World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Comparison of alkaline phosphatase assay using two different buffer systems: AMP and DEA

Rajeswari S[1], Gandhi M [2], Arun Kumar D [3], Dhananjayan R[4] and Swaminathan S[5]

[1]Junior Technical Officer, Department of Biochemistry, Apollo Speciality Hospitals, Ayanambakkam, Chennai 600 095.
[2]Lab Technologist, Department of Biochemistry, Apollo Speciality Hospitals, Ayanambakkam, Chennai 600 095.
[3]Senior Lab Technologist, Department of Biochemistry, Apollo Speciality Hospitals, Ayanambakkam, Chennai 600 095.
[4]Consultant, Department of Biochemistry, Apollo Speciality Hospitals, Ayanambakkam, Chennai 600 095.
[5]Senior Consultant and Head, Department of Biochemistry, Apollo Speciality Hospitals, Ayanambakkam, Chennai 600 095.

Received: 11-06-2015 / Revised: 23-06-2015 / Accepted: 29-06-2015

ABSTRACT

Alkaline Phosphatase (ALP) enzyme estimation is used for the diagnosis in Liver Function. It is mainly assayed in the differential diagnosis of jaundice. Although many different substrates and buffers are used in the estimation, IFCC recommends one of the two buffer systems viz., Amino Methyl Propanol (AMP) and Diethanolamine (DEA) buffers with para Nitro Phenyl Phosphate (PNPP) as the substrate. The reference ranges for ALP differs mainly due to the buffers used and it is has been observed that the normal ranges are higher with DEA buffer compared with AMP buffer. This research work is an attempt to find a quantitative relationship between the results obtained for about 250 patient's samples using the same analyser, same company reagents but with different buffer systems. We have found out a factor of 2.47, which should be used to convert the value obtained using AMP buffer to get the value of ALP assayed with DEA buffer.

Key words: ALP, AMP, DEA, Buffer

INTRODUCTION

ALPs are a group of isoenzymes that split off a terminal phosphate group from an organic ester in alkaline solution. The process of removal of phosphate group is called dephosphorylation. The optimum pH for the measurement of ALP is usually around 10, but this varies with the particular substrate and buffers used. It is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. It is present in a number of tissues including liver, bone, intestine, and placenta. Estimation of serum ALP is used in the diagnosis of hepatobiliary and bone diseases associated with increased osteoblastic activity. There are different types of buffers used for the measurement of ALP and the normal values are also different due to such buffer enhancement of the reaction. Whatever may be the buffer used, the normal value for a patient by one method should fall within the normal value for another method. The transphosphorylating accepter buffers AMP; N-methyl-D-glucamine (MEG), DEA and 2-ethylaminoethanol (EAE), have been widely used for the measurement of serum total ALP activity in clinical laboratories, and the individual isoenzyme are activated differently by respective buffers [1].

In a study, serum samples when preincubated with AMP buffer a loss of activity was observed in 4 out of the 6 buffers. Four human isoenzymes showed varying inactivation during preincubation with AMP buffers. However, no loss of activity was observed when the preincubation was done with the six DEA buffers. These results indicate that the purity of the commercially-available buffers is quite unsatisfactory [2]. ALP levels show great variation with age and sex in children and adolescents. Additionally, different buffers used

***Corresponding Author Address:** Dr. S. Swaminathan,Senior Consultant and Head, Department of Biochemistry, Apollo Speciality Hospitals,No. 64, Vanagaram to Ambattur Main Road, Ayanambakkam, Chennai – 600 095, South India. Email: glorynathan@gmail.com*

even in the same method cause variable results. This detail is not usually taken into account in the method evaluation studies. Establishing normative data will provide a basis for better evaluation of ALP levels determined by a new method. Furthermore, use of z-scores gives a more precise assessment of changes in ALP levels in rickets and other bone disorders [3].

