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A study of correlation of tumor markers with Ca^{2+} and HCO_3^- during chemotherapy in lung cancer patients

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Abstract
Background and Aims: The study was conducted on 20 patients with lung cancer going through chemotherapy to aim to correlate two inorganic ions HCO_3^- and Ca^{2+} with three tumor markers LDH, CK and AFP. In a few past studies these two ions have been found to play a well-defined role in development and progression of cancers. Hence, hereunder it was tried to define the behavior of both ions if they can be correlated with different levels of some tumor markers like CK, LDH and AFP to use this correlation as a predictive diagnostic tool to know the direction of progression or prognosis of the disease.
Methods: The tumor markers of interest were evaluated by chemiluminescent immunometric assays, while as Calcium was estimated colorimetrically and HCO_3^- was measured by blood gas analyzer. All evaluated before chemotherapy, after 1st cycle & after 2nd cycle. The estimated values at three different stages were compared and evaluated statistically (by 'p' & 't values), both Ca^{2+} and HCO_3^- were correlated with all other three tumor markers and statistical evaluations done to calculate 'p', 't '& 'r'(correlation coefficient).
Results: The average values of two tumor markers CK and AFP and both ions were found to be moving in their normal range beside only LDH was found to be elevated at all three stages. In all three stages the measured average values of all parameters were found in decreasing pattern as shown i.e. evaluated values were statistically significant by showing $p<.0001$.Further as evaluated Calcium was in negative correlation in all three CK, AFP, LDH and in positive correlation ($p<.0001$) with HCO^3- (r= +0.1184) before starting chemotherapy which turned positive for all markers including bicarbonates after chemotherapy ($p<.0001$).Bicarbonates (HCO_3^-) when correlated with all markers found to be in negative correlation ($p<.0001$) with all only leaving Calcium(r=+0.1184) where it was positive but after chemotherapy all CK and AFB also turned positive correlation ($p<.0001$) while as LDH only remained in negative correlation (r=-0.0094).
Conclusion: In the present study the altered pattern of correlations of both Ca2+ and HCO_3-, specially moving within their normal range can be assumed to be showing a good degree of protection measure taken by cells themselves in altered metabolic environment in order to shifting towards normalization.

Keywords: Correlation, chemotherapy, metastasis, tumor markers etc

Introduction
Worldwide, the lung cancer remains an important common cancer diagnosed, which accounts for 17% and 9% of all cancers in men and women, respectively, and represents 19% of all cancer-related deaths [1].
Calcium is an essential dietary mineral that can be obtained from food and supplements. Most of the research results have shown a relationship between long term [2] higher intake of calcium causing risks of various cancers like, lung cancer [3] breast cancer and colorectal cancer.
Contrarily, on the other hand, because results are not always consistent, high intakes of dietary calcium, and supplemental calcium, to correct its deficiency, were found to be associated with an approximately 20% lower risk of cancer among women [4] and estimated lower values of Vitamin D and Calcium are known to cause risk to develop cancers [5].

It is notable that studies regarding concentration of Calcium (Ca^{2+}) in blood circulation (serum) during the chemotherapy lacks sufficient data. Moreover, it hasn't been found being correlated with tumor markers present in blood circulation of lung cancer patients in past decades leaving a few researches. Bicarbonate is a well-known member of blood buffer to maintain blood pH. It is also known to affect TME (Tumor Micro Environment) and found that a lower pretreatment bicarbonate in patients is potentially correlating with an acidic and hypoxic TME. The acidic TME is found to be associated with both the progression and drug resistance of cancer [6]. Recently, many researchers seemed very much interested in the therapeutic use of sodium bicarbonate to reduce tumor cell invasion and metastases *in vitro*, as well as in the rate of involvement of lymph nodes *in vivo*. The least known is the evaluation of bicarbonate ion concentrations and their correlation with tumor markers during chemotherapeutic cycles [7].

