ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

ENZYMES: LACTATE DEHYDROGENASE AND ALKALINE PHOSPHATASE IN LUNG CANCER PATIENTS BEFORE AND AFTER TREATMENT.

Nirensingh Koch[1], Th. Ibetombi Devi[2], L. Jaichand[3], Bharat Kumar Gupta[4].

1. Assistant Prof, Department of Biochemistry, Subharti Medical College, Meerut.
2. Prof. Department of Biochemistry, Regional Institute of Medical Sciences, Imphal.
3. Prof. Department of Radiotherapy, Regional Institute of Medical Sciences, Imphal.
4. Prof. Department of Biochemistry, Subharti Medical College, Meerut.

Manuscript Info

Manuscript History:

Received: 14 December 2015
Final Accepted: 19 January 2016
Published Online: February 2016

Key words:
LDH, ALP, Lung cancer, NSCLC, SCLC.

Corresponding Author

Nirensingh Koch.

Abstract

Background:- Cancer produces profound biochemical changes in the body of the host among which alterations in the enzyme levels in serum or plasma is a remarkable one.

Objectives of the study:- The present study aims to assess the status of LDH and ALP in lung cancer patients before and after treatment.

Materials and Methods:- A total of 50 lung cancer patients clinically staged and confirmed by histopathological examination were included in the present study. Another 30 healthy age and sex matched subjects free from any systemic diseases were taken as control. Serum LDH and ALP levels were estimated by Calorimetric method as described by Henry RJ et al. (1960) and Bowers GN et al. (1976) respectively.

Results:- Mean serum LDH (439.42±145.31 IU/L) and ALP (299.60±144.2 IU/L) were significantly higher in lung cancer patients as compared to control group (LDH= 221.06±34 IU/L and ALP=162.30± 20.61IU/L) respectively, p value<.001. Pre treatment serum LDH and ALP levels show positive correlation with stages of disease. Mean serum LDH decreased to 356.84±150.39 IU/l and ALP to 227.30±107.66 IU/l after treatment and difference between pretreatment and post treatment values were statistically significant.(p value<.001)

Conclusion:- Measurement of serum LDH and ALP activity would be useful tool in the diagnosis and assessment of prognosis in lung cancer patients. The pretreatment serum LDH and ALP has positive correlation with stage of disease, tumor mass and extend of disease. However the post treatment values of the enzymes were inconsistent. Further studies are recommended in order to evaluate the significance of post treatment values of these enzymes

Introduction:-

The incidence of lung cancer and its mortality in the world have been increasing in epidemic proportions. According to National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) 2010 report, lung cancer is the second most common cancer worldwide, in both males (accounting 15% of all cancer) and females (accounting 14% of all cancer) and it is the most common cause of cancer death worldwide.[1]

Epidemiological study shows that there is increased prevalence of lung cancer in Indian population.[2] Currently it is the fourth largest cause of cancer mortality in India and accounts for nearly 8% of all cancer related deaths. In males it is the leading cause of cancer mortality accounting for 13% of all cancer death.[3]

In India, approximately 63,000 new lung cancer cases are reported each year.[4] The latest National Cancer Registry programme (NCRP) 2010, has reported the highest age-adjusted incidence rates (AARs) of lung cancer in north-eastern region of India: Aizawl district (18.2/100000) followed by Imphal west district Department of Radiotherapy, Regional Institute of Medical Sciences, Imphal, India (18/100000) for males and AAR of 26.2/100000 in Aizawl district and 18/100000 in Imphal west district for females. According to the Hospital Based Cancer Registry (HBCR) 2012, lung cancer was the most common cancer in both males (19.7% of all cases) and females (15.1% of all cases). Lung cancer has been the most common malignancy in both males and females in this state for the past decade contributing approximately 16-20% of all malignancies.

The measurement of biochemical markers especially serum enzymes are being gradually increasing in general practice for early diagnosis and monitoring the progress in a variety of malignant diseases, as the process of transformation of normal cells into malignant cells often leads to abnormal serum enzyme synthesis, even before changes in tumor morphology. [5] The enzymes have also been useful in following the effect of treatment. Therefore, enzyme studies have recently received widespread attention. Among these serum enzymes, the clinical importance of Lactate dehydrogenase (LDH) and Alkaline phosphatase (ALP) in malignancies have been reported by number of workers. [6-8]

Cancer produce profound biochemical changes in the host among which alterations in the enzyme levels in serum or plasma is a remarkable one. The biochemistry of cancer involves a complex multisystem carcinogenic process during which several regulatory circuits are altered. These alterations manifest themselves through changes in the enzymatic patterns. The most prominent biochemical feature of malignant cells is the production of large quantity of lactic acid. LDH is key enzyme in anaerobic glycolysis that catalyzes the process of production of lactic acid from pyruvate.

There is aberrant expression of ALP genes in cancer cells which could be involved in tumorigenesis. In healthy adults it is mainly derived from the liver, bones and in lesser amounts from intestines, placenta, kidneys and leukocytes.[9] Serum levels ALP have also been used in the clinical evaluation of numerous diseases, including malignancies, for half a century. It is a classical marker of bone formation especially in cancer patients.[10] Lung cancer commonly causes destructive bony metastases and bone isoenzyme of ALP is significantly higher in patients with bone metastases than those without bone metastases.[11,12]Another isoenzyme, High molecular weight ALP is a useful marker of hepatic metastases.[13] and ALP with heat sensitivity characteristics similar to that of bone type is found in serum of patients suffering from lung cancer.[14]
Survival of patients with malignant disease may be improved with early diagnosis and treatment. But the symptoms of metastasis appear late. The radiological diagnostic procedures such as x-rays; ultrasound and CAT scans although sensitive are costly.

The estimation of these enzymes in serum is a simple laboratory technique which could help in early detection of liver and bone metastasis in oncological patients. Moreover, although extensive work has been done on these enzymes in other countries, only few reports are available in India. It is with this view as well as most predominance of lung cancer in this part of the country that this study has been undertaken to assess the activity of LDH and ALP in lung cancer patients before and after treatment to evaluate the significance of these enzymes in diagnosis and prognosis as well as in monitoring therapy.

Aims and objectives:-

1) To estimate and compare the serum LDH and ALP in lung cancer cases and control subjects
2) To compare the LDH and ALP values in lung cancer cases before and after treatment.

Materials and methods:-

The study was conducted in the department of biochemistry Regional Institute of Medical Sciences, Imphal, Manipur from July 2010 August 2011, after obtaining ethical clearance from the institutional ethical committee. A total of 50 lung cancer patients suffering from different stages of the disease were selected for the study. All patients

were freshly diagnosed and clinically staged. A total of 30 age, sex matched apparently healthy individuals were taken as the "control group."Serum LDH and ALP were measured in both cases and controls. Serum LDH and ALP were estimated once before starting treatment both in control and cases and follow up second samples from lung cancer patients were taken within 3-4 weeks of completion of treatment. Cutoff value for LDH was taken as 240 IU/L and for ALP 160 IU/L.

Exclusion criteria:- Patients having concurrent illness like hepatobiliary disease especially obstructive type, renal disease, pancreatic disease, cardiac disease, bone diseases as well as parathyroid disease were excluded while selecting study group.

Sample collection:- 2 ml blood samples were collected in plain vials from patients once at the time of diagnosis and once after the treatment. From the control subjects blood samples were also collected only once. Hemolysed and lipemic blood samples were not included in the study because of possible interferences.

