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Elimination of bilirubin interference by photolysis in the analysis of creatinine, glucose and alkaline phosphatase

PM Abraham Sam Rajan

Associate Professor, Saveetha Medical College, Thandalam, Chennai

Email: salomi.jayasam@gmail.com

Abstract

Introduction: Clinical biochemistry laboratory uses many techniques for measuring the concentration of specific biochemical substances. All these techniques are subject to interference from variety of sources. The aim of our study is to see the changes in concentration of serum bilirubin on exposure to special blue light of 440nm for six hours and to study the usefulness of photolysis for the elimination of bilirubin interference in the estimation of creatinine, glucose, and alkaline phosphatase.

Method: We have taken 218 samples and the samples were analyzed for the estimation of total bilirubin, direct bilirubin, creatinine, glucose and alkaline phosphatase before and after photolysis.

Results & Conclusion: There was significant decrease in the concentration of total and direct bilirubin after photolysis. There was a significant negative interference of creatinine and glucose and significant positive interference of alkaline phosphatase with an increasing concentration of bilirubin which was also eliminated by photolysis.

Keywords: Interference, Photolysis, Analytes, Isomerization.

Introduction

Analysis of body fluids in clinical laboratory is subjected to a number of interferences that affect the analytical accuracy. The interferences arise from exogenous sources like drugs and additives as well as endogenous sources like lipemia, hemolysis and icterus. Interference can be defined as" The effect of substance present in the sample that alters the correct value of the result, usually expressed as a concentration or activity for an analyte".[1] When interference is present, it is important to determine whether it depends or does not depend on the analyte. An interferant or the product of the interferant reacting with reagent may absorb light at the wavelength of the reaction. This type of interference is independent of the concentration of analyte and referred independent. If the interferant may react directly with the analyte or it may interfere in the chemical reaction, then this interference is analyte dependent where the degree of interference changes as the concentration of the analyte is altered.[2,3]

Bilirubin, a degradation product of heme is found to interfere in the estimation of various analytes, like creatinine, glucose, ALP, uric acid, cholesterol, triglycerides, total protein and few enzymes. This interference may be probably by two mechanisms; one is the spectral interference resulting from the absorbance spectra of bilirubin and of the chromophore produced in the reaction. Second is the chemical interference occurring when a portion of the reaction intermediate is being destroyed by bilirubin, thereby decreasing the amount of chromophore formed. The aim of our present study is to observe the change in concentration of bilirubin in the serum on exposure to special blue light of 440nm for six hours, to study the usefulness of phototherapy for the elimination of bilirubin interference in the estimation of creatinine, glucose and alkaline phosphatase and to arrive at the concentration of bilirubin at which photolysis is useful.

Materials and Methods

The study group comprises of 218 samples obtained from the clinical central laboratory located at the Sri Ramachandra Medical College Hospital and Research Institute. All serum samples were the leftover aliquots of blood draw collected from patients in serum tubes that were submitted to the laboratory for routine chemistry analysis was collected. The specimens were used as anonymous samples irrespective of factors like age, sex, medication, pathological problems etc. The samples were analysed for the estimation of total bilirubin, direct bilirubin, creatinine, glucose and alkaline phosphatase. After the estimation, the samples were transferred into glass tubes and were subjected to photolysis under the blue light at 440nm for 6hrs duration for photolysis. The samples were divided into seven groups based on the total bilirubin concentrations as group I with total bilirubin value 0-1mg/dl, group II with 1.1-3mg/dl, group III with 3.1-6mg/dl, group IV with 6.1-9mg/dl, group V with 9.1-12mg/dl, group VI with 12.1-15mg/dl and group VII with bilirubin value >15mg/dl respectively. After photolysis, the samples were again analysed for total bilirubin, direct bilirubin, creatinine, alkaline phosphatase and glucose. Due permission was obtained from the Institutional ethical committee for this study.

