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RESEARCH ARTICLE

Evaluation of Bilirubin Degradation in Plasma Specimen Exposed to Room Light at Room Temperature

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ABSTRACT

Background: Delay in handling blood samples lead to inaccurate results because they may be exposed to light causing analyte degradation. The photodegradation of bilirubin will result in clinically significant changes in concentrations of bilirubin, this change might affect the clinical circumstances for which the test was requested. This study was done to determine the effect of light on plasma bilirubin concentration.

Methods: Blood samples of normobilirubinemia and hyperbilirubinemia were drawn from neonates and adults, tested for total and direct bilirubin concentration at different time intervals after exposure to laboratory light using DMSO photometric method.

Results: the study showed that, total, direct and indirect bilirubin levels are decreased after 2-hours and then the levels were markedly decreased after 4 and 6-houres. In adults with hyperbilirubinemia, the maximum decrease after 6-hours delay was 5.8mg/dl, 2.8mg/dl and 2.33mg/dl in total, direct and indirect bilirubin respectively. In adults with normal bilirubin levels the maximum decrease after 6 hours delay was 0.39mg/dl, 0.14mg/dl and 0.24 mg/dl in total, direct and indirect bilirubin respectively. In neonates with normal bilirubin, the maximum decrease after 6- hours was 1.8mg/dl, 0.53mg/dl and 1.3 mg/dl in total, direct and indirect bilirubin respectively. In neonates with hyperbilirubinemia, the maximum decrease in total bilirubin was 3.3mg/dl after 6-hours, in indirect bilirubin the maximum decrease was 2.4mg/dl while in direct bilirubin the maximum decrease was 0.79mg/dl after 6-hours delay. There was significant difference observed in mean results of all samples except for adult indirect hyperbilirubinemic samples after 2-hours exposure (P = 0.308).

Conclusion: This study confirms that exposure of blood samples to laboratory lighting lead to statistically significant lowering of plasma bilirubin level.

Keywords: Photolysis, Bilirubin, Room Light, Conjugated bilirubin.

1. INTRODUCTION:

Bilirubin is the catabolic product of heme metabolism, which is formed by the breakdown of heme present in hemoglobin, myoglobin, cytochromes, catalase, peroxidase, and tryptophan pyrrolase [(1)].

Bilirubin is a substance absorbing light within the visible spectrum and it is well recognized to undergo both isomerization and oxidation in serum exposed to visible light, resulting in decreased measured bilirubin values [(2)]. In spite of that the sensitivity of bilirubin to light is a problem inside the laboratories, but this is the rationale for exposing neonates who have clinical significant jaundice to light in order to reduce plasma unconjugated bilirubin concentrations "phototherapy" [(3)].

Accurate measurement of serum bilirubin level is important, especially in the treatment of neonatal jaundice since bilirubin level often dictates decision to

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initiate or discontinue treatment [4].

Sometimes, inadvertent delay in measurement may be possible either due to heavy work load or to confusion arising from low work load, leaving the samples exposed to light. Also delay in samples transportation to laboratories from wards and nursery units is a very common problem in Sudan, leading to erroneous results which are not matching the clinical marks of patients [5-7]. So, this study aimed to find out the actual changes in bilirubin results in different time intervals and to look for correction formula as to solve these problems instead of samples rejecting.

2. MATERIALS AND METHODS

The study was conducted at Omdurman Maternity Hospital at special care nursery and Ibn-Sena Hospital from May 2013 till June 2013. The study was approved by Alneelain University Ethics Committee and all subjects gave informed consent (Based on Helsinki Declaration). Signed consents were obtained from study groups, jaundiced adult patients and neonate's parents.

The samples divided as: Fifty normobilirubinemic samples: (25 samples from neonates and 25 samples from adults). Fifty hyperbilirubinemic samples: (25 samples from neonates and 25 samples from adults).

Samples were collected by syringe (5 ml) using venipuncturing from antecubital vein or other visible vein in the forearm for adults and femoral vein for neonates. In a case of obese persons dorsal vein is a one of choice. Two and half ml of whole blood collected in heparin containers without samples haemolysis. The containers were enclosed with aluminum foil and transported immediately to the laboratory. The plasma was separated from whole blood by centrifugation and transferred into foil enclosed plain containers.

