Evaluation of Alkaline phosphatase (ALP) and Heat Stable-ALP (HS-ALP) in Chronic Obstructive Pulmonary Disease (COPD)

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Abstract

Background: COPD (Chronic Obstructive Pulmonary Disease) is defined as a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases. Tobacco smoke remains the main etiological agent. COPD inflammation initiated by tobacco smoke remains exuberant long after smoking cessation. Functional impairment of lung organ is reflected by impairment in FEV1% predicted. This is the result of underlying remodeling and fibrosis in small airways. Type 2 alveolar cells are involved in this process of remodeling and repair. Many of the studies have showed that heat stable alkaline phosphatase (HS-ALP) is released from these type 2 alveolar cells.

Objectives: To correlate serum activity of ALP, HS-ALP, FEV1% predicted and BMI in various stages of COPD patients.

Method: This study tried to evaluate correlation between serum activity of ALP, HS-ALP and FEV1% predicted and BMI in various stages of COPD. Total 45 patients of stage 2, 3, 4 of COPD were studied.

Result: We found there is unanimous increase in total ALP activity in all the stages of COPD. HS- ALP is increased in all the stages of COPD with no difference in between the stages. There is no relation between ALP and HS-ALP and between FEV1% predicted and BMI. We find that HS-ALP once considered as carcino-embryonic antigen is increased in lung inflammation of COPD.

Conclusion: Serum ALP and HS-ALP which can be very easily measured could be the one of the laboratory marker for diagnostic and prognostication of COPD patients.

Keywords: COPD, ALP, Heat stable-ALP

Running Title: ALP and HS-ALP in COPD

Introduction

COPD (Chronic Obstructive Pulmonary Disease) is an increasing health problem worldwide. Global prevalence is estimated to be 8.34/1000. Similarly, COPD is an increasing health problem even in India where prevalence is found to be 4.1%.[1,2] COPD is defined as "a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases".[3] Tobacco smoke remains the most typical etiological factor for COPD. Inflammation initiated by the external noxious stimuli remains even after cessation of the stimulus, this persistence of inflammation leads to progression and fibrosis which is reflected as functional respiratory impairment. This is measured in terms of reduced FEV1 % predicted.[4] The present system of staging does not include any of the laboratory inflammatory indicators. There is now renowned interest in finding some cellular and biochemical markers to monitor the inflammation in various respiratory disorders including COPD.[5,6,7] Alkaline phosphatase (ALP) is one such enzyme which is evaluated in differentiation of various kinds of pleural effusion and is a component of Light's criterion,.[8,9] ALP is nonspecific and ubiquitous enzyme found in the serum. It is membrane bound extracellular enzyme and has 5 isoforms, which are; Bone; Liver; kidney; intestinal and placental. Bone, kidney and liver isoforms are encoded by one distinct genetic locus which differs in their carbohydrate content; all of these isoforms are heat labile. Other two, intestinal and placental isoforms are encoded by another distinct genetic locus and these isoforms are heat stable in nature. Normally, Placental and placental like isoforms are not present in the serum of adult males and adult non-pregnant females.[10] Elevation in the heat stable ALP is seen in the sera of patients with certain malignant diseases. These carcinoplacental isoenzymes (e.g. Regan isoenzyme) appear to result from the derepression of the placental ALP gene. The presence of these isoenzymes can be readily detected in serum by their heat stability.[10]

Alkaline phosphatase fractions derived from lung tissue essentially belongs to placental and placental like isoforms. In the lungs, ALP is extracellular enzyme bound to type 2 alveolar cells[11] the cells which are involved in remodeling process of the lung tissue. Secretory activity of type2 alveolar cells is shown by ALP which is heat stable placental or placental like isoform. Thus, ALP-HS specifically being the product of type 2 alveolar cells may indicate the activity of remodeling and fibrosis in the lung tissue.[6] Also, COPD is a chronic systemic inflammatory disease,[7,12,13] which results in decreased Body mass index (BMI). So the aim of this study was to evaluate serum levels of ALP and HS-ALP in various stages of COPD and to correlate the values with FEV 1 % predicted and BMI (as an indicator of systemic involvement) and to know the potential of this parameter to encompass the inflammatory face of COPD.