The analytes total bilirubin and direct bilirubin were measured using commercial kits from Siemens Healthcare Diagnostics Ltd, from Point Scientific for creatinine, from Randox Laboratories for Alkaline phosphatase and glucose in fully automated Biochemistry analyzer, RXL Dimension from Dade Behring. Special blue tube light of 2 feet length was

purchased from Nice Neotech Medical systems Pvt. Ltd which emit light in the range of 400-550nm(peak wavelength 450-475nm). This range corresponds to the spectral absorption of light by bilirubin and is considered most effective for the degradation of bilirubin which was mounted on a wooden frame. The light provides an average irradiance of 12-13µw/cm^2/nm at a distance of 40cm and the irradiance was checked by Photoradiometer.[4]

All the parameters were compared to see the Interference of bilirubin on the estimation of creatinine, alkaline phosphatase and glucose, before and after exposure to light. The samples were analysed statistically using paired 't' test / Wilcoxon Signed Ranks test using SPSS for windows (version 17). Statistical significant considered to be $p<0.05$.

Results

Table 1: Represents the statistical data of Total bilirubin, Direct bilirubin, Alkaline phosphatse, Creatinine & Glucose in Group I with total bilirubin 0-1mg/dl before and after exposure to light

Parameter	Before light exposure	After light exposure	p-Value
	Mean ± SD	Mean ± SD	
Total bilirubin	0.521 ± 0.3094	0.236 ± 0.1531	0.000***
Direct bilirubin	0.160 ± 0.1013	0.78 ± 0.0647	0.000***
ALP	211.731 ± 142.4835	210.583 ± 142.2676	0.000***
Creatinine	1.314 ± 2.6368	1.325 ± 2.6353	0.164NS
Glucose	139.602 ± 73.2606	140.852 ± 73.1309	0.001***

***p<0.001; NS: Not significant

From Table 1, there is a statistical significant difference between before and after light exposure in total bilirubin, direct bilirubin, alkaline phosphatse & glucose at $p<0.001$. There is no statistical significant difference in creatinine at $p>0.05$ before and after exposure to light.

Table 2: Represents the statistical data of Total bilirubin, Direct bilirubin, Alkaline phosphatse, Creatinine & Glucose in Group II with total bilirubin 1.1-3mg/dl before and after exposure to light

Parameter	Before light exposure	After light exposure	p- Value
	Mean ± SD	Mean ± SD	
Total bilirubin	1.871 ± 0.5597	0.957 ± 0.3551	0.000***
Direct bilirubin	0.710 ± 0.4908	0.359 ± 0.2714	0.000***
ALP	296.00 ± 235.3003	294.549 ± 234.4564	0.001***
Creatinine	0.531 ± 0.3641	0.553 ± 0.3443	0.012**
Glucose	114.667 ± 54.2154	115.510 ± 54.2444	0.006**

***p<0.001; **p<0.01

From Table 2, there is a statistical significant difference between before and after light exposure in total bilirubin, direct bilirubin, alkaline phosphatse at $p<0.001$ and creatinine & glucose at $p<0.01$.

Table 3: Represents the statistical data of Total bilirubin, Direct bilirubin, Alkaline phosphatse, Creatinine & Glucose in Group III with total bilirubin 3.1-6mg/dl before and after exposure to light

Parameter	Before light exposure	After light exposure	p-Value
	Mean ± SD	Mean ± SD	
Total bilirubin	3.776 ± 0.7035	1.871 ± 0.4681	0.000***
Direct bilirubin	2.324 ± 1.1406	1.219 ± 0.5930	0.000***
ALP	455.762 ± 272.0121	451.714 ± 268.7525	0.004**
Creatinine	0.471 ± 0.5331	0.571± 0.5159	0.002**
Glucose	105.0 ± 38.8677	107.429 ± 39.0635	0.001**

***p<0.001; **p<0.01

From Table 3, there is a statistical significant difference between before and after light exposure in total bilirubin, direct bilirubin, glucose at $p<0.001$ and creatinine & alkaline phosphatse at $p<0.01$.