Biomarkers for cancer are known as tumor markers. They provide insight into histogenesis, interrelationships, and biological behavior of lung tumors. Hence, their importance cannot be denied as they are the most preferred among many other diagnostic techniques [8].

Tumor markers are used alone or in combination with other indices [9] because they are important in monitoring response to chemotherapy along with other therapies. They are used in early detection of tumor reactivation to establish treatment strategies and secondary prevention [10]. Some interesting lung cancer biomarkers are-CYFRA 21-1, NSE, ProGRP, SCC, CEA, AFP, Tumor M2-PK, as well as other markers in clinical application such as CRP, LDH, tumor-suppressor genes and oncogenes, CK, CA-125, CgA, NCAM, and TPA [11].

Various methods are used to cure lung cancer such as surgery, chemotherapy, radiotherapy, laser therapy, immunotherapy and photodynamic therapy [12]. Chemotherapy of lung cancer is generally, the choicest treatment in well-established lung cancer [13]. Major chemotherapeutic drugs used in this study were Carboplatin, Adriamycin/Doxorubicin, Docetaxel and Cisplatin etc.

A few of the important markers of lung cancer i.e. AFP [14], CK [15] & LDH are included in the study because of ease of their estimation as well as they can be correlated also with inorganic ion markers like Ca^{2+} and HCO^{3-} in this study. Interestingly in view of that, they have already been studied extensively in various aspects in cancers, but the synchronization among all organic and inorganic markers are with treatment has not been given much importance. So, it is expected that the alterations in these tumor markers may show some interesting outcomes if they are correlated with calcium and bicarbonate ion concentration during the chemotherapeutic cycles in the study on basis of changes in cell and blood environment during stress induced upon them during chemotherapy.

Hence, this study is an effort to find out the effects of chemotherapy by evaluating variation in calcium and bicarbonate level in patients' blood and correlating them with tumor markers like LDH, AFP and CK along with monitoring the hematological parameters also.

In this study, It is desired to establish a correlation if, there is any, among variation of tumor markers with calcium and bicarbonate values of the lung cancer patients during ongoing chemotherapeutic cycles. The study is an effort in order to establish a better understanding of clinical diagnosis and management of lung cancer.

Materials and Methods

The entire study was carried out on 20 Lung cancer patients admitted in Oncology department of SMS Medical College Jaipur. The basis of study was purely biochemical. All required prerequisites and permissions were taken from the concerned departments. No animals were harmed for this study. The study was a follow up study and comprised of duration of three cycles of chemotherapy in various types of cancers. Standard serological methodology [16, 17] was used as required. The demographic criteria were meeting the expected physical characteristics.

Blood Samples (5-7 ml) were collected in dry plain vials for serum assays. Storage required -20 ^{o}C for longer periods and 2-8 ^{o}C was required for 7 days storage. Serum was used for assays. All general Biochemical Parameters were analyzed on Olympus AU-400, AU-680.

AFP: It was analyzed on Immulite 2000 [18, 19, 20] through a solid phase two site sequential chemiluminescent immunometric assay [19]. Chemical Kit and components were matched set for all tumor markers. Provided kits required barcodes. Evaluations of results were based on reference range of the kits based on observational population studies carried out. Medians and 95th Percentiles for relevant subgroups were provided, where percentiles were determined non-parametrically [18, 19, 20]. Samples were assayed under various dilutions. AFP calibration range was 300 IU/ml and analytical sensitivity was 0.2 IU/ML, no high dose hook effect up to 53400 IU/ml. There was negligible impact of bilirubin and hemolysis.

CK: Measured by non-radioactive colorimetric assay on AU-680. Enzyme CK catalyzes phosphorylation of ADP and generates ATP, who with enzyme glucokinase converts glucose into glucose-6-phosphate that reacts with NADP in water to produce NADPH, formation of NADPH is kinetically measured at 340nm. NAC acts as a coenzyme in the system. Activity of CK in U/L =ΔA/min×4127.