Reflotron ALP was compared with three different wet chemistry procedures using different buffer compounds: N-methyl-D-glucamine or diethanolamine or 2-amino-2-methyl-1-propanol. In samples containing predominantly ALP isoforms not of liver origin, the measurements with N-methyl-D-glucamine buffer gave the best fit with respect to Reflotron. The accuracy related to an ALP method using diethanolamine buffer was good. The systematic differences for ALP in samples from pregnant women and adolescents have to be taken into account. The assay is well suited for differential diagnosis of hepatic diseases in decentralized testing [4]. In this paper, we have measured ALP by using the two most commonly used buffers, AMP and DEA and we have found out a factor of 2.47, which can be used to convert ALP assayed with AMP buffer to get the value of ALP assayed with DEA buffer. We further checked the factor which we derived with those derived from the available EQAS provider's data.

MATERIALS AND METHODS

The study population consisted of 250 non-hospitalised patients, both males and females in the Age group of 27 to 92 years, who underwent routine Master Health Check Up. As the sole aim of this study was to find out a numerical relationship between two methods for measuring ALP, we made use of laboratory results available for the above patients using the routinely used ALP method employing AMP buffer and compared the results obtained using DEA buffer for the same patients. The source of kits for both buffer systems was from the same company viz., Dia Lab. All measurements were done using fully automated chemistry analyser DIRUI CS 400.

The accuracy of the results obtained was validated by the use of Bio-Rad accuracy controls at two levels. For Statistical analysis of data, a software download from the website **http:// www.graphpad.com** was used to calculate correlation coefficient (r), Student 't' distribution (t) and probability (p) between two methods of ALP measurements. Inclusion or exclusion criteria were not followed as this study was to compare two analytical methods.

RESULTS

Table I shows the mean results obtained for all patients, males and females using both methods and the factor obtained to convert AMP values to DEA buffer values. The average factor obtained for all the groups was found to be 2.47. Figures I, II and III gives the correlation coefficients in the form of regression graphs between the two methods. The mean factor obtained from these graphs for converting AMP values to DEA method values was also found to be 2.47. The mean R2 is 0.963, and the probability is < 0.0001 indicating good correlation between the two methods.

DISCUSSION

The reference ranges for ALP differs from each and every buffers used and it is also been observed in our study that the ranges are in higher side with DEA buffer compared with AMP buffer. Similarly, in an earlier study, the mean ALP levels obtained were within the reference range, in which DEA buffer was used and higher than the values in the references using AMP as a buffer [5].

Most of the clinical laboratories in the western countries use ALP methods similar to that proposed by the International Federation of Clinical Chemistry (IFCC), based on the use of AMP buffer. Biases were predominantly due to differences in reagents rather than analyser characteristics. Compared to a reagent system prepared exactly as described by the IFCC, Bayer was sub-optimal and Dade and Boehringer methods produced results higher than the IFCC method. Reference ranges and results on patient's samples by the various methods showed large differences but no clinically significant difference was observed in EQAS either between Bayer and Boehringer or against method means. Apparently similar methods produce different results in patient's sera and EQAS are not useful in highlighting these differences [6].

Compared to many other enzyme measurements, the methods available for the measurement of ALP are diverse involving many substrates with many buffers. Starting from the earliest King-Amstrong, Kind-King methods which employed β-glycerophosphate and disodium phenyl phosphate as substrates and bicarbonate as the buffer, all modern methods now employ a common substrate, p-nitro phenyl phosphate but uses many buffer systems numbering almost one dozen.