Table 4: Represents the statistical data of Total bilirubin, Direct bilirubin, Alkaline phosphatse, Creatinine & Glucose in Group IV with total bilirubin 6.1 - 9mg/dl before and after exposure to light

Parameter	Before light exposure	After light exposure	p-Value
	Mean ± SD	Mean ± SD	
Total bilirubin	7.506 ± 0.9344	3.859 ± 0.5209	0.000***
Direct bilirubin	4.288 ± 2.4367	2.218 ± 1.3083	0.000***
ALP	427.765 ± 223.0786	396.059 ± 211.7922	0.000***
Creatinine	0.635 ± 0.9772	1.265± 0.9810	0.000***
Glucose	101.235 ± 57.1068	111.471 ± 58.2195	0.000***

***p<0.001

From Table 4, there is a statistical significant difference between before and after light exposure in total bilirubin, direct bilirubin, alkaline phosphatse, creatinine & glucose at $p<0.001$.

Table 5: Represents the statistical data of Total bilirubin, Direct bilirubin, Alkaline phosphatse, Creatinine & Glucose in Group V with total bilirubin 9.1 - 12mg/dl before and after exposure to light

Parameter	Before light exposure	After light exposure	p- Value
	Mean ± SD	Mean ± SD	
Total bilirubin	10.386 ± 1.0946	5.286 ± 0.7883	0.018**
Direct bilirubin	4.414 ± 2.9002	2.186 ± 1.4781	0.018**
ALP	356.714 ± 107.5743	322.571 ± 104.9855	0.018**
Creatinine	0.386 ± 0.2545	1.143± 0.2299	0.018**
Glucose	85.571 ± 22.8900	99.0 ± 24.6103	0.018**

**p<0.01

From Table 5, there is a statistical significant difference between before and after light exposure in total bilirubin, direct bilirubin, alkaline phosphatase, creatinine & glucose at p<0.01.

Table 6: Represents the statistical data of Total bilirubin, Direct bilirubin, Alkaline phosphatse, Creatinine & Glucose in Group VI with total bilirubin 12.1 - 15mg/dl before and after exposure to light

Parameter	Before light exposure	After light exposure	p- Value
	Mean ± SD	Mean ± SD	
Total bilirubin	13.114 ± 0.7734	6.771 ± 0.8731	0.018**
Direct bilirubin	5.729 ± 4.1129	2.771± 1.9704	0.018**
ALP	320.857 ± 181.9619	293.00 ± 172.1579	0.018**
Creatinine	0.686± 0.6669	1.857± 0.6079	0.018**
Glucose	82.714 ± 14.6483	97.857 ± 15.9523	0.018**

**p<0.01

From Table 6, there is a statistical significant difference between before and after light exposure in total bilirubin, direct bilirubin, alkaline phosphatse, creatinine & glucose at p<0.01.

Table 7: Represents the statistical data of Total bilirubin, Direct bilirubin, Alkaline phosphatse, Creatinine & Glucose in Group VII with total bilirubin >15mg/dl before and after exposure to light

Parameter	Before light exposure	After light exposure	p- Value
	Mean ± SD	Mean ± SD	
Total bilirubin	18.771 ± 3.5631	9.357 ± 2.3373	0.018**
Direct bilirubin	11.314 ± 6.1012	6.014± 3.3598	0.018**
ALP	510.00 ± 493.3305	467.429 ± 473.1226	0.018**
Creatinine	1.457± 1.4536	3.157± 1.3843	0.018**
Glucose	119.714 ± 61.0920	140.00 ± 62.3110	0.018**

**p<0.01

From Table 7, there is a statistical significant difference between before and after light exposure in total bilirubin, direct bilirubin, alkaline phosphatse, creatinine & glucose at p<0.01.