Material and Methods

Total 45 patients of stable COPD were studied attending the internal or chest medicine outpatient department of (Govt. medical College), Aurangabad. PFTs, oxygen saturation were measured and patients were classified according to the GOLD criterion into stage 2, 3, 4.[2] Patients were stable without any active exacerbation at the time of study, and patients with any other major disease were excluded based on history and clinical examination. Guidelines laid by the

institutes' ethical committee were followed. Total 40 age and sex matched controls were selected; attending Medicine OPD without having COPD or any other respiratory system's diagnosis were included in the study. ALP and HS-ALP were estimated on semi auto chemistry analyzer. ALP was estimated by the kinetic assay kit manufactured by Reckon diagnostics which utilizes stabilized p-NPP. This method is based on the recommendations by German society of clinical chemistry.[14] For estimation of the heat stable component of ALP, serum was incubated at 56^0 C for half an hour followed by measurement of ALP activity in the serum.

Observation and results

Almost all the patients showed ALP more than the upper normal value (which was 56-140 IU/L based on manufacturer's recommendation). Normal range of Hs ALP is up to 7-21 IU/L.(10) In our study, 17 patients out of 45 (33 %) showed increase in the HS-ALP, maximum up to 114IU/L. When compared with the control group, activity of total ALP and HS-ALP is significantly higher in COPD group. (Table 2) Table 1 depicts results obtained after categorizing patients according to GOLD stages. Total ALP increased in the later stages of COPD (stage 3 and stage 4) when compared with stage 2 ($p<0.05$). But HS-ALP doesn't differ in between the COPD GOLD stages.

ALP has negative but insignificant relation with FEV1% predicted. ($r=-0.08$). There is no consistent relation with BMI. When the results are viewed in the context of smoking status, Values of ALP and HS-ALP are more in the smoker group but this is not a statistically significant finding. HS-ALP values maximum up to 114 IU/L are found in some of the smoker COPD patients.

Discussion

Type II alveolar cells are important in the repair of alveolar epithelium after any kind of cellular insult and injury and response to it.[15,16] Broncho alveolar lavage fluid (BALF) is the fluid that is near to the lung tissue and recovered after bronchial washing. Various biomarkers are assayed in the BALF to assess the pathologic process of lung parenchyma. Normally, type II cells are not present in BALF. The ALP activity, a marker of type II cell damage and/or proliferation was reported to be increased in BALF after exposure to pneumotoxicants and associated with progression of fibrosis.

In smokers, ALP activity is found to be increased in BALF.[17] In our study, we found raised value of serum total ALP and HS-ALP activity. In the light of exclusion of other sources of ALP, it can be said that raised serum total ALP and HS-ALP activity could be the result of proliferation of type 2 alveolar cells involved in remodeling process of small airways in COPD. Capelli et al hypothesized that ALP could be a marker of evolving fibrosis process of diffuse interstitial lung disease (DILD).[16] In our study, activity is seen more in Ex-smokers group than never smoker COPD group may suggest that disease process is more pronounced in smokers. Interestingly, individuals in the Ex-smoker group had quit the smoking at least 5 years back which suggests

that the exaggerated remodeling process which may be initiated by smoke far back is still continuing. The ALP activity does not show any correlation with BMI (Indicator of systemic involvement). Going further, these findings need to be ascertained in the COPD patients having rapid lung volume loss. This may help to establish ALP and HS-ALP as a marker of fibrosing disease process of COPD which may be helpful for early detection of rapid lung volume losers in diagnosed COPD patients and also in smokers.

Conflict of Interest: None declared.

References:

1. Jindal SK, Aggarwal a N, Chaudhry K, Chhabra SK, D'Souza G a, Gupta D, et al. A multicentric study on epidemiology of chronic obstructive pulmonary disease and its relationship with tobacco smoking and environmental tobacco smoke exposure. The Indian journal of chest diseases & allied sciences [Internet]. 2005;48(1):23–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16482948

2. Global strategy for the Diagnosis, Management, and Prevention of COPD [Internet]. 2010; Available from: http://www.goldcopd.org/

3. Rogerio Rufino JR, Lapa Silva. cellualr and biochemical bases of Chronic obstructive pulmonary Disease. J Bras Pneumol. 2006;32(3):241–8.

4. Reilly JJ, Silverman EK, Shapiro SD. Chronic obstructive pulmonay disease. In: Kasper Dl, Braunwald E, Fauci AS, Hauser SL, Longo Dl, Jameson JL, editors. Harrison's Principles of internal medicine. 16th Ed.New York City, McGraw-Hill; 2005. 1548-52.

5. MacNee W, Rennard SI, Hunt JF, Edwards LD, Miller BE, Locantore NW, et al. Evaluation of exhaled breath condensate pH as a biomarker for COPD. Respiratory medicine [Internet]. 2011 Jul 1 [cited 2012 Apr 29];105(7):1037–45. Available from: http://www.resmedjournal.com/article/S0954-6111(11)00058-8/abstract

6. Cobben NA, Drent M, Schols AM, Lamers RJ, Wouters EF, Van Dieijen-Visser MP. Serum lactate dehydrogenase pattern in ex-coalminers and its isoenzyme. Respir Med. 1997: 616–23.

7. Nillawar A, Bardapurkar S, Bardapurkar J. High sensitive C-reactive protein as a systemic inflammatory marker and LDH-3 isoenzyme in chronic obstructive pulmonary disease. Lung India [Internet]. 2012 [cited 2012 Jan 30];29(1):24. Available from: http://www.lungindia.com/text.asp?2012/29/1/24/92358.

8. Light RW. Clinical practice Pleural effusion. N Engl J Med [Internet]. 2002;346(25):1971-7. Available from: http://www.nejm.org/doi/full/10.1056/NEJMcp010731

9. Jadhav AA, Bardapurkar JS, Jain A. Alkaline phosphatase: Distinguishing between tuberculous and nontuberculous pleural effusion. Lung India : official organ of Indian Chest Society [Internet]. 2009 Jul [cited 2012 Apr 29];26(3):77–80. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2862511&tool=pmcentrez&rendertype=abstract

10. Mauro P, Bais R, Wouter W. van Solinge In:Burtis CA, Ashwood ER, Bruns De. Editors. Tietz textbook of clinical chemistry and molecular diagnostics.4thed. St.Louis, MO: Elsevier Saunders. 2004.

11. Edelson JD, Shannon JM, Mason RJ. Alkaline phosphatase: a marker of alveolar type II cell differentiation. The American review of respiratory disease [Internet]. 1988 Nov [cited 2012 May 1];138(5):1268–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2462386

12. Man SFP, Connett JE, Anthonisen NR, Wise RA, Tashkin DP, Sin DD. C-reactive protein and mortality in mild to moderate chronic obstructive pulmonary disease. Thorax [Internet]. 2006 Oct [cited 2012 May 1];61(10):849–53. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2104755&tool=pmcentrez&rendertype=abstract

13. van Eeden SF, Yeung A, Quinlam K, Hogg JC. Systemic response to ambient particulate matter: relevance to chronic obstructive pulmonary disease. Proceedings of the American Thoracic Society [Internet]. 2005 Jan [cited 2012 Apr 29];2(1):61–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16113470

14. Recommendations of the German Society for Clinical Chemistry: Standardization of Methods for the estimation of Enzyme Activity in Biological Fluids. J. Clinical Chemistry, Clinical Biochemistry. 1972;8:182–92.

15. Henderson RF, Scott GG, Waide JJ. Source of alkaline phosphatase activity in epithelial lining fluid of normal and injured F344 rat lungs. Toxicology and applied pharmacology [Internet]. 1995 Sep [cited 2012 May 1];134(1):170–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7676452

16. Capelli A, Lusuardi M, Cerutti CG, Donner CF. Lung alkaline phosphatase as a marker of fibrosis in chronic interstitial disorders. American journal of respiratory and critical care medicine [Internet]. 1997 Jan [cited 2012 Apr 29];155(1):249–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9001320

17. McLaughlin PJ, Twist AM, Evans CC, Johnson PM. Serum placental type alkaline phosphatase in cigarette smokers. Journal of clinical pathology [Internet]. 1984 Jul [cited

2012 May 1];37(7):826–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=498819&tool=pmcentrez&rendertype=abstract

Table 1: Serum total ALP, heat stable ALP in control and COPD patients; GOLD stage wise

	Reference value	Control	Complete Cohort of COPD	Stage 2 COPD	Stage 3 COPD	Stage 4 COPD
No		40	45	12	17	15
Total ALP (IU/L)	54-130	88.7±52.51	215.7±9.033*	186.6 ± 17.09	224.2 ± 15.12	225.1 ± 14.43
ALP HS	0-20	0.48±0.78	21.87±4.3*	22.50 ± 8.051	21.12 ± 6.578	23.60 ± 9.034

**p=0.00*

Table 2: Patients grouped and analyzed according to their BMI

	BMI< 19	BMI>19
Total ALP	205.0 ± 13.40 N=22	226.0 ± 12.06 N=23
ALP hs	18.23 ± 4.925 N=22	25.35 ± 7.193 N=23
	P=NS	

Table 3: ALP and HS-ALP according to smoking status

Parameter	Smokers	Non smokers	P value
ALP total	220.63±63	202.25±53.56	0.37
ALP-HS	44.62±26.25	22.25±38.01	0.95
FEV1% predicted	44.62±15.51	39.54±15.73	0.33
BMI	19.01±4.15	20.52±4.82	0.30