Reagents used were of analytical grade. The organic solvents were redistilled before use. Blood samples were centrifuged at 700×g for10 minutes and the sera thus separated were analyzed in the same day for estimations of serum LDH and ALP in semi auto analyzer Statfax 3300 with reagents from Human diagnostics, Germany. Serum LDH was estimated according to method of Henry RJ et al. (1960) [15] Serum ALP was assayed by method of Bowers GN and Mc Comb RB (1975).[16]

Statistical analysis:-

Data were expressed as Mean± SD, percentages and continuous variables of the 2 groups were compared by student's t test wherever suitable. These statistical analyses were performed using SPSS version 16 for windows. P value of < 0.001 is considered as statistically significant.

Result:-

The mean age of the study populations were comparable in both males and females and majority of the study populations were in the age group of >60-70 years. 88% of the patients had elevated LDH and 78% of patients had elevated ALP at the time of diagnosis (Table 3 and 4). Serum LDH and ALP levels in lung cancer cases were significantly higher than the control subjects (Table 1). The pretreatment and post treatment difference of serum LDH and ALP were statistically significantly (Table 4, 5 and 6). The maximum decrease in LDH was observed in limited stage disease of SCLC stage and maximum decrease in ALP was observed in extensive stage disease of SCLC. The results and observations are given in Table-1-6.

Table 1: Serum LDH and ALP in control and lung cancer cases.

Serum enzymes	Control (n=30) Mean±SD	Lung cancer cases (n=50) Mean±SD	t	df	P value
LDH (IU/L)	**221.06±34.00**	**439.42±145.31**	**.079**	**8**	**<0.001**
ALP (IU/L)	**162.30±20.61**	**299.60±144.29**	**.167**	**8**	**<0.001**

Legend 1:- Lung cancer patients have significantly higher levels of LDH and ALP at the time of diagnosis than controls.(P value<0.001)

Table 2: Distribution of lung cancer patients before and after treatment based on normal cutoff value of LDH (240 IU/L).

Pre treatment	Post treatment		
	Remained Normal/Decreased to normal	**Decreased but above normal**	**Increased**
Normal (<240) =6 (12%)	4	____	2
Abnormal (>240) =44 (88%)	11	29	4
Total	15 (30%)	29 (58%)	6 (12%)

Legend 2:- In 12% LDH was normal before treatment and in 88% Abnormal values before treatment. In the normal group, LDH level remained normal in 4 patients and increased to abnormal levels in 2 patients after treatment. In the

abnormal group, LDH returned to normal in 11 patients, decreased but above normal in 29 patients and increased in 2 patients after treatment

Table 3: **Distribution of lung cancer patients before and after treatment based on normal cutoff value of ALP (160 IU/L).**

Pre treatment	Post treatment		
	Remained Normal/Decreased to normal	**Decreased but above normal**	**Increased**
Normal (<190)=11(22%)	9	___	2
Abnormal (>190) =39 (78%)	16	19	4
Total	25(50%)	19(38%)	6 (12%)

Legend 3:- In 22% ALP was normal before treatment and in 78% Abnormal values before treatment. In the normal group, ALP level remained normal in 9 patients and increased to abnormal levels in 2 patients after treatment. In the abnormal group, ALP returned to normal in 16 patients, decreased but above normal in 19 patients and increased in 4 patients after treatment

Table 4: Comparison of pre and post treatment value of Serum LDH by mode of Treatment.

Mode of Treatment	Number	Serum LDH level (Mean ± SD)		P value
		Pre Treatment (IU/l)	Post Treatment (IU/l)	
Chemotherapy	26	453.69±138.26	390.76±145.20	p<0.001
Chemo-RT	18	432.27±124.30	317.89 ±145.51	p<0.001
RT	6	399.00±235.86	326.66±178.06	p<0.001
Total	50	439.42±145.31	356.84±150.39	(t=6.473,df=49), p<0.001

Legend 4:- Comparison of mean pre treatment and post treatment values of LDH according to mode of treatment. It is significant in all the categories of treatment and maximum decrease was observed in the group receiving chemotherapy followed by radiotherapy.