The use of p-nitro phenyl phosphate and two main buffers AMP and DEA are now universally accepted and these methodologies have been recommended both by WHO and IFCC. The differences in normal values are solely due to the buffers used and it is important that each lab should establish their own normal values and compare with published works [3, 5]. However, the two organizations has left to the individual laboratories to decide the method of choice among the AMP and DEA buffer system methods and the use of appropriate normal values [4,6]. Two methods for measuring ALP, one makes use of phenyl phosphate, carbonate-bicarbonate buffer, and continuous-flow methodology; the other uses p-nitro phenyl phosphate, DEA buffer, and reaction-rate analysis correlated well over a wide range of values. A factor can therefore be applied to convert results by one method into those that would be obtained by the other. The possibility that the presence of different proportions of isoenzymes in the plasma will affect this factor should be considered [5]. As per the outcome of this research work, a factor of 2.47 is to be used to convert ALP buffer based method to get values in DEA buffer based method. It is recommended that every lab should establish their own conversion factors if they switch from one buffer based method to another buffer based methods.

CONCLUSION

The use of 2.47 as a factor to convert ALP measured using AMP buffer to get the value of ALP using DEA buffer has been established in this study using a large number of patient's samples. Therefore, the outcome presented in this study will enable clinical laboratories to establish such factors and to use it for conversions when they suddenly switch over from one buffer to another in case kits routinely used with a particular buffer is not available. Similar works should be undertaken by biochemists to compare the results when many different buffer systems are used to measure ALP.

Acknowledgement

The authors would like to thank Dr. Mitra Ghosh, Chief of Laboratory Services at Apollo Speciality Hospital, Ayanambakkam, Chennai, Tamilnadu for giving us permission to undertake this study.

Table I: ALP VALUES (AMP vs DEA BUFFER) (Results for all patients, Males and Females)

Analytes		ALP-AMP	ALP-DEA	DEA/AMP
All patients n=250	MEAN	93	231	2.52
Male patients n=150	MEAN	100	245	2.50
Female Patients n=100	MEAN	78	186	2.4

Figure I

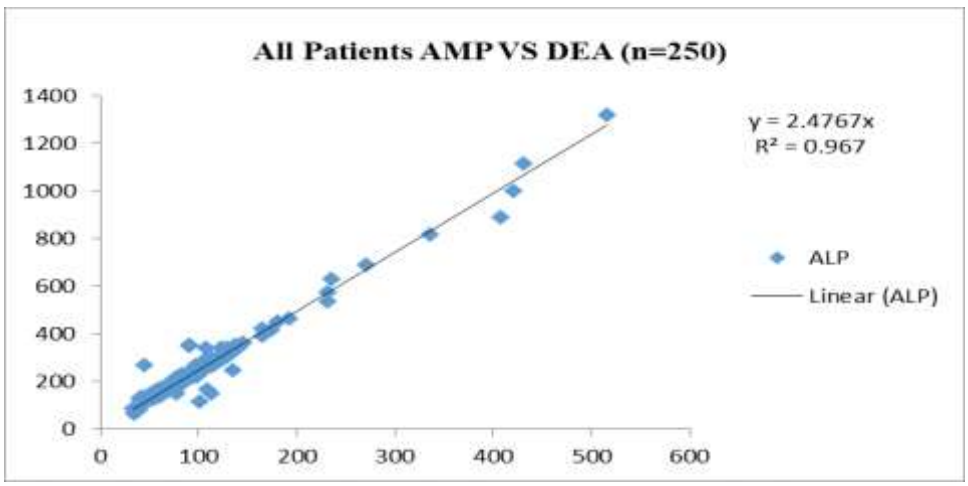


Figure II

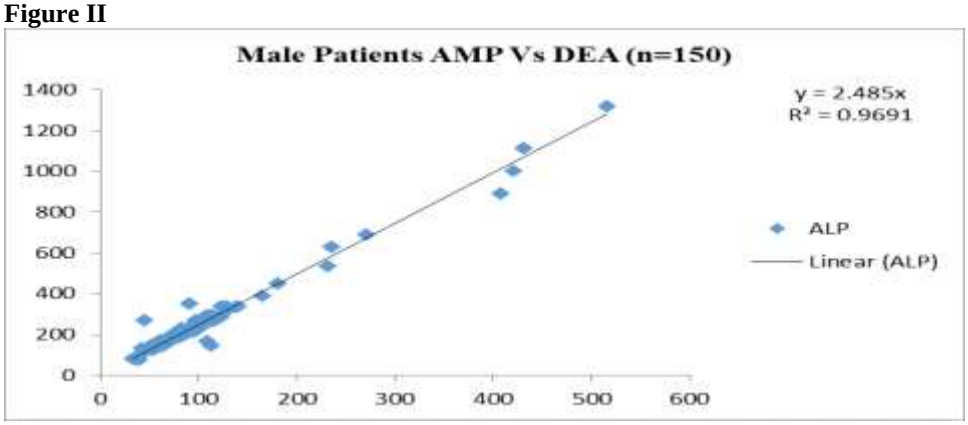
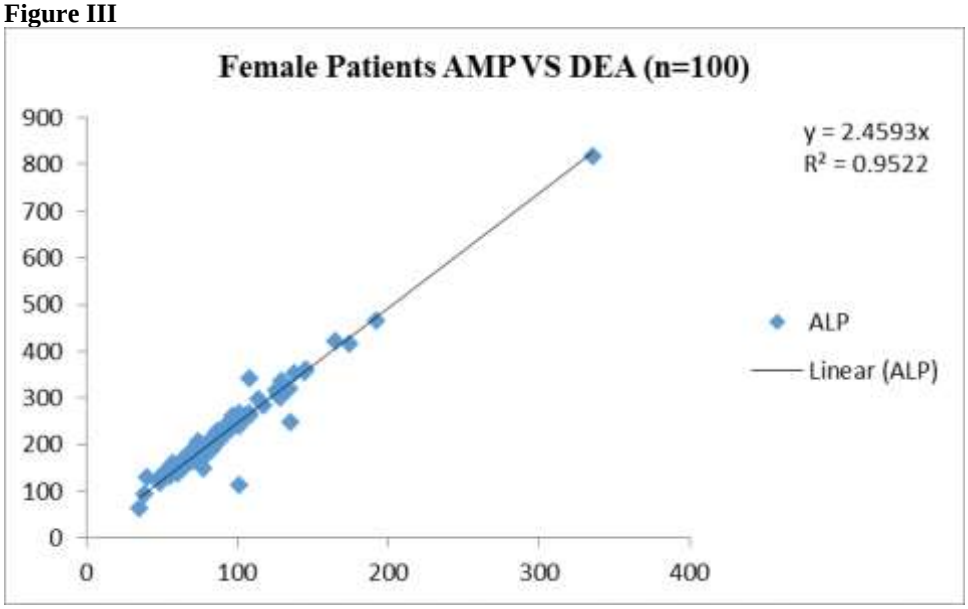


Figure III



REFERENCES

1. Matsushita M et al. The effect of different buffers and amounts of intestinal alkaline phosphatase isoforms on total alkaline phosphatase activity. Clin Chim Acta, 2002 7; 319(1): 49-55.
2. Pekelharing JM et al. Initial activity and inactivation of alkaline phosphatase in different lots of buffer. Clin Chim Acta 1978 May 2; 85(3):335-8.
3. Serap Turan et al. Serum Alkaline Phosphatase Levels in Healthy Children and Evaluation of Alkaline Phosphatasez-scores in Different Types of Rickets. J Clin Res Pediatr Endocrinol, 2011; 3(1): 7-11.
4. Schumann G et al. Alkaline phosphatase activity: new assay for the Reflotron system. Results of the evaluation in eight clinical laboratories. Clin Chem Lab Med, 2001; 39(1): 71-8.
5. Eaton RH. Plasma alkaline phosphatase assay: interconversion of results by two methods. Clin Chem, 1977; 23(11): 2148-50.
6. Lamb EJ et al. Alkaline phosphatase activity measurement in the UK by AMP-buffered methods: an appraisal of current practice. Ann Clin Biochem, 1998; 35 (1): 120-7.