LDH: Measured by non-radioactive colorimetric assay on AU-680. LDH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565nm. Intensity of the purple color is directly proportional to enzyme activity. LDH activity = ODS25-ODSO×Reaction Volume (µl).

Calcium (Ca^{2+}): It was colorimetrically measured on AU-680 by Arsenazo-III method. Intensity of blue color formed is proportional to the calcium concentration.

Bicarbonate (HCO_3^-): It is the second largest fractions of anions in blood plasma. It was measured by ABG Analyzer Radiometer. It is calculated from pH and pCO_2 in whole blood (primarily arterial) by ABG analyzer (make-Radiometer). Its determination actually includes dissolved CO_2 [21]. Calculated values are advantageous as not affected by electrolyte exclusion effects and reflects HCO_3^- measured with an ISE. Different ABG analyzers display different HCO_3^- upper and lower limits. Formula to calculate directly is $cHCO_3^-$ (mmol /L) = 24.1 × pCO_2 mmHg/10-9-pH & $cHCO_3^-$ = 0.03 × pCO2 10 pH-6.1. It can also be get by

software calculations [22].

Table 1: Normal Range of Parameters

S. No.	Parameter	Normal Range	Unit
1.	Creatine Kinase	22-198	U/L
2.	AFP	10-20	ng/ml
3.	LDH	140-280	U/L
4.	Bicarbonate	23-30	mmol /L
5.	Calcium	8.5-10.2	mg/dl

Table 2: Average values of markers in Pre (before), after 1st cycle & after 2nd cycle of chemotherapy

S. No.	Parameter	Stage	No. of cases (n)	Mean ± S.D.
1.	Creatine Kinase (CK)	Pre	20	33.25±31.68
		1st	20	25.2±20.351
		2nd	20	15.7±10.03
2.	Alpha Feto Protein (AFP)	Pre	20	6.5±1.65
		1st	20	4.625±1.644
		2nd	20	3.457±1.619
3.	LDH	Pre	20	383±122.06
		1st	20	363±122.06
		2nd	20	424.6±145.5
4.	Bicarbonate	Pre	20	27.9±3.193
		1st	20	23.6±2.91
		2nd	20	20.3±2.341
5.	Calcium	Pre	20	9.675±0.494
		1st	20	8.95±0.5898
		2nd	20	8.49±0.4140

Table 3: Statistical significance of correlation(r) of Calcium with other markers before chemotherapy

S. No.	Marker	'n'	't'	'p'	'r'	'p'
1.	CK	20	3.3276	<0.0020	-0.17711	<0.2743
2.	AFP	20	8.1425	<0.0001	-0.0341	<0.8345
3.	LDH	20	16.74	<0.0001	-0.1599	<0.3243
4.	HCO_3^-	20	25.225	<0.0001	+0.1184	<0.4668

Table 4: Statistical significance of correlation(r) of Calcium with other markers after chemotherapy

S. No.	Marker	'n'	't'	'p'	'r'	'p'
1.	CK	20	3.22	<0.0026	+0.1107	<0.4965
2.	AFP	20	13.46	<0.0001	+0.1049	<0.2281
3.	LDH	20	12.7	<0.0001	+0.4022	<0.0101
4.	HCO_3^-	20	22.21	<0.0001	+0.3126	<0.0495

Table 5: Statistical significance of correlation (r) of Bicarbonate with other markers before chemotherapy

S. No.	Marker	'n'	't'	'p'	'r'	'p'
1.	CK	20	0.7514	<0.457	-0.1043	<0.5219
2.	AFP	20	26.627	<0.0001	-0.4947	<0.0012
3.	LDH	20	15.92	<0.0001	-0.2103	<0.1927
4.	Ca	20	25.22	<0.0001	+0.1184	<0.4668

Table 6: Statistical significance of correlation of Bicarbonate with other markers after chemotherapy