Table 5: Comparison of pre and post treatment values of Serum ALP by mode of Treatm

Mode of Treatment	Number	Serum ALP level (Mean± SD)		P value
		Pre Treatment (IU/L)	Post Treatment (IU/L)	
Chemotherapy	26	313.46±156.52	251.80±126.33	P<0.001
Chemo-RT	18	273.37±73.20	197.77±52.31	P<0.001
RT	6	318.23±243.78	209.66±133.64	P<0.001
Total	50	299.60±144.29	227.30±107.66	(t=6.174, df=49),P<0.001

Legend 5: Comparison of mean pre treatment and post treatment values of ALP according to mode of treatment. It is significant in all the categories of treatment and maximum decrease was observed in the group receiving radiation therapy.

Table 6: Mean Serum LDH and ALP levels in different stages of lung cancer both before and after treatment. NSCLC (IIIa, IIIb and IV) and SCLC (Limited disease and extensive disease).

Stages of lung cancer	Number	Percent (%)	Pre treatment		Post treatment		P value (LDH)	P value (ALP)
			LDH (Mean±SD) (IU/L)	ALP (Mean±SD) (IU/L)	LDH (Mean±SD) (IU/L)	ALP (Mean±SD) (IU/L)		
III a	7	14	353±93.47	213.42±67.53	261.57±108.22	164.71±36.74	<0.001	<0.001
III b	13	26	413.46±116.04	268.37±70.49	333.69±139.73	223.53±67.03	<0.001	<0.001
IV	22	44	469.00±167.44	336.03±172.36	381.81±146.64	249.18±138.98	<0.001	<0.001
Limited Disease	5	10	392.20±109.09	260.34±32.80	289.80±113.06	176.20±49.08	<0.001	<0.001
Extensive disease	3	6	615.33±6.65	434.26±273.01	608.00±50.47	314.33±91.68	<.001	<.001
Total	50	100	439.42±145.31	299.60±144.29	356.84±150.39	227.30±107.66	(t=6.473 df=49), <0.001	t=6.174, df=49 p<.001

Legend 6: Pre-treatment LDH and ALP level shows positive correlation with clinical stage of the disease. Mean serum values of LDH and ALP decreases after treatment and the difference are statistically significant.(p<0.001).

Discussion:-

In the present study serum LDH and ALP levels were analyzed in 50 lung cancer patients and 30 control subjects. For lung cancer cases estimation was done twice, once before initiating treatment at the time of diagnosis and repeated once after treatments were over. For control subjects these enzymes were analyzed once and compared with lung cancer cases to see any possible relationship that could be useful in early diagnosis, in monitoring therapy and in prognostication.

In our present study it was found that LDH levels were high in 88% of patients and ALP in 78% of patients at diagnosis and 90% of patients belonged to stage III (40%), stage IV (44%) of NSCLC and extensive stage (6%) disease of SCLC. It is evident that there is increased serum LDH levels in neoplasia, both in humans and animals [17,18] Some authors reported that 62% of bronchogenic carcinoma patients have high LDH [19] at diagnosis while others reported as 82% [20], but these studies failed to give any account on the metastatic status of the patients.

They also observed that the highest values were seen in association with extensive metastasis particularly to liver reflecting the total tumor mass [21] but in contrary one study reported that even in the absence of metastasis in small number of patients there were increased serum LDH. [22]

Maestu I et al (1997)[23] reported high serum ALP levels in 68.78% of cases and the majority of cases (75.63%, i.e. 326 out of 431) belonged to stage III and IV, which is similar to the findings of present study. In the early stage of disease with less number of metastatic sites the serum ALP level was found to be low.[24,25] But during the last month of life serum ALP is elevated suggesting tumor metastases.[26,24] This elevation suggested that majority of the patient were presented in advanced stage of the disease and had adverse prognosis. [27]

