Evaluation of Serum Thymidine Kinase 1 Level as a prognostic factor of non-Hodgkin's lymphoma patients under Therapy

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Abstract: This study was performed to examine possible use of thymidine kinase 1 concentration in serum (STK1) as a prognostic factor of non-Hodgkin's lymphoma patients following chemotherapy treatment. **Methods**: The STK1 levels of 50 patients were determined by Enzyme linked immunosorbent assay before chemotherapy, and after start of the treatment. **Results**: Enzyme linked immunosorbent assay of TK1 in serum showed high specificity and sensitivity. The mean STK1 level of the non Hodgkin's lymphoma patients was significantly higher compared to healthy persons ($p < 0.001$). The mean STK1 level increased significantly ($p < 0.001$) Before therapy and after therapy declined, reaching values corresponding to those of healthy persons. The mean STK1 values before treatment and after start of the treatment also correlated significantly with five-year survival. **Conclusion**: Although the number of patients was limited in this study, TK1 in serum might possess an important in this study, TK1 in serum might possess an important reference value in the evaluation of treatment and prognosis of non-Hodgkin's lymphoma following chemotherapy.

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1. Introduction

Lymphomas are cancers involving the cells of the lymphatic system. The majority of lymphomas involve the lymph nodes and spleen but the disease may also affect other areas within the body. Non-Hodgkin's lymphoma (NHL) is a classification of all lymphomas except Hodgkin's disease. Thus NHL is a mixed group of diseases that is characterized by the malignant increase in specific cells of the immune system (B or T lymphocytes). B-cell lymphomas are more common than T-cell lymphomas, accounting for about 85% of all cases of NHL (**(American Cancer Society, 2017).**

NHL is more common among people who have abnormal or compromised immune systems, such as those with inherited diseases that suppress the immune system, individuals with autoimmune disorders and people taking immunosuppressant drugs following organ transplants. Genetic predisposition (e.g., inherited immune deficiencies) only accounts for a small proportion of NHL cases **(Muller and Scherr, 2006).** Thymidine kinase (TK) is involved in nucleic acid synthesis and is very low in non proliferating cells, but increases dramatically at late G1 to late S-phase/early G2 phase during the cell-cycle in proliferating cells and tumor cells. This makes TK-1 an interesting marker for cell proliferation and tumor growth **(Wu etal., 2009).** TK1 is a cell cycle-dependent enzyme of the pyrimidine salvage pathway, catalyzing the phosphorylation of thymidine to thymidine monophosphate. TK1 concentration in the cell is low in the G1 phase of the cell cycle, increases duringthe S/G2 phases and decreases in thelate G2/M phase. TK1 is low or absent in non-proliferating cells, with some exceptions. Transcriptional and translational mechanisms control the expression of TK1 **(Aufderklamm etal., 2012).**

The degradation of TK1 in the M phase is due to ubiquitin-proteasome- related mechanisms Singling out TK1 as useful biomarker for cell proliferation, and thus for malignancy. Using the thymidine analogue5-iodo-2-deoxyuridineasasubstrate, a STK1 assay was established for human tumors in the early 1980s. The STK1 assay is useful for the prognosis of survival and the monitoring of tumor treatment mainly in patients with leukaemia, as well as Hodgkin's and non-Hodgkin's lymphoma **(James, 2002).**

The potential power of TK as a tumor marker for identifying early neoplasia and will aid in determining an appropriate course of treatment. In determining appropriate treatment, it should be recognized that higher levels of TK may hinder the effects of some chemotherapeutics and radiation **(Thomas et al.,1995).**

The study was designed to evaluate of serum thymidine kinase 1 as a prognostic marker of non-Hodgkin's lymphoma patients before and after therapy.

2. Subjects and Methods

Subjects

Thirty primary non-Hodgkin's patients were studied (15women and 15men). The mean age (± SD) of these patients was 54.3 (± 7.6) years range (39-68). Subjects who had a Hodgkin's lymphoma, Non smoldering CLL, Myelodysplastic syndrome, Small cell carcinoma of the lung, Prostate cancer, Breast cancer. Other malignancies: Squamous cell head and neck cancer (oral cavity, oropharynx and hypopharynx cancers, (Non cancer) factors: pernicious anemia and acute stages of herpes simplex virus induced disease and Infection and inflammations were excluded from the study. Healthy age-matched subjects (n=20, 10 women and 10 men) served as controls. The mean age (± SD) of these subjects was 40.0 (±7.0) years (range 28-55). All subjects gave written, informed consent, which was approved by the Ethical Committee of Medical Faculty.

Methods

All included patients were submitted to full history, general examination, laboratory tests included; (Complete blood count, ESR, BM examination, Immunophenotyping and lymph node biopsy), radiological investigation and Thymidine kinase1 by Enzyme Linked Immunosorbent Assay (ELISA).

Blood sampling:

5 ml of blood were withdrawn with minimal stasis. Blood was divided into 3 tubes as follows:

1. 1ml was carried into tube containing K-EDTA for complete blood count.

2. 0.8ml was carried into tube containing sodium citrate (3.8%) for ESR by westergren.

3. The rest of the blood was delivered into a plain tube, left to clot and then centrifuged within 10-20 minutes. The separated serum was divided into two capped tubes, one stored at - 20°C until assayed for Thymidine kinase 1 and the other used for routine investigations.

STK1 Assay

Serum-coagulation were collected before chemotherapy and after chemotherapy The ELISA assay was performed according to the manufacturer's protocol (Mitochondrial (sTK1) ELISA KIT FOR RESEARCH USE ONLY. Not for clinical DIAGNOSIS USE CATALOG #:10135. www.glorybios.com.

Blood sample were collected at room temperature for 10-20 min，centrifuge at the speed of 2000-3000 rpm for 20-min. Remove supernatant, if precipitation appeared, Centrifuge again. The standard was diluted by Pipette 50µl standard dilution in each tube, 40uL of sample diluent were dispensed into testing sample well then 10µl of sample were dispensed into wells, without touching the well wall as far as possible, and mix gently for 5 seconds, then incubated and covered by adhesive strip for 30 minutes 37C temperature, The wells were washed fives times with buffer. 50uL of HRP-Conjugate reagent were dispensed into each well except blank well then incubated for30 minutes at 37C temperature, The wells were washed fives times with buffer. Chromogen Solution a 50ul and Chromogen Solution B were dispensed into to each well, avoiding the light for 15 min at 37C, 50uL of stop solution was added to stop reaction and read at 450 nm with a micro well reader within 15 minutes.

Statistical analysis

The mean values of the STK1 levels were calculated by mean ± standard deviation program (Microsoft Excel). The statistical differences were calculated by Student's t test and Kruskal–Wallis test. The survival curves were of Kaplan– Meier type and the statistics was calculated by the log-rank test. The regression analysis was done by Spearman test The analysis of variance was done by ANOVA test with the data processing program Stata 8.0. Statistical differences were considered to be significant when the p value was less than 0.05.

3. Results

In the study, males represent 50% of all studied populations, and females represent 50%. In the p study, mean age of the study group 54.3±7.6 years and mean age of the control group 40.0±7.0years. The study has shown statistically significant linear relationship of degree of thymidine kinase 1 with age (P value 0.001). In study, ESR was significantly increased in study group when compared to control group (P value<0.001). In addition, there was a positive and linear correlation between ESR and thymidine kinase1. In study, LDH was significantly increased in study group when compared to control group (P value<0.001). In addition, there was a positive and linear correlation between ESR and thymidine kinase1. In study, white blood cells was significantly increased in study group when compared to control group (p= 0.003). In study, Platelets was significantly increased in study group when compared to control group (p= 0.002). In study, SGPT was significantly increased in study group when compared to control group (p= 0.004). In study, SGOT was significantly increased in study group when compared to control group (p= 0.003). In this study, we exam whether TK1 concentration in serum can be used for monitoring the effect of chemotherapy in patients with non-Hodgkin's lymphoma. The patients were treated. We found that the STK1 concentration significantly distinguished between patients with non-Hodgkin's lymphoma and healthy persons.

Table 1: Statistical significance analysis of patients before, after start of chemotherapy treatment and control. with different levels of STK1

Variable (s)	Study group (Before)	Study group (After)	Control group	P value
Age (Years)	54.3± 7.6	54.3± 7.6	40± 7	**0.001 S**
TK1(ng/dl)	685.87 ±193.8	152.49±70.4	54.95 ±16.99	**<0.001* V.H.S**
LDH (U/L)	648.8 ±99.2	409.87 ±55.59	267.75 ±44.52	**<0.001* V.H.S**
ESR (mm)	71.17±8.6	33.7±4.86	10.4±1.98	**<0.001* V.H.S**
HB (g/dl)	10.66±0.71	10.07±0.67	13.34 ±1.05	**0.06 NS**
RBCS (m/cmm)	4.47 ±49007	3.71 ±0.22644	4.852 ±28993	**0.07 NS**
WBCS ((C/mm)	19806±8022	7103±2028	6710±1557	**0.003 S**
PLT ((C/mm)	115600±30320	145166±20688	262800±79290	**0.002 S**
SGPT (U/L)	53.6±16.64	38.4±8.91	24.55±6.25	**0.004 S**
SGOT (U/L)	50.9 ±12.57	32.37 ±5.83	23.5 ±5.84	**0.003 S**

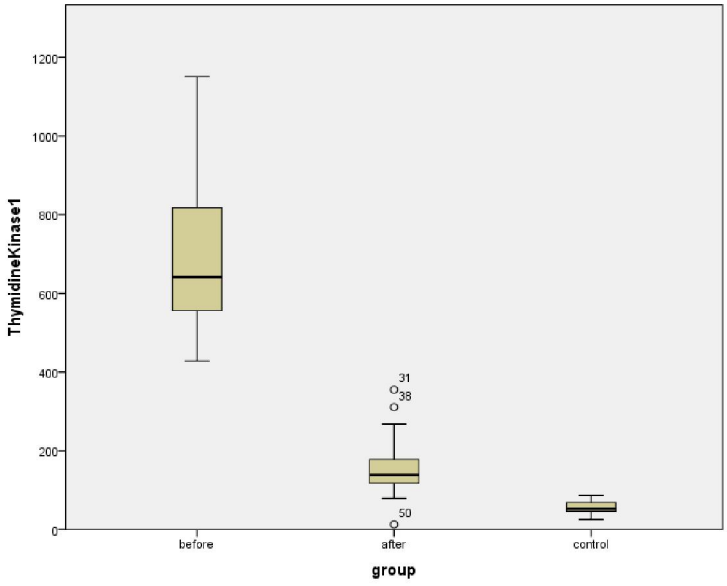

Fig. 1 a STK1 concentrations before of chemotherapy in

Patients with non-Hodgkin's lymphoma (n = 30). **b** STK1 concentration after of chemotherapy in patients with non-Hodgkin's lymphoma **c** concentration in healthy control.

4. Discussion

Lymphomas are neoplasms of T, B or natural killer (NK) lymphoid cells and their precursors. Although having different characteristics from their normal counterparts, the neoplastic cells of many lymphomas have the features of lymphoid cells at a particular stage of differentiation. In addition, lymphoma cells can have the characteristics of lymphocytes that normally reside in a particular organ or tissue **(Howlader et al.,2014).**

Non-Hodgkin's lymphomas (NHLs) represent a heterogeneous group of malignancies that arise from the lymphoid system. Recent advances in molecular genetics have significantly deepened our understanding of the biology of these diseases. The introduction of gene expression profiling especially has led to the discovery of novel oncogenic pathways involved in the process of malignant transformation **(Morton et al.,2006).**

Several potential biomarkers are available at present and each marker has its own clinical characteristics. Thymidine kinase 1 activity in serum (STKa) is a cell-proliferating marker that has been used for the prognosis and monitoring of treatment, follow-up and survival in patients with lymphoma and leukaemiasince1983 **(Wu et al., 2003).**

Human thymidine kinase 1 (TK1) has a narrow specificity, phosphorylates only deoxythymidine (dT) and deoxyuridine, and is strictly cell-cycle regulated. TK1 activity is low or absent in resting cells, starts to occur in late G1 stage, increases in S-phase (coinciding with the increase in DNA synthesis), and disappears during mitosis, by degradation through an

ubiquitin–proteasome degradation mechanism **(Ke and Chang 2004).**

The TK1 levels in serum of patients with malignancies are significantly higher than those of healthy persons, and thus, TK1 is useful as a proliferation marker for malignancies, that is, for assessment of tumor progression and monitoring therapy (**Xu et al. 2008; Zhang et al. 2006).**

A reasonable assumption from the knowledge of TK1 regulation during the cell cycle of normal cells is that no or little release of TK1 to the body Xuids occurs, since normal cells die in G1, where the concentration of TK1 is low, or that TK1 is degraded in mitosis. However in some cases of healthy persons TK1 levels show minor elevations, for example, in blood donors and during menstruation, in Xammations and infections **(He et al. 2005).**

It should be mentioned that other (non cancer) factors will also result in an elevated level of TK in the serum. The main causes for this transient elevation are: pernicious anemia and acute stages of herpes simplex virus induced disease. Both conditions are easily identified and therefore do not give false results when analyzing samples for s-TK levels (**Al-Nabulshi et al.,2004).**

In this study, we exam whether TK1 concentration in serum can be used for monitoring the effect of chemotherapy in patients with non-Hodgkin's lymphoma. We found that the STK1 concentration significantly distinguished between patients with non-Hodgkin's lymphoma and healthy persons (negative control).

We also found that the STK1 concentration in patients increased significantly before start of the treatment, then decreased significantly to values closer to healthy persons, due to a successful treatment. STK activity was also found to be useful for prognosis and monitoring responses of treatment effect in adult patients with non-Hodgkin's lymphoma.

From the present study **it is recommended that:** Use serum TK1 as useful marker for prognosis and monitoring the outcome of chemotherapy of patients with non-Hodgkin's lymphoma before start of therapy and after therapy.

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