G1, and no significant different in PTT level in G2 when compared with control, in addition to a highly significant different in PTT level between two groups.

Unlike factors VIII and IX, the platelet clotting factor is not available until platelets are acted upon by thrombin. In thrombocytopenia, the flawed prothrombin utilization is clear yet the absence of platelets is frequently muddled by a subjective absence of their thickening component. The prothrombin utilization time yields more noteworthy data, in this way, than does the platelet check [21]. The thickening time isn't uncovering since it is ordinary in thrombocytopenia and factor VII inadequacy yet notably drawn out in the Hageman attribute, in which hemostasis once in a while is by all accounts traded off. The one-arrange prothrombin time is ordinary in thrombopathy and hemophilias an and B, while the Iowa two-organize prothrombin test is typical in thrombocytopenia and in factor VII inadequacy [22]. The thrombocytopenia was convoluted by thrombopathy, since the platelets were notably lacking in the platelet thickening element (pcf) [23]. The coagulation anomalies specifically owing to intense leukemia and its treatment are in this manner multifactorial. Potential reasons for drain rely upon pretreatment factors, for example, kind of intense leukemia,

introducing impact cell check, and seriousness of thrombocytopenia. The one-organize prothrombin time (PT) and initiated incomplete thromboplastin time (PTT) are perpetually drawn out in these disorders [24].

The outcomes in **Table 2.** demonstrated an exceedingly critical increment (p < 0.005) in plasma thymidine kinase-1(TK-1) levels in both idiopathic thrombocytopenic purpura patients (G1) and thrombocytosis caused by leukemia patients (G2), separately, when contrasting and control group, while there was no noteworthy distinctive in TK-1 between two gatherings.

In the flow contemplate, the level of TK-1 in youngsters patients with thrombocytosis that caused by leukemia (G2) was higher than that in kids patients with idiopathic thrombocytopenia (G1); in this manner kids patients with thrombocytosis that caused by leukemia might be more inclined to malignancy infections than that in kids patients with idiopathic thrombocytopenia , in light of the fact that thymidine kinase (TK) action in plasma is a tumor development related marker which has been utilized for forecast and observing of treatment of lymphoma and leukemia [25]. TK1 is synthesized by the cell during the S phase of cell division. After cell division is finished, TK1 is debased intracellularly and does not go to body liquids after ordinary cell division [26].

Investigation of Aufderklamm and et al. [27] shows that TK1 has all the earmarks of being a decent all inclusive marker for growth among both strong and hematological malignancies. Earlier examines on STK1, affirm that TK1 in serum isn't helpful for assessment of tumor movement but at the same time is a valuable instrument for assessment of the impact of tumor therapy [28].

To the extent as far as anyone is concerned, there is no information in the writing concerning the plasma level of thymidine kinase-1 chemical in youngster's patients with idiopathic thrombocytopenic purpura and with thrombocytosis that caused by leukemia. Accordingly, the present investigation was the primary that planned to reveal an insight into the part and level of TK-1 and its association with TAC in such patients, which could investigate at any rate partially the commitment of this catalyst to the pathogenesis of the illness .

The outcomes in **Table 2.** uncovered no noteworthy height in plasma add up to cell reinforcement limit (TAC) levels in youngster's patients with idiopathic thrombocytopenic purpura patients (G1) and an exceptionally huge rise in kids' patients with thrombocytosis caused by leukemia patients (G2), individually, when contrasting and control gathering, while there was a critical diverse in TAC levels between two gatherings.

As of late, a few confirmations have recommended that oxidative pressure assumes a vital part in the pathogenesis of immune system maladies, and it is likewise associated with the improvement of ITP. These findings might provide a new hypothesis for the initiation of abnormal autoimmunity and a possible novel therapeutic approach [29]. Sinan Akgun and et al. in their investigation, they reason that an addition in serum MDA, TOS, and OSI levels, and a decrement in TAC levels were found in patients with intense and unending ITP. Based on these discoveries, they recommend that free oxygen radicals may affect the basic and utilitarian harm of platelets, and on the system of thrombocytopenia in both intense and unending ITP, notwithstanding a positive relationship between's thrombocyte check and TAC, in patients with intense and ceaseless ITP [30]. The platelet obliteration and draining may assume significant part on rise of lipid peroxidation and diminishment in cancer prevention agent limit in patients with ITP, additionally examines on oxidant and cell reinforcement status of ITP are likewise expected to confirm these outcomes [31].

The findings of (C. - Q. Jin and et al.) [32] think about is proposes that oxidative pressure may affect the auxiliary and practical harm of platelets and on the system of thrombocytopenia in unending ITP. Thusly, oxidative pressure is thought to assume a part in thrombocyte harm [32]. The expanded aggregate cell reinforcement limit may avert oxidative damage prompted by exercise and change in platelet reactions to preparing might be identified with enhanced cancer prevention agent status [33].

In this study, **Figures 1.** and **2.** were showed a highly significant positive correlation between thymidine kinase -1 and total antioxidant capacity levels in both G1 (r = 0.675) and G2 (r = 0.820).

Table 1. Levels of diagnostic parameters in control, children patients with idiopathic thrombocytopenia purpura (G1), and with thrombocytosis caused by leukemia (G2) groups.

Groups / Parameters	Control No.(30)	G1 No. (30)	G2 No.(30)	P-value (C &G1)	P-value (C &G2)	P-value (G1&G2)
Platelet (10^3/μL)	290 ± 53.55	23.67 ±4.43	778.60 ±142.20	*HS*	*HS*	*HS*
PT (Sec)	11.73±2.14	15.52±2.83	12.58 ± 2.29	*S*	*S*	*NS*
APTT (Sec)	32.36±5.90	41.45±7.56	32.60 ± 5.95	*HS*	*NS*	*HS*

Table 2. Levels of thymidine kinase -1(TK-1) and total antioxidant capacity (TAC) in control, children patients with idiopathic thrombocytopenia purpura (G1), and with thrombocytosis caused by leukemia (G2) groups.

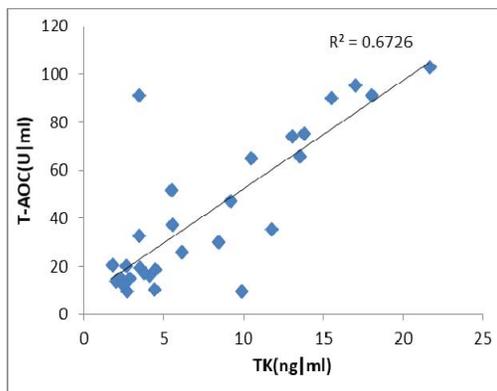| Groups / Parameters	Control No.(30)	G1 No.(30)	G2 No.(30)	P-Value (C & G1)	P-Value (C &G2)	P-Value (G1& G2)
TK-1 (ng/ ml)	2.99 ± 0.54	6.81 ± 1.24	7.42 ± 1.35	*HS*	*HS*	*NS*
TAC (U/ml)	20.49 ± 3.74	27.08 ± 4.60	40.70± 7.43	*NS*	*HS*	*S*

Figure 1. Correlation between TK-1 and TAC in children patients with ITP.
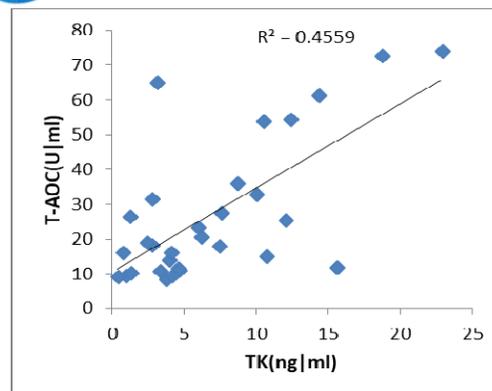

Figure 2. Correlation between TK-1 and TAC in children patients with thrombocytosis.

4. Conclusions

This study was the first in the determination of plasma thymidine kinase -1 (TK-1) level as tumor marker in the children patients with thrombocytopenia and with thrombocytosis. Current study proved a highly significant elevation in TK-1 levels in the Iraqi children patients with platelet count disorder; therefore, TK-1 may be a novel biomarker for this disease and there was a probability of expose these patients for tumor diseases. Plasma total antioxidant capacity (TAC) levels were increase in Iraqi children patients with platelet count disorder, while there was a significant different in TAC level in two patient's groups. There was a highly significant positive correlation between TK-1 and TAC levels in both Iraqi children patients with thrombocytopenia and with thrombocytosis.

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