In the present study it was found that the lung cancer patients have significantly higher levels of serum mean± SD LDH and ALP **(Table 2)** than the control group and the mean differences were statistically significant (P value <0.001). These findings are consistent with the findings of **Gault MH et al** (1967).[26] In the present study we observed that elevated levels of serum LDH were more in extensive stage disease of SCLC which was similar to the findings of previous authors. [28,29] In SCLC patients serum LDH is a significant independent pretreatment

prognostic factor and it correlates with stage of disease, response to treatment, and survival [28] and direct indicator of clinical stage and tumor burden in patients with NSCLC.[30]

Malignant cells have a distinctive type of metabolism in which the glycolytic sequence and the tricarboxylic acid cycle are poorly integrated, leading to excessive production of lactate by the lactate dehydrogenase enzyme.[31] The extent of disease and tumor mass also have a significant correlation with serum LDH levels.[32-34]

Our present study reports elevated ALP in lung cancer patients and it is supported by the fact that there is progressive elevation of serum ALP in metastatic bronchogenic carcinoma.[35] Increase in ALP or its bone isoenzyme particularly reflects metastatic bone involvement.[10,12] High activity was also found in the cerebrospinal fluid of a patient with intracranial metastasis from lung cancer.[36] Another variant of ALP with heat sensitivity characteristics similar to that of bone type is also found in the serum of patient suffering from lung cancer.[14]

It was also observed in the present study that with increasing stage or disease extent there is increase in mean serum ALP levels. This finding is in agreement with findings of **Stokkel MPM et al** (1998) [34] and **Bacci G et al.** (1993) [37] **Mohan A et al.** (2006)[27]who observed a positive correlation between serum ALP and extend of the metastatic lung disease. **Rotenberg Z et al.** (1988) [30] and **Chen Y et al**.(2006) [38] observed a direct correlation between clinical stage and serum LDH level in non small cell lung cancer.

In the present study different modalities of treatment (**Table-5 and 6**) were used either single or in combination depending on various factors such as age, sex, stage of the disease and other comorbidity factors for the treatment of lung cancer patients. Treatment modalities included chemotherapy, radiotherapy and combination of chemotherapy with radiotherapy. The post treatment serum LDH and ALP decreased in all modalities of treatment and the differences of pre and post treatment values were statistically significant ($p<0.001$).When the differences were considered irrespective of the modalities of treatment was also found to be statistically significant ($p<0.001$). **Gault MH et al.**(1967**)** [26] had observed that there is transient increase in LDH activity in patients responding to chemotherapy, with rapid return to pre-therapy levels, even during treatment. ALP also shows variable increase in activity, but then declines like LDH in that order and the timing was usually similar for both. Marked increase in enzyme activity is specially seen in oat cell carcinoma of the lung.

In our present study it was observed that both LDH and ALP increased in 12% of cases (Table 3 and 4) after the treatment. LDH returned to normal in 30% of cases and decreased but remained above normal in 58% of cases. The serum ALP returned to normal in 25% of cases and decreased but remained above normal in 38% of cases.

The cause for this increase in LDH after treatment could not be ascertained in the present study as to whether this increase was transient in nature due to response to therapy or due to disease progression as there was no serial sample collection and analysis after treatment. **Johnson PW et al.(1993)** [39] reported in his study that in some patients the LDH levels initially fell during treatment and in some rose again despite continued chemotherapy and explained that this might be due outgrowth of a chemoresistant tumor sub-clone and indicated that such patients have a shorter duration of remission than those with steady or falling levels. In contrast **Couskun SH et al(2005)** [40] found no statistically significant difference between pre treatment and post treatment serum LDH levels and the enzyme level also did not differ significantly between limited and extensive disease.

Moreover ALP also increased in 12% of cases but this study could not interpret these findings probably because of the drawbacks of the present study that only a single sample was collected 3-4 weeks after the treatment. More serial samples should have been examined to rule out the cause. It is commonly observed that there is transient increases in ALP in patients who ultimately response to treatment. These changes are called flare phenomenon and were first reported by Greenberg et al.(1972)[41] Few authors have reported flare phenomenon in lung cancer.(Killian et al.,1981)[42]; Cosolo et al.,1988[43];Vogel et al.,1995;[44] Amaroso et al.,2007)[51]. Although the frequency of flare phenomenon in lung cancer is unknown, treatments involving the use of molecular target drugs seem to yield more reports of these phenomena compared to those involving standard cytocidal chemotherapies. Moreover no accounts of drug details as to what drugs were administered, not recorded in our study.

