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Using Buffer Saline in High Dilution to Measure the Concentration of Conjugated and Total Bilirubin in Newborn

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ألخلاصه:

في هذه الدراسة تم حساب البلروبين المقترن و الكلى فى خمسين طفل حديثى الولاده باستخدام كميه ضئيله من الدم 10 , مايكرولتر ,حيث تعتبر هذه الطريقه مهمه جداً بالنسبه للاطفال حديثى الولادة والمصابون بمرض اليرقان وذلك لصعوبة سحب كميه كبيره من الدم ,وبالتالي تجنب تعرض الطفل لمخاطر سحب الدم من الوريد العنقى.

تعتمد هذه الطريقه بشكل أساسي على إستخدام محلول ملحي منظم لتخفيف الكميه الضئيله من دم الطفل حديث الولاده لغرض قياس تركيز البلروبين الكلي والمقترن طيفياً باستخدام طريقة Van der Bergh حيث لوحظ بان محلول الملح المنظم ليس له أي تأثير سلبي على الدم.

Abstract:

In this work conjugated and total bilirubin in infants were measured using microliter of blood. Such measurments are quite valuable in jaundice of newborn when very small volumes of serum can be used (about 10µl). This method relys on using buffer saline to dilute small amount of infant's blood in order to measure the concentration of conjugated and total bilirubin. Such method has the advantage of preventing exposing the newborns the risks of blood aspiration.

Introduction:

Bilirubin is a wasteproduct derived fromthe breakdown ofhaemoglobine. It hasa chemical formula $C_{33}H_{36}O_6N_4$, with four pyrrole rings. Bilirubin circulates in low concentration in the plasma mainly attached to albumin and referred to as unconjugated bilirubin. It is not water soluble and can enter the liver cells by active carrier-mediated mechanism and then undergoes conjugation with two molecules of glucuronic acid by ester formation within the smooth endoplasmic reticulum forming diglucuronides. Once conjugated the bilirubin is water soluble, which referred to as direct bilirubin.

Conjugated bilirubin is water soluble but dissolves poorly in chloroform. It is trasported to the biliary capillary where it passes into the intestine through the biliary ducts. Gut bacteria then convert the bilirubin to urobilinogen which is colourless. Most urobilinogen becomes oxidised to the brown pigment urobilin within the gut and is excreted in the faeces. A small amount of urobilinogen is absorbed from the gut into portal blood. However, hepatic uptake of this is not complete with the result that small amount of urobilinogen reaches the urine. In health, plasma bilirubin is almost entirely unconjugated and because of its protein binding, filtaration in the glomerulus does not occur. Hence bilirubin is not found in urine. Bilirubinuria is always abnormal and is due to an increase in the amount of conjugated bilirubin in the plasma.

jaundice is a yellowish discolouration of tissues due to deposition of bilirubin. This can be detected clinically when plasma bilirubin is greater than 2.9 mg/dL. Jaundice may be classified as prehepatic, hepatic and post hepatic.

Patients with haematological conditions causing haemolysis or ineffective erythropoiesis, for example sickle cell disease and thalassaemia, are often slightly jaundiced. Gilbert's disease is a common condition characterised by mild, fluctuating jaundice due to increased unconjugated bilirubin in plasma. It is usually asymptomatic and is often revealed by a blood test taken for an intercurrent illness.

As many as half of all normal babies develop physiological jaundice after 48 houres of age which is essentially due to immaturity of the hepatic conjugating system. The excess bilirubin is unconjugated and if its cosncentration risres very high it can cross the blood-brain barrier and deposited in the brain causing a specific form of brain damage (kernicterus). If neonatal jaundice persists from more than ten days further inveestigationas should be do done, particularly determination of wether the hyperbilirubinaemia is conjugated or unconjugated.

Any how, jaundice and its underlying hyperbilirubinemia are considered pathologic if their time of appearance, duration, or pattern of serially determined serum bilirubin concentrations varies significantly from that of physiologic jaundice; or if the course is compatible with physiologic jaundice but other reasons exist to suspect that the infant is at special risk from the neurotoxicity of unconjugated bilirubin. It may not be possible to determine precisely etiology for an abnormal elevation of unconjugated bilirubin. Many of these infants have associated risk factors such as Oriental race, prematurity, breast-feeding, or weight loss; hence the terms exaggerated physiologic jaundