



Original Research Article

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## The effect of light on the specimens of serum bilirubin in clinical laboratory

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### Abstract

**Background:** Laboratories can influence the stability of bilirubin by the type of light used in the laboratory which has direct impact on the quantitative estimation of the bilirubin. **Objective:** To compare the levels of total and direct bilirubin in moderately hyperbilirubinemic to normobilirubinemic vs. Hyperbilirubinemic serum specimens before and after exposure to the room light. **Materials and Methods:** This was an experimental laboratory based study conducted in the department of clinical laboratory services (CLS), Birat Medical College and Teaching Hospital, Morang, Nepal. An early morning blood samples were collected from 50 patients who visited the CLS for their bilirubin and liver function test analysis. To evaluate the effect of light on the kinetics of bilirubin changes, each serum specimen was transferred into two microtubes with one tube completely enclosed in aluminum foil as a control in dark. Specimens were exposed to room light on a laboratory bench at room temperature for a set time interval of five hours. All specimens were assayed in duplicate directly from the microtubes for direct and total bilirubin before and after a set time interval of five hours by the diazo reaction. **Result:** In our present study, in normobilirubinemic / moderately hyperbilirubinemic specimen, measured values of total bilirubin decreased by an average of 55.9 % while the decline in total bilirubin for the hyperbilirubinemic specimen was smaller 32.4% when exposed to light but in contrary, direct bilirubin decreased by slightly more amounts, 47.4% and 49.7% respectively, for the normobilirubinemic / moderately hyperbilirubinemic specimen and hyperbilirubinemic specimen when exposed to light over a period of five hours. **Conclusion:** The practical consequences of this finding is that accurate measurement of bilirubin in specimens with normal or low bilirubin concentrations requires even greater care in the protection of specimens from light than for specimens with high bilirubin concentrations.

**Keywords:** Bilirubin, Room Light, Photodegradation, hyperbilirubinemic, normobilirubinemic.

### Introduction

Bilirubin is a yellowish pigment found in bile, a fluid produced by the liver. Since long time, the primary goal of estimating serum bilirubin concentration in clinical laboratory is for the

detection of increased bilirubin concentration (1, 2). Increased bilirubin concentration in serum is a marker of hepatic dysfunction or increased red cell turnover and potentially toxic substance in

newborn(3). However, increased interest in the measurement of low and normal bilirubin concentrations has been stimulated by recent findings that bilirubin is one of the major antioxidants in the circulation. Bilirubin appears to be protective against cardiovascular disease and other pathophysiology resulting from generation of free radicals (4, 5).

Sometimes delay in the measurement of serum specimens due to heavy workload or delay in samples transportation to laboratories from wards and nursery units is common problem in most of the hospitals setups of developing countries(6). In the clinical laboratory, it is well recognized that bilirubin concentrations decrease when specimens are exposed to light(7)but very few studies has been done to compare the effect of light on different concentration of bilirubin at different interval of time.

Thus, the aim of this study was to compare the rate of photodegradation of direct and total bilirubin in normobilirubinemic and hyperbilirubinemic specimens exposed to room light too see whether the photodegradation of bilirubin will result in a clinically significant change in concentration of bilirubin or not. Also, our ultimate aim was to find out the criteria on sample handling to eliminate clinically significant photolysis of bilirubin.

## Materials and Methods

This study was conducted at Birat Medical College and Teaching Hospital, Morang, Nepal by the Department of Physiology along with the coordination of Department of Clinical Laboratory Services. The study was approved by ethical clearance board and signed informed consent was taken from all the subjects.

An early morning blood sample were collected from patients in serum tubes that were submitted to laboratory of Birat Medical College for routine chemistry analysis over a period of one month from 1<sup>st</sup> January, 2016 to 31<sup>st</sup> January, 2016. The collected samples were divided into two groups: 26 samples of hyperbilirubinemic serum (total bilirubin > 2mg/dl) and 24 samples of moderately hyperbilirubinemic (total bilirubin = 1-2mg/dl) /

normobilirubinemic serum (total bilirubin < 1mg/dl) specimen (i.e. total 50 serum specimen).

## Inclusion criteria:

Direct and total bilirubin analysis and liver function test requested blood sample for routine chemistry analysis were taken as selection criteria.

## Exclusion Criteria:

- Blood samples other than bilirubin analysis for routine chemistry analysis were excluded.
- Photolysis of bilirubin in specimens during collection and processing was not taken into consideration.

## Processing Technique:

Individual serum aliquots (1 ml) were transferred into plastic tubes after centrifugation of blood sample. To evaluate the effect of light on the kinetics of bilirubin changes, each serum specimen was transferred into two microtubes with one tube completely enclosed in aluminum foil as a control in dark. Specimens were exposed to room light on a laboratory bench at room temperature for a set time interval of five hours.

The specimens were kept in the room that gets a decent amount of light of mean intensity of about 300 Lux. The window in the laboratory was 3 feet wide by 4 feet high. The room had also a door on the opposite wall with 2.5 feet wide by 7 feet high.

All specimens were assayed, in duplicate, directly from the microtube for direct and total bilirubin before and after a set time interval of five hours by the diazo reaction (Modified Jendrassik-Grof Method)(8)

## Statistical Method

Measurements of variable were normal in distribution and thus parametric tests were used for statistical analysis. Variables were compared in two groups of sample before and after the exposure of light and dark and statistical

significance were tested with paired t test. All analysis were calculated with SPSS 16 software with value <0.05 considered significant.

**Results**

In the present study, 50 blood samples were analyzed for total and direct bilirubin using spectrophotometer at an interval of five hours. Analysis of changes in total and direct bilirubin exposed to laboratory light vs. exposed to dark was assessed in two groups: 26 hyperbilirubinemic and 24 moderately

hyperbilirubinemic / normobilirubinemic specimen.

Normobilirubinemic/ moderately hyperbilirubinemic specimens showed greater percentage of photodegradation of bilirubin than that of hyperbilirubinemic specimen before and after exposure of light or in dark over a period of five hours (Fig 1 & 2). Also, we can notice that the protection of bilirubin sample from light drastically retards the process of photodegradation but this protection is not sufficient for a period of five hours since, there is clinically significant decrease in bilirubin concentration (Table 1)

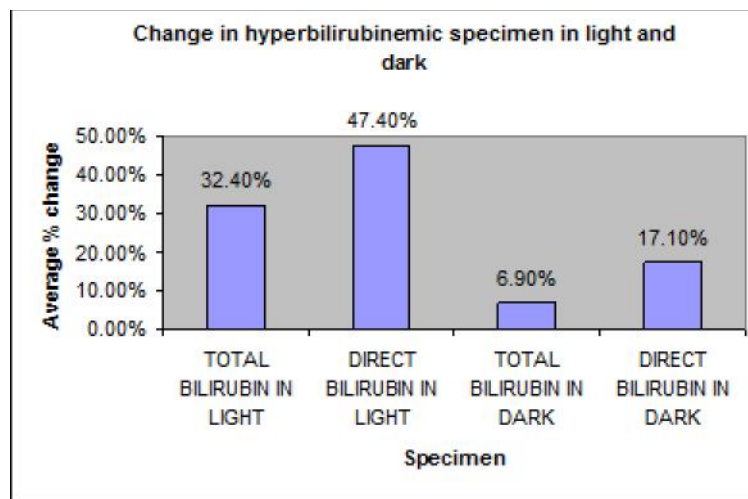


Fig 1: Average percentage change of total and direct bilirubin concentration in hyperbilirubinemic specimen in light and dark.

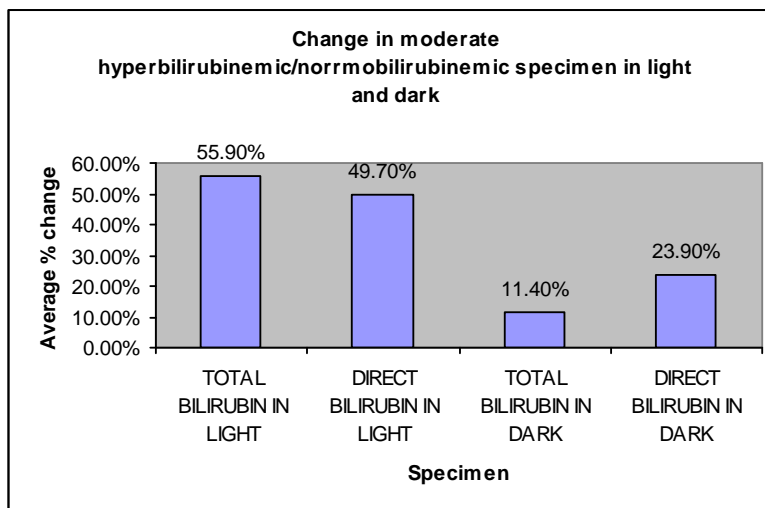


Fig 2: Average percentage change of total and direct bilirubin concentration in Normobilirubinemic to moderately hyperbilirubinemic specimen in light and dark

Table 1: Test of significance in hyperbilirubinemic and normobilirubinemic / moderately hyperbilirubinemic specimen kept in light and dark over a period of five hour

	Type	(Mean $\pm$ S.D)	(C.V= S.D/MEAN*100)	Paired t test =[(Mean) / (S.D/ n)]	
Hyperbilirubinemic specimen	Total bilirubin in light	(2.81 $\pm$ 2.32)	82.56 %	t = 6.2, P<0.001	Significant decrease
	Direct bilirubin in light	(3.22 $\pm$ 2.39)	74.22 %	t = 6.85, P<0.001	Significant decrease
	Total bilirubin in dark	(0.63 $\pm$ 0.54)	85.71 %	t = 6.3, P<0.001	Significant decrease
	Direct bilirubin in dark	(1.14 $\pm$ 0.94)	82.45 %	t = 6.33, P<0.001	Significant decrease
Normobilirubinemic/ Moderately Hyperbilirubinemic	Total bilirubin in light	(0.63 $\pm$ 0.32)	50.79 %	t = 9.7, P<0.001	Significant decrease
	Direct bilirubin in light	(0.33 $\pm$ 0.17)	51.51 %	t = 9.7, P<0.001	Significant decrease
	Total bilirubin in dark	(0.12 $\pm$ 0.07)	58.33 %	t = 8.57, P<0.001	Significant decrease
	Direct bilirubin in dark	(0.14 $\pm$ 0.09)	64.28 %	t = 7.78, P<0.001	Significant decrease

## Discussion

In the clinical laboratory, it is well recognized that bilirubin concentrations decrease when specimens are exposed to light but very few studies has been done to compare the effect of light on different level of bilirubin concentration in different interval of time. In our present study, in normobilirubinemic / moderately hyperbilirubinemic specimen, measured values of total bilirubin decreased by an average of 55.9 % while the decline in total bilirubin for the hyperbilirubinemic specimen was smaller 32.4% when exposed to light but in contrary, direct bilirubin decreased by slightly more amounts, 47.4% and 49.7% respectively, for the normobilirubinemic / moderately hyperbilirubinemic specimen and hyperbilirubinemic specimen when exposed to light. Likewise, analysis of changes in measured total and direct bilirubin over a period of five hour kept in the

dark showed change in total bilirubin of only about 6.9% and 11.4% in hyperbilirubinemic and normo bilirubinemic / moderately hyperbilirubinemic specimen respectively but in contrary slightly increased change in direct bilirubin of about 17.1% and 23.9% in hyperbilirubinemic and normobilirubinemic / moderately hyperbilirubinemic specimen respectively.

A study by Rehak et al.(9) agrees with our study in term that there is increased rate of photodegradation of bilirubin in normo bilirubinemic / moderately hyperbilirubinemic serum specimens than hyperbilirubinemic serum specimens. In a study by Rehak et al(9), photolysis of serum bilirubin exposed to 9 hour laboratory light revealed that for the normobilirubinemic specimens, measured values of total bilirubin decreased by an average of 59%.

The decline in total bilirubin for moderately hyperbilirubinemic specimens was smaller—41%. Direct bilirubin decreased by slightly smaller amounts 38% and 31%, respectively, for the normobilirubinemic and moderately hyperbilirubinemic specimens. In the similar type of study by Landt et al(10), total bilirubin significantly decreased in serum stored for 24 hour in the clear samplette (average decrease 16%) and the decrease was greatest when starting bilirubin concentrations were low. However, compared to our study, the Amber samplette values were essentially unchanged (average decrease 2%) after 24-h storage, and were similar to values for foil-covered containers. Further, the study by McDonagh et al(11), found that samples being collected for direct bilirubin analysis should be "promptly cooled to 4°C" and minimally kept at room temperature to prevent their degradation from artificially inflating the unconjugated bilirubin concentration.

An applied aspect of this finding is that accurate measurement of bilirubin in specimens with normal or low bilirubin concentrations requires even greater care in the protection of specimens from light than for specimens with high bilirubin concentrations since, normobilirubinemic serum specimens showed greater percentage of photodegradation of bilirubin than that of hyperbilirubinemic serum specimen. Therefore, exposure of specimens to normal laboratory light should be limited to short interval of time as possible in order to limit significant losses of bilirubin. Hence, protection of specimen tubes from light during processing and storage should be indicated, where amber rather than clear samplettes should be used for specimen collection.

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### Conflict of Interest

Authors declare no conflict of interest.

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