Limitations:-

Post treatment estimation of serum samples were done only once and the time of sample collection were not defined to see the desired results. Serial sample estimation of the enzymes should have been done in order to evaluate whether the elevation or decrease of enzyme levels after treatment were due to response to treatment or due to disease progression.

Conclusion:-

Measurement of serum LDH and ALP activity will be a useful tool in the diagnosis and in the assessment of prognosis in lung cancer patients. The pretreatment serum LDH and ALP has positive correlation with stage of disease, tumor mass and extend of disease. However the post treatment values of the enzymes were inconsistent. Further studies are recommended in order to evaluate the significance of post treatment values of these enzymes in prognosis.

Acknowledgement:-

I highly appreciate Prof B.K. Gupta, Professor, Biochemistry, Subharti Medical College, Meerut, for his immense contribution in preparation of the manuscript and Dr Suraj Senjam, Assistant Professor, Community Medicine, AIIMS, New Delhi for statistical assistance.

Reference:-

1. Longo DL . Approach to the patient with cancer. In:Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J, editors. Harrisons principles of internal medicine.18th ed. New York: McGraw Hill Medical;2012.p. 646
2. Jagadish R, Girish S, Dushyant G. Clinico-pathological profile of lung cancer in Uttarakhand. Lung India2009; 26: 74-6.
3. GLOBOCAN 2002, 2008 database, International Agency for Research on Cancer, World Health Organization. Available at http://www-dep.iarc.fr/, last accessed January 27, 2010. Available at http://globocan.iarc.fr/. Last accessed august 10, 2010.
4. Ganesh B, Sushama S, Monika S. A case-control study of risk factors for lung cancer in Mumbai, India. Asian Pac J Cancer Prev2011; 12: 357-62.
5. Cascante M, Centelles JJ, Veech RL. Role of thiamin (vitamin B1) and transketolase in tumor cell proliferation. Nutr Cancer 2000;36:150-154.
6. Bardawill C, Chang C. Serum lactate dehydrogenase, leucine amino peptidase and 5'-nucleotidase activities. Observations in patients with carcinoma of the pancreas and hepatobiliary disease. J Canad Med Ass 1963; 89:755-61.
7. Rao YN, Singh GP, Chakravorty M, Khanna NW. Serum lactate dehydrogenase, leucine amino peptidase and alkaline phosphatase in the diagnosis of cancer. Ind J Cancer 1978;15:39-44.
8. Khan N, Tyagi SP, Ullah S. Estimation of serum cholinesterase and lactate dehydrogenase levels for diagnosis and prognosis in lymphoreticular malignancy. Indian J Med Res 1985;82:141-45.
9. Friedman LS, Martin P, Munoz SJ. Liver function tests and the obstructive evaluation of the patient with liver disease. In: Zakim D, Boyer TD, editors. Hepatology: A Textbook of Liver Disease. Philadelphia:WB Saunders;1996.p.791-833.
10. Body JJ, Dumon JC, Blocket D, Darte C. The bone isoenzyme alkaline phosphatase in hypercalcaemic cancer patients. Eur J Cancer 1997Sep;33(10):1578-82.
11. Arugal A, Koizumi M, Hotta R, Takahashi S, Ogata E. Usefulness of bone metabolic markers in the diagnosis and follow-up of bone metastasis from lung cancer. Br J Cancer 1997;76(6):760-4.
12. Alatas F, Alatas O, Metintas M, Colak O, Erginel S, Harmanci E, et al. Usefulness of bone markers for detection of bone metastases in lung cancer patients. Clin Biochem 2002 Jun;35(4):293-6.
13. Ben-Arie A, Hagay Z, Ben-Hur H, Open M, Dgani R. Elevated serum alkaline phosphatase may enable early diagnosis of ovarian cancer. Eur J Obstet Gynecol Reprod Biol 1999 Sep;86(1):69-71.
14. Miyanaga M, Sugimoto H, Komoda T, Nosjean O, Honma K, Nemoto K, et al. A variant of alkaline phosphatase detected in patient with lung cancer. Enzyme Protein 1996;49(5-6):313-20.
15. Henry RJ, Chaiamori N, Golube OH, Berkman S. Revised spectrophotometric methods for the determination of Glutamic pyruvate oxaloacetate transaminase, Glutamic pyruvic transaminase and Lactic dehydrogenase. Am J Clin Path 1960;34:381-98.