S. No.	Marker	'n'	't'	'p'	'r'	'p'
1.	CK	20	2.00	<0.0523	+0.0511	<0.7542
2.	AFP	20	26.51	<0.0001	+0.1296	<0.4254
3.	LDH	20	12.42	<0.0001	-0.0094	<0.9541
4.	Ca	20	22.21	<0.0001	+0.3126	<0.0495

Discussion

The study was carried out at SMS Medical College and attached Hospitals Jaipur on diagnosed and established lung cancer patients going through chemotherapy. Study was conducted to find out the correlation among different tumor markers [23] with both Calcium and Bicarbonate being inorganic and ionic molecules.

As depicted by previous studies these two ions play significant role in progression, reduction and defining the cancer cell metabolism as well as the environment in that the tumors are surrounded by (TME) [24]. To serve the purpose, other markers (CK, LDH, AFP) with Ca^{2+} and HCO_3^- were estimated before chemotherapy (Pre), during chemotherapy (1st Cycle) and after chemotherapy (2nd Cycle). Their normal reference range is shown in Table 1.

Table '2' shows the average values of all parameters with respective SDs at each stage as estimated. In this table (2) all markers including Calcium (Ca^{2+}) and Bicarbonate (HCO_3), the estimated values were found keeping themselves between their normal reference range specifically AFP in normal range was a surprise being specific for lung cancer. Per say, It may be exhibiting poor progression and good prognosis of the disease. All were in decreasing pattern i.e. from pre to after 2nd cycle of chemotherapy, leaving only LDH that was decreased after 1st cycle but again increased after 2nd cycle as a surprise. The possible cause for this may be stressful hypoxic metabolic cell environment [24] that could lead to increase activity of LDH enzyme (383>363<424.6) which was not able to be compensated by normal metabolic activity by normal cells to approach towards homeostasis.

Table '3' & '4' shows calculated correlation values of Calcium with other four markers as well as the values of their statistical significance predictors 'p' & 't'. It is well known that cellular calcium signaling plays important roles in several signal transduction pathways that control proliferation, differentiation and apoptosis [25]. In this study it is noted that CK and HCO_3^- were exhibiting a positive correlation with Calcium while as AFP and AFB were exhibiting a negative correlation before treatment cycles. This pattern is not followed after chemotherapy and this correlation turned positive for all markers including HCO_3^- i.e. increasing or decreasing along with Calcium. The correlation pattern was supported by the respective 'p' & 't' values showing the results mostly significant ('p'<.0001). The probable cause and explanation for this correlation pattern may be the change brought about by struggling normal cells in need of energy for survival where variation in Calcium ion concentration helps in opening and closing of Calcium channels for some enzymatic and hormonal actions [26] as well as for supply of micronutrients to struggling cells harmed by chemotherapy [27]. Furthermore, as we proceed to Table '4' &'5' we see the variation in correlation pattern between HCO_3^- ion and other markers including Calcium ion. Maintenance of cellular pH homeostasis is fundamental to life. A number of key intracellular pH (pHi) regulating systems including the Na^+/H^+ exchangers, the proton pump, the monocarboxylate transporters, the HCO_3^- transporters and exchangers and the membrane-associated and cytosolic carbonic anhydrases cooperate in maintaining by hypoxia (low oxygen tension) [28]. Hypoxia has a vital role in tumor angiogenesis, metastasis, apoptosis in addition to oncogene activation and transformation, it is also responsible for inducing acidosis through a shift in cellular metabolism that generates a high acid load in the tumor microenvironment

[24]. However, hypoxia and oncogene activation also allow cells to adapt to the potentially toxic effects of an excess in acidosis. As the table shows before treatment HCO_3^- was in negative correlation with all markers leaving Calcium ion that was in positive correlation showing that HCO_3^- and Calcium were synergistically working towards changed cell and metabolic environment related to compensate the prospective incidence of metabolic acidosis (or alkalosis) during progression and chemotherapeutic cycles. This action of both ions being positively in correlation was followed after chemotherapy that included all other organic markers i.e. AFP, LDH and CK and moreover, their correlation also turned positive after the treatment. This shows the impact of HCO_3^- ions on cell environment exhibiting itself as a marker for indicating the change decrease or increase in acidic TME before, during and after the chemotherapy.

As per the results of the study it has been found that both ions Calcium and Bicarbonate have a definite role in checking carcinogenesis and its metastasis. Although they cannot be perfect cancer markers of diagnostic values but metabolically they are involved and show different types of correlation with CK, LDH and AFP to define their role more precisely during development of tumor as well as chemotherapeutic treatments.

Conclusion

The study was planned to find out the action pattern of inorganic ions like Calcium and HCO_3^- during carcinogenesis, in cancer/tumor cells and in normal cells. Both of them modulate and regulate lots of metabolic reactions and actions in the body. Calcium acts as a secondary messenger for channeling various cascade actions. HCO_3^- acts to maintain acid-base balance by means of blood pH buffer system.

Lung carcinoma is the most common malignant tumor in the world. Calcium is a ubiquitous cellular signal, and found crucial in cancer. The expression of specific Ca^{2+} channels and Ca^{2+}-binding proteins is supposed to be altered in lung carcinoma specially and it has been mostly found to be a characteristic feature of lung cancer in various studies. Role of Calcium cannot be denied in tumor progression as it also regulates cell signaling pathway leading to cell proliferation or apoptosis. In lung cancer chemo resistance is observed most often. Ca^{2+} homeostasis is altered in endoplasmic reticulum of lung cancer cells and can be correlated with drug resistance during chemotherapy. In view of the role of HCO_3^- ion it has been noted that acidosis is a common feature of cancer and is essentially caused by hypoxia (low oxygen tension). Hypoxia is responsible for inducing acidosis in addition to oncogene activation and transformation. This causes a major shift in cellular metabolism in turn generating a high acid load. Thus, the tumors microenvironment experiences hypoxia that is known to play a vital role in tumor angiogenesis, metastasis and apoptosis. In view of synergistic role of calcium together with HCO_3^-, the Ca^{2+} channels are open induced by hypoxia i.e. related to HCO_3^- and causes the increase of Ca^{2+} influx ultimately causing tumor growth. In epithelial cells calcium signaling is initiated mainly by calcium release from endoplasmic-reticulum-associated intracellular calcium pools. Because calcium is accumulated in the endoplasmic reticulum by sarco/endoplasmic reticulum calcium ATPases (SERCA), these enzymes play a critical role in the control of calcium-dependent cell activation, growth and survival creating a pHi (internal pH) that is permissive for cell survival. A common feature of tumours is acidosis that induced the carcinogenesis. The metastasis of cancer is a result of combination of lots of erroneous actions and reactions, whether they are at molecular level or metabolic level, ultimately they harm the body and disturb harmony and environment at cellular, tissue and organ level. HCO_3^- reduces free Ca^{2+} concentration and decreases the amount of Ca^{2+} that remains associated with mucins. Present study was an effort to find out if any synchronicity (correlation with other lung cancer/tumor markers CK, LDH and AFP is present in actions of both ions to check carcinogenesis and to regulate pathological changes i.e. tumor growth in the body. In the present study the altered pattern of correlations of both Ca^{2+} and HCO_3^-, specially moving within their normal range can be assumed to be showing a good degree of protection measure taken by cells themselves in altered metabolic environment in order to shifting towards normalization. Furthermore, it is also showing the fraction of destruction of normal and tumor cells when the marker values are observed increased and in during the chemotherapeutic treatment. More studies in future are required with more specific and sensitive statistical evaluations than correlation coefficient in various combination of tumor markers with Calcium and HCO_3^- is required.

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