Zero time bilirubin concentrations were measured immediately and then the foil was removed leaving the samples on laboratory bench exposed to light with an intensity of 908.65 Lux (84.1 Foot Candles) for the specific time intervals (2, 4, and 6 hours). Adult samples were collected from Ibn-Sena hospital and transported in ice bag to Omdurman Maternity hospital laboratories immediately after separation and protection from light.

Bilirubin was determined by DMSO colorimetric method instead of the DIAZO method (gold method) because Results of bilirubin obtained at zero time were used as the baseline value against which the bilirubin concentrations at future time points were compared. The data were analyzed using a computerized (Stata) program version 10 for statistical analysis. many laboratories in Sudan use it as a routine method. So, results will be practicable for all [8]. Differences in total bilirubin, direct bilirubin and indirect bilirubin levels between the 0-hour and 2,4 and 6-hours delay intervals were determined by a paired t-test.

3. RESULTS

This study included 100 blood samples which were analyzed for total plasma bilirubin, direct plasma bilirubin and indirect plasma bilirubin using spectrophotometer at different time intervals: zero time, 2-hours, 4-hours and 6-hours from sample collection. The analysis was done under certain condition (Illuminance = 908.65 Lux).

Reading of control materials (both normal and pathological) were within the accepted levels. The mean, standard deviation, changes in percentage and the rate of changes per hour are presented in tables (1 – 9).

There was a significant difference in the total, direct and indirect bilirubin measurements of hyperbilirubinemic neonatal samples observed after the first 2-hours exposed to laboratory light in comparison with 0-hour samples (P. value < 0.05), the maximum decrease after 6-hours light exposure was 24% , 40% and 21.3% respectively, tables (1, 2,3)

Time interval	Mean (SD)	Mean difference (95%)	P.value	Change (%)	Rate of change (%/h)
0 hour	13.78 (2.72)	-	-	-	
2 hour	11.99 (2.96)	1.78 (0.92 to 2.65)	0.0003	-13.1%	-6.5%
4 hour	11.11(2.69)	2.66 (1.74 to 3.59)	0.0000	-18.9%	-4.7%
6 hour	10.41(2.87)	3.36 (2.35 to 4.38)	0.0000	-24%	-4%

Table 1: Total bilirubin levels of neonatal hyperbilirubinemic samples in different delay interval (*n =25*):

Time interval	Mean (SD)	Mean difference (95%)	P.value	Change (%)	Rate of change (%/h)
0 hour	2.01 (2.11)	-	-	-	
2 hour	1.65 (2.03)	0.36 (0.15 to 0.57)	0.0015	-20%	-10%
4 hour	1.33 (1.81)	0.67 (0.15 to 0.57)	0.0008	-35%	-8.7%
6 hour	1.21(1.78)	0.79 (0.32 to 1.26)	0.0018	-40%	-6.6%

Table 2: Direct bilirubin levels of neonatal hyperbilirubinemic samples in different delay intervals (*n =25*):

Time interval	Mean (SD)	Mean difference (95%)	P.value[#]	Change (%)	Rate of change (%/h)
0 hour	11.74 (2.96)	-	-	-	
2 hour	10.31 (3.29)	1.43 (0.32 to 1.26)	0.0033	-11.9%	-5.9%
4 hour	9.73 2.71)	2.00 (0.32 to 1.26)	0.0001	-17%	-4.2%
6 hour	9.26 (2.95)	2.48 (0.32 to 1.26)	0.0000	-21.3%	-3.5%

#Comparison with zero hour.

Table 3: Indirect bilirubin levels of neonatal hyperbilirubinemic samples in different delay intervals (*n =25*):

There was also a significant difference in the total, direct and indirect bilirubin measurements of normobilirubinemic neonatal samples observed after the first 2-hours exposed to laboratory light in comparison with 0-hour samples (P value < 0.05), the maximum decrease after 6-hours light exposure was 27.9% , 45% and 22.8% respectively, tables (4, 5, 6).

Time interval	Mean (SD)	Mean difference (95%)	P.value#	Change (%)	Rate of change (%/h)
0 hour	6.84 (3.09)	-	-	-	
2 hour	6.18 (2.89)	0.66 (0.32 to 1.26)	0.000	-10.2%	-5.1%
4 hour	5.66 (2.82)	1.17 (0.32 to 1.26)	0.000	-17.6%	-4.4%
6 hour	4.97 (2.64)	1.86 (1.46 to 2.27)	0.000	-27.9%	-4.6%

Table 4: Total bilirubin levels of neonatal normobilirubinemic samples in different delay intervals (*n =25*):

Time interval	Mean (SD)	Mean difference (95%)	P.value#	Change (%)	Rate of change (% / h)
0 hour	1.09 (0.65)	-	-	-	
2 hour	0.75 (0.49)	0.33 (1.46 to 2.27)	0.0005	-25%	-12.5%
4 hour	0.65 (0.48)	0.43 (1.46 to 2.27)	0.0001	-35%	-8.7%
6 hour	0.55 (0.47)	0.53 (1.46 to 2.27)	0.000	-45%	-7.5%

Table 5: Direct bilirubin levels of neonatal normobilirubinemic samples in different delay intervals (*n =25*):

Time interval	Mean (SD)	Mean difference (95%)	P.value#	Change (%)	Rate of change (% / h)
0 hour	5.73 (2.89)	-	-	-	
2 hour	5.41 (2.84)	0.32 (0.11 to 0.53)	0.0044	-5.2%	-2.6%
4 hour	5.12 (2.61)	0.60 (0.11 to 0.53)	0.0066	-10.5%	-2.6%
6 hour	4.41 (2.59)	1.32 (1.04 to 1.60)	0.0000	-22.8%	-3.8%

#Comparison with zero hour

Table 6: Indirect bilirubin levels of neonatal normobilirubinemic samples in different delay intervals (*n =25*):

For adult's hyperbilirubinemic samples, the photolysis of total and direct bilirubin fractions significantly takes place after the 2-hours delay in comparison with 0-hour samples (p value < 0.05), the maximum decrease after 6-hours light exposure was 38.1% and 32.5% respectively, tables (7 and 8).

Time interval	Mean (SD)	Mean difference (95%)	P.value#	Change (%)	Rate of change (%/h)
0 hour	15.26 (11.84)	-	-	-	
2 hour	13.34 (10.08)	1.92 (0.94 to 2.90)	0.0004	-12.5%	-6.2%
4 hour	10.27 (6.69)	4.99 (1.99 to 7.99)	0.0022	-32.8%	-8.2%
6 hour	9.42 (6.36)	5.83 (2.64 to 9.03)	0.0009	-38.1%	-6.3%

Table 7: Total bilirubin levels of adult's hyperbilirubinemic samples in different delay intervals (*n =25*):

Time interval	Mean (SD)	Mean difference (95%)	P.value#	Change (%)	Rate of change (%/h)
0 hour	8.95 (6.84)	-	-	-	
2 hour	8.24 (6.26)	0.71(0.40 to 1.01)	.0001	-7.8%	-3.9%
4 hour	6.57 (4.25)	2.38 (0.78 to 3.98)	.0053	-26.9%	-6.7%
6 hour	6.09 (4.17)	2.86 (1.20 to 4.52)	.0016	-32.5%	-5.4%

Table 8: Direct bilirubin levels of adult's hyperbilirubinemic samples in different delay intervals (*n =25*):

The indirect bilirubin fraction didn't show a significant decrease after the first 2-hours delay (P value = 0.308), the percentage of decrease was 8.9%. The significant decrease observed after 4-hours light exposure (P value = 0.0053) in which the percentage of decrease was 35.7% versus the initial sample and the maximum decrease after 6-hours light exposure was 41%, table (9).

Time interval	Mean (SD)	Mean difference (95%)	P.value#	Change (%)	Rate of change (%/h)
0 hour	5.66 (4.88)	-	-	-	
2 hour	5.10 (4.00)	0.55 (-0.54 to 1.65)	.308 *	-8.9%	-4.4%
4 hour	3.69 (2.84)	1.96 (0.64 to 3.29)	.0053	-35.7%	-8.9%
6 hour	3.33 (2.41)	2.33 (0.92 to 3.73)	.0022	-41%	-6.8%

#Comparison with zero hour.

*Insignificant difference observed (P > 0.05).

Table 9: Indirect bilirubin levels of adult's hyperbilirubinemic samples in different delay intervals (*n =25*):

For adult's normobilirubinemic samples, the photolysis of total, direct and indirect bilirubin fractions significantly takes place after the 2-hours delay in comparison with 0-hour samples (P value < 0.05), the maximum decrease after 6-hours light exposure was 57.1%, 68.1% and 53.1% respectively, tables (10, 11, 12).

Time interval	Mean (SD)	Mean difference (95%)	P.value#	Change (%)	Rate of change (% / h)
0 hour	0.70 (0.33)	-	-	-	
2 hour	0.53 (0.28)	0.17(0.10 to 0.23)	0.000	-24.2%	-12.1%
4 hour	0.44 (0.27)	0.25 (0.19 to 0.31)	0.000	-37.1%	-9.2%
6 hour	0.30 (0.16)	0.39 (0.26 to 0.53)	0.000	-57.1%	-9.5%

Table 10: Total bilirubin levels of adult's normobilirubinemic samples in different delay intervals (*n =25*):

Time interval	Mean (SD)	Mean difference (95%)	P.value[#]	Change (%)	Rate of change (% / h)
0 hour	0.22 (0.15)	-	-	-	
2 hour	0.15 (0.12)	0.07 (0.04 to 0.10)	.000	-31.8%	-15.9%
4 hour	0.10 (0.11)	0.11 (0.08 to 0.15)	.000	-54.5%	-13.6%
6 hour	0.07 (0.10)	0.14 (0.10 to 0.19)	.000	-68.1%	-11.3%

Table 11: Direct bilirubin levels of adult's normobilirubinemic samples in different delay interval (*n =25*):

Time interval	Mean (SD)	Mean difference (95%)	P.value[#]	Change (%)	Rate of change (% / h)
0 hour	0.47 (0.28)	-	-	-	
2 hour	0.38 (0.27)	0.09 (0.03 to 0.14)	.0017	-19.1%	-9.5%
4 hour	0.33 (0.25)	0.13 (0.08 to 0.19)	.000	-29.7%	-7.4%
6 hour	0.22 (0.12)	0.24 (0.13 to 0.36)	.0002	-53.1%	-8.8%

#Comparison with zero hour.

Table 12: Indirect bilirubin levels of adult's normobilirubinemic samples in different delay intervals (*n =25*):

4. DISCUSSION

There are a little bit studies around the world regarding the effect of light on bilirubin concentration, in Sudan this study might be the first one concerning the problematic effects of the photolysis on bilirubin levels. Our study showed a statistically significant difference after the first 2-hours of light exposure in the all groups of study except for bilirubin levels of adult's hyperbilirubinemic samples.

The results of hyperbilirubinemic samples of adults and neonates showed a statistically significant decrease in total, direct and indirect plasma bilirubin levels except the indirect fraction of adults- at 2, 4 and 6-hours compared with the initial results (P <0.05), while the indirect fraction of adults showed significant decrease at 4 and 6-hours, these findings agree with the study done by Rehak N *et al*. 2008 [(1)].

The results of normobilirubinemic samples of adults and neonates showed a statistically significant decrease in total, direct and indirect plasma bilirubin levels at 2,4 and 6-hours compared with the initial results (P <0.05), this is in accordance with Rehak *et al* findings [(1)].

In our study, the results of indirect bilirubin fraction of adult's hyperbilirubinemic samples contradicts with Rehak N *et al* results, in our study there were insignificant decrease observed at 2-hours (P >0.05) on indirect bilirubin fraction of adult's hyperbilirubinemic samples. This finding disagrees with Rehak N *et al* results. Also Leung C *et al* findings did not agree with ours for neonatal hyperbilirubinemic samples, they observed the first significant decrease after 6-hours being exposed to light [(9)], this disagreement may be due to the difference in sample type been used (capillary blood samples vs. plasma samples) as red cells might provide some limited protection of bilirubin in whole blood specimens compared to the plasma samples which might be more vulnerable to photolysis.

Categorization of plasma bilirubin concentrations to be able to apply a correction formula for each category was not possible in our study due to erratic differences in bilirubin results when exposed to light.

This study is limited by tow facts, firstly, we did not examine the photolysis of bilirubin in specimens during collection and processing, secondly, we did not study the effect of temperature on samples, however it had been recommended that samples being collected for direct bilirubin analysis should be promptly cooled to 4^0C and minimally kept at room temperature to prevent their degradation from artificially inflating the unconjugated bilirubin concentration [(10)].

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6. CONCLUSION

In conclusion, exposure of blood samples to room lighting can lead to statistically significant lowering of plasma bilirubin level. So it is indicated to measure plasma bilirubin concentration immediately after sample collection, otherwise it should be kept in a dark place.

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