16. Bowers GN, McComb RB. Determination of serum alkaline phosphatase activity. Clin chem 1975;21:1988-95.
17. Kornberg A, Polliak A. Serum lactic dehydrogenase (LDH) levels in acute leukaemia marked elevations in lymphoblastic leukaemia. Blood 1980;56:351-5.
18. Starkweather WH, Schoch HK. Some observation on the lactate dehydrogenase of human neoplastic tissue. Biochem Biophys Acta1962;62:440-2.
19. Zimmerman HJ, Weinstein HG. Lactic dehydrogenase activity in human serum. J Lab clin Med 1956;48:607-609.
20. Gold JA. Serum enzymes in bronchogenic carcinoma and other pulmonary diseases. Dis Chest 1961;39:62-7.
21. West M, Zimmerman HJ. A.M.A. Arch Intern Med 1958;102:103.
22. Hinton JM. Serum lactacte dehydrogenase in bronchial carcinoma. Thorax 1965;20:198-9.
23. Maestu I, Pastor M, Gomez-Codina J, Aparicio J, Oltra A, Herranz C, et al. Pretreatment prognostic factors for survival in small cell lung cancer: A new prognostic index and validation of three known prognostic indices on 341 patients. Annal Oncol 1997;8:547-53.
24. Kawahara M, Fukuoka M, Saijo N, Nishiwaki Y, Ikegami, H Tamura T, et al. Prognostic Factors and Prognostic Staging System for Small Cell Lung Cancer. Jpn J Clin Oncol 1997;27(3):158–165.
25. Jacob W, Quantin X, Boher JM, Andre f, Moreau L, Gainet M, et al. Brain metastases at the time of presentation of non-small cell lung cancer: a multi-centric AERIO analysis of prognostic factors. Br J Cancer 2001;84(7):903-9.
26. Gault MH, Cohen MW, Kahana LM, Leelin J FT, Meakins J F, Aronovitch M. Serum Enzymes in Patients with Carcinoma of Lung: Lactic-Acid Dehydrogenase, Phosphohexose Isomerase, Alkaline Phosphatase and Glutamic Oxaloacetic Transaminase. J Canad Med Ass 1967 Jan;96:87-94.
27. Mohan A, Goyal A, Singh P, Singh S, Pathak AK, Bhutani M, et al. Survival in small cell lung cancer in India: Prognostic utility of clinical features, laboratory parameters and responses to treatment. Indian J Cancer 2006;43:67-74.
28. Sagman U, Feld R, Evans WK, Warr D, Shepherd FA, Payne D, et al. The prognostic significance of pretreatment serum lactate dehydrogenase in patients with small-cell lung cancer. J Clin Oncol 1991 Jun;9(6):954-61.
29. Quoix E, Purohit A, Faller-Beau M, Moreau L, Oster JP, Pauli G. Comparative Prognostic value of lactate dehydrogenase and neurone specific enolase in small cell lung cancer patients treated with platinum based chemotherapy. Lung Cancer 2000 Nov;30(2):127-34.
30. Rotenberg Z, Welnberger I, Sagle A, Fuchs J, Davidson E, Sperling O, et al. Total serum lactate dehydrogenase and its isoenzymes in serum of patients with Non Small Cell Lung Cancer. Clin Chem 1988;34(4):668-70.
31. Hormonal Regulation and integration of mammalian metabolism. In: Michael MC, Nelson DL, editors. Lehninger Principles of Biochemistry. 5th ed. New York: WH Freeman and Company; 2008.p.901-39.
32. Tas F, Aydiner A, Demir C, Topuz E. Serum lactate dehydrogenase levels at presentation predict outcome of patients with limited stage small cell lung cancer. Am J clin Oncol 2001Aug;24(4):376-8.
33. Von-Eyben FE, Blaabjerg O, Madsen FL. Serum lactate and tumor volume are indicators of response to treatment and are predictors of prognosis in metastatic testicular germ cell tumor. Eur J Cancer 1992;28:410-15.
34. Stokkel MPM, Van Eck-Smit BLF, Zwinderman AH, Willems LNA, Pauwels EKJ. Pre treatment serum lactate dehydrogenase as additional staging parameter in patients with small cell lung carcinoma. J Cancer Res Clin Oncol 1998;124:215-219.
35. Fishman WH, Inglis NI, stolbach LL. Serum alkaline phosphatase isoenzyme of human neoplastic cell origin. Cancer Res 1968;28:150-4.
36. Hoshino T, Kumasaka K, Kawano K, Koyama I, Arai-Fujimori Y, Yamagishi F, et al. Abnormal alkaline phosphatase of hepatic type in cerebrospinal fluid of a patient with intracranial metastasis from lung cancer. J Clin pathol 1993 Nov;46(11):1059-61.
37. Bacci G, Picci P, Ferrari S, Orlandi M, Ruggieri P, Casadei R, et al. Prognostic significance of serum alkaline phosphatase measurements in patients with osteosarcoma treated with adjuvant or neoadjuvant chemotherapy. Cancer 1993;71:1224-30.
38. Chen Y, Zhang H, Xu A, Li N, Liu J, Liu C, et al. Elevation of serum I-lactate dehydrogenase B correlated with the clinical stage of lung cancer. Lung Cancer 2006 Oct;54(1):95-102.

39. Johnson PWM, Joel SP, Love S, Butcher M, Pandian MR, Squires L, et al. Tumor markers for prediction of survival and monitoring of remission in small cell lung cancer. Br J Cancer 1993;67:760-6.
40. Couskun HS, Olzem ER, Altinbas M, Ozkan M, Soyuer S. Serum tumor markers in small cell lung carcinoma patients treated with cyclophosphamide, epirubicin and vincristine combination.Turk J Cancer 2005;35(2):81-87.
41. Greenberg EJ, Chu FCH, Dwyer AJ, Ziminiski EM, Dimich AB, Laughlin JS, et al. Effects of radiation therapy on bone lesions as measured by 47 Ca and 85SR local kinetics.J.Nucl.Med.1972;13:747-751.
42. Killian CS, Vargas FP, Pontes EJ, Beckley S, Slack NH, Murphy GP et al. The use of serum isoenzyme of alkaline and acid phosphatase as possible quantitative markers of tumour load in prostate cancer. Prostate 1981;2:187-206.
43. Cosolo W, Morstyn G, Arkles B, Zimet AS, and Zalcberg JR. Flare response in small cell carcinoma of the lung.Clin. Nucl. Med.1988;13:13-16.
44. Vogel CL, Schenfelder J, Shemano I, Hayes DF, and Gams RA. Worsening bone scan in the evaluation of anti-tumour response during hormonal therapy of breast cancer. J. Clin. Oncol.1995;13:1123-1128.
45. Amoroso V, Pittiani F, Grisanti S,Valcamonico F, Simoncini E, Ferrari VD et al. Osteoblastic flare in a patient with advanced gastric cancer after treatment with pemetrexed and oxaliplatin: implication for response assessment with RECIST criteria. BMC Cancer 2007;7:94.