Discussion

In the present study, there is statistically significant decrease in the value of total bilirubin after exposure to light in all the seven groups. In the same way direct bilirubin values were also found to decrease after exposure to light which is highly statistically significant in all the seven groups. The decrease in the values of total bilirubin and direct bilirubin were because of the substantial changes in the bile pigment composition of the sample because of photoisomerization reactions, which is an unimolecular processes that occurs many orders of magnitude faster.[5] Nadja N. Rehak et al have shown in their the measured values of total bilirubin in normobilirubinemic specimens decreased by an average of 59% and that for moderately hyperbilirubinemic specimens decreased by 41%, the direct bilirubin values decreased by 38% for normobilirubinemic and decreased by 31% for moderately hyperbilirubinemic specimens, when these samples were exposed to fluorescent lighting at ambient temperature approximately 6 feet below a light fixture for 9 hours.[6] The wavelength as well as the intensity of light is an important factor in photoisomerisation of bilirubin, although most of the available data is for the in vivo clearance of bilirubin in patients exposed to phototherapy rather than in vitro stability of serum specimens exposed to light.[7] Photoisomerisation of bilirubin is enhanced by exposure to shorter wavelengths of light, near bilirubin absorbance maximum of about 455nm, at the blue end of the visible spectrum. Tan reported that the decline of bilirubin is most rapid with special blue lights and duration of exposure is also less, when compared to fluorescent day light lamps.[8] High intensity blue light phototherapy

have been found twice as effective as standard day light.[9] In our study the substantial decrease in the values of total bilirubin and direct bilirubin may be because of exposure of the samples to special blue light of 440nm in the visible range which was placed only at a distance of 45cm from the samples. The decrease in the values was almost same irrespective of the concentration of bilirubin.

Analyzing the values of creatinine by Jaffe procedure by the interference of bilirubin, it is found that in group I there was no statistical significant changes in the values of creatinine after exposure to light. Whereas groups II, III, IV, V, VI, VII showed statistically significant increase in creatinine after exposure to light. From the analysis, the negative interference caused by bilirubin in the estimation of creatinine increases as the concentration of bilirubin increases and the increase is more pronounced at the concentration of 6mg/dl onwards. Some studies showed that there is negative interference of bilirubin at a concentration ≥5mg/dl in the estimation of creatinine.[10] This negative interference mechanism has been explained by both spectral and chemical effects. The spectral interference can be minimized by using sample blank, bichromatic analysis and kinetic analysis. Reaction intermediates effect rapid oxidation of bilirubin to biliverdin, thereby removing interferences in Jaffe procedure.[11] Joseph D et al showed in their study that bilirubin oxidase an enzyme that catalyses the oxidation of bilirubin to biliverdins.[12] In our study, the elimination of bilirubin interference was achieved by exposing the samples to special blue light at a wavelength of 440nm that causes photoisomerization which can be structural or configurational isomerization of bilirubin and decrease in creatinine value that was statistically significant. Bilirubin isomers are water soluble and they do not get estimated in the analysis of creatinine.

In our study, statistical significant increase was observed in the glucose after exposure to light in groups I, II, III, IV, V, VI and VII showing that there is negative interference by bilirubin in glucose estimation. Pretreating sample with titanium IV complex seems to reduce negative interference by an unknown mechanism.[13] The degree of interference of bilirubin in glucose oxidase method varies between different analyzers. These potential areas of inaccuracy are particularly important in neonatal samples, where substantial amounts of bilirubin are present which can lead to artificially low plasma glucose values. In the current study the negative interference of bilirubin in the estimation of glucose was eliminated by exposing the sample to blue light at 440nm in the visible range.

Analyzing the changes in the values of alkaline phosphatse after exposure to light, it was found there was significant decrease in all groups after exposure to light. This shows a positive interference by bilirubin in the estimation of alkaline phosphatse at increasing concentration of total bilirubin. Morgenstren. S. et al showed in their study over estimation of alkaline phosphatse in the increasing concentration of bilirubin[14] which correlates with our study. This positive interference may be due to spectral interference by bilirubin to strongly chromogenic – yellow-p-nitrophenoxide anion at alkaline pH formed by cleavage by alkaline phosphatase which is measured at 405nm.[15] Interference can be handled by blank correction and photolysis. Alkaline phosphatase method may be susceptible to positive interference when serum bilirubin concentrations are high. In the current study the positive interference by bilirubin in the estimation of alkaline phosphatse was eliminated by exposing the samples to blue light at 440nm in the visible range.

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The authors deny any conflicts of interest related to this study.

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