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INTERFERING EFFECT OF BILIRUBIN ON THE MEASUREMENT OF ALKALINE PHOSPHATASE ACTIVITY

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ABSTRACT
Introduction: Analytical interference is the systematic error of measurement caused by a sample component which does not by itself produce a signal in the measuring system. **Objective:** This study was designed to evaluate whether the high concentration of bilirubin is able to interfere the determination of alkaline phosphatase (ALP). **Methods:** Clinical tests evaluating the interfering substance of bilirubin on the determination of ALP were conducted based upon the Clinical and Laboratory Standards Institute (CLSI) document EP7-A2, the most recent guideline on interference testing approved in 2005. **Results:** The interference judgment is made by comparing the observed systemic error (bias) with the amount of error that is allowable for the test. There is no interfering effects of bilirubin at this cocentration on the determination of different ALP concentrations. **Conclusion**: We need high concentration of bilirubin to detect the interfering effects can cause false measurement of ALP levels, probably interfering with the clinical prognosis of liver diseases.

KEYWORDS: Alkaline phosphatase, bilirubin, interference.

INTRODUCTION
Analytical interference is the systematic error of measurement caused by a sample component which does not by itself produce a signal in the measuring system.[1,2]

Interference by endogenous and exogenous substances with assays for clinical analytes is a common problem in laboratory analyses.

The major exogenous sources of interference are drugs prescribed for the patient; and there are several excellent compendia of the effect of drugs on clinical laboratory tests. There are four major endogenous compounds that consistently interfere with laboratory results: hemoglobin, bilirubin, lipids, and paraproteins.

A variety of previous investigations have proven that high concentrations of bilirubin exert significant interference on the determination of multiple biochemical substances.[3,4]

Bilirubin causes a negative interference with the Jaff, enzymatic method for creatinine.[5,6,7] also interferes with determinations of uric acid, cholesterol, and triglycerides that use peroxidase- coupled reactions.[8]

Alkaline phosphatase (ALP) is a routine laboratory test used to screen for liver disease and elevated levels are often the frst indication of chronic hepatic lesions, which is also an important index for evaluating the severity and prognosis of liver illnesses.[9] Recent data also indicated that elevated serum alkaline phosphatase levels are associated with increased mortality in patients with metabolic syndrome and they also suggested that higher levels of serum alkaline phosphatase is apredictor of mortality independent of the baseline prevalence of metabolic syndrome.[10]

This study is designed to investigate whether high levels of bilirubin are able to pose interference upon the detection of ALP in patients with hyperbilirubinemia.

MATERIAL AND METHODS
This study designed as analytical experimental study, conduct in Sudan, Khartoum state, from October 2017 to December.

Methods
The end-point method was performed for the measurement of ALP and total brilirubin on the biochemical analyzer.

Testing procedure
Selection of 3patient sample with different level of alkaline phosphatase divided into 5sample, add bilirubin standard with 4.88mg/dl (83.44µmol/L) concentration, each sample measuring duplicate, calculate the interference to each level of different bililrubin, test sample –base line against total allowable error within 10% according to CLLA88.

Preparation of basic samples
The concentration of the test specimens should be designed to include high, low and medical decision levels. Fresh serum was obtained from healthy individuals who had not taken medication in the past 3 days. The serum samples should have no signs of hemolysis, jaundice or lipemia.

Since bilirubin is an endogenous metabolite, therefore, the final concentration of bilirubin may be excessively high, which probably influences the credibility of the experimental outcomes.

The final concentrations of ALP and bilirubin in the mixed serum were determined by laboratory instruments.

Statistical analysis
All data will be analyzed statistical according to document EP7-A2.

Ethical consideration
This study was approved by faculty of medical laboratory sciences, AlNeelain University, Khartoum, Sudan, and ethical clearance was obtained from ministry of health. All participants signed an informed consent before sample collection.

RESULTS
Evaluation of bilirubin interference effect on the different concentrations of alkaline phosphatase (ALP)
The interference judgment is made by comparing the observed systemic error (bias) with the amount of error that is allowable for the test.

ALP test is supposed to be correct to within +/- 30% according to the CLIA proficiency testing criteria for acceptable performance. The allowable error would be 45 U/L.

Interpretation of result
Interference (bias) > allowable error (45U/L) = interference
Interference (bias) ≤ allowable error (45 U/L) = No interference

Calculation of bilirubin Interference effect on high ALP concentration
Average concentrations of high ALP test = 418 mmol/l
Average concentrations of high ALP control = 418 mmol/l
Bias = average test concentration – average control concentration
= 418 – 418 = 0 U/L (Table1).
0 U/L < 45 U/L = No interference. (Figure 1).

Calculation of bilirubin Interference effect on high ALP concentration
Average concentrations of low ALP test = 55 mmol/l
Average concentrations of low ALP control = 49mmol/l
Bias = average test concentration – average control concentration
= 55– 49 = 6U/L (Table 1).
6 U/L < 45 U/L = No interference. (Figure 1).

Calculation of bilirubin Interference effect on high ALP concentration
Average concentrations of normal ALP test = 125 mmol/l
Average concentrations of normal ALP control = 127 mmol/l
Bias = average test concentration – average control concentration
= 125– 127= -2 U/L. (Table 1).
-2 U/L < 45 U/L = No interference. (Figure 1).

Table 1: Evaluation of the interfering effects of total bilirubin on the determination of different ALP concentrations.

Level	Average test concentrations (mmol/l)	Average control Concentrations (mmol/l)	Bias (U/I)	CLIA Allowable error (U/L)	Interference judgment
High	**418**	**418**	**0**	**45**	**No**
Low	**55**	**49**	**6**	**45**	**No**
Normal	**125**	**127**	**-2**	**45**	**No**

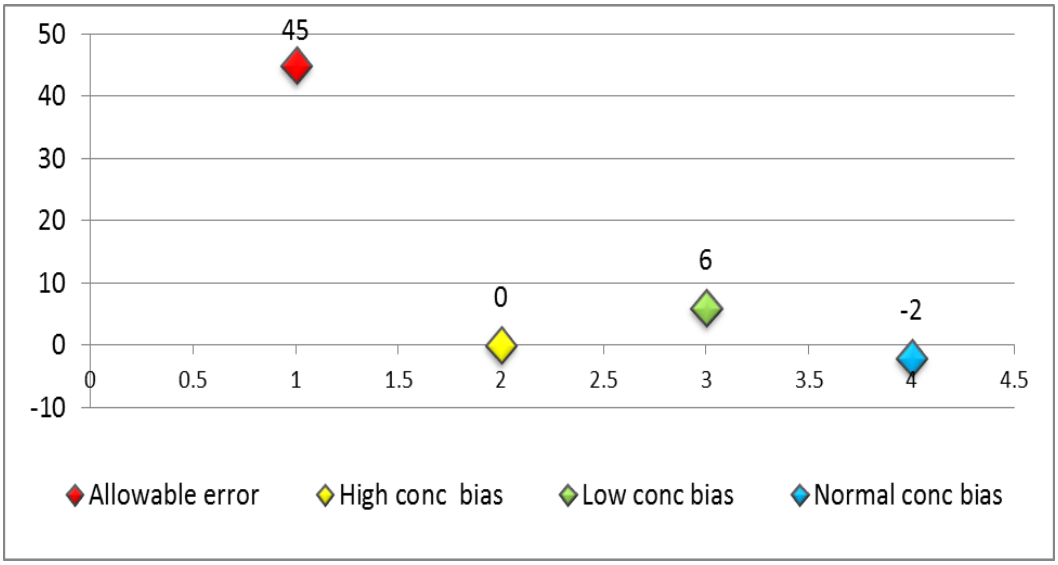

Figure 1: Comparing the observed systemic error (bias) with the amount of error that is allowable for the test.

DISCUSSION

Alkaline phosphatase is a routine laboratory test used to screen for liver diseas and elevated levels are often the first indication of chronic hepatic lesions which is also an important index for evaluating the severity and prognosis of liver illnesses.[1] Hyperbilirubinemia is also one of the commin symptoms encountered by patients with liver diseases.

Avariety of previous investigations have proven that high concentration of bilirubin exert significant interference on the determination of multiple biochemical substances such as alkaline phosphates.[3,4]

This study is designed to investigate the interference of bilirubin upon the detection of alkaline phosphatase. The laboratory procedures of the interference test were conducted strictly according to the clinical and laboratory standard institute (CLSI).

The results of the present study showed that the observed systemic error (bias) in all alkaline phospahtase concentration was lower with the amount of error that is allowable for the test which is 45 U/L according to CLSI. This is in agreement with the findings of many studies in other parts of the world.

These results suggest that when patients were clinically diagnosed with hyperbilirubinamia, alkaline phospahtase alone should not be directly utilized as a diagnostic parameter if the interfering effects of bilirubin can not be confirmed. Bilirubin probably inhibits the enzyme activity, especially by exerting negative interfering effect since bilirubin is a type of reducing agentnwhich is able to weaken electron and proton transmission.[11]

This study indeed certain limitations as below: The substance of bilirubin supplemented to the serum samples may differ from the endogenous bilirubin yielded within human body and the concentration of the test samples may be excessively low or high.

REFERENCES

1. IFCC provisional recommendation on quality control in clinical chemistry. J Clin Chem Clin Biochem, 1976; 14: 270.
2. Buttner J, Borth R, Boutwell JH, Broughton PMG, Bowyer Re. IFCC approved recommendation (1978) on quality control in clinical chemistry. Part I. General principles and terminology. J Clin Chem Clin Biochem, 1979; 18: 69-77.
3. Hullin DA, McGrane MT. Effect of bilirubin on uricase-peroxidase coupled reactions. Implications for urate measurement in clinical samples and external quality assessment schemes Ann Clin Biochem, 1991; 28: 98-100.
4. Cobbaert CM, Baadenhuijsen H, Weykamp CW. Prime Time for Enzymatic Creatinine Methods in Pediatrics. Clin Chem, 2009; 55: 549-558.
5. Jay DW, Provask D. Characterization and mathematical correction of hemolysis interference in selected Hitachi 717 assays. Cim Chem, 1993; 39: 1804-10.
6. Blijenberg BG, Liesting EC, Zwang L. Creatinine and automatic analyzers in relation to icteric specimens. Eur J Cim Chem Biochem, 1992; 30: 779-84.
7. Crocker H, Shephard MDS, White GH. Evaluation of anenzymatic method for determining creatinine in plasma. J Clin Pathol, 1988; 41: 57681.
8. Spain MA, Wu AHB. Bilirubin interference with determination of uric acid, cholesterol, and triglycerides in commercialperoxidase-coupled assays, and the effect of ferrocyanide. CkeChem, 1986; 32: 518-21.
9. Yingwu Y, Yusan W, Ziyu S. National Clinical Laboratory Procedures [M] 3rd edition Nanjing: Southeast University Press, 2006; 80: 476,485490.

10. Krishnamurthy VR, Baird BC, Wei G, Greene T, Raphael K, Beddhu S. Associations of Serum Alkaline Phosphatase with Metabolic Syndrome and Mortality, Am J Med, 2011; 124: 566.e1-566.e7.
11. Pyles LA, Stejskal EJ, Einzig S. Spectrophotometric measurement of plasma 2-thiobarbituric acid-reactive substances in the presence of hemoglobin and bilirubin interference. Proc Soc Exp Biol Med, 1993; 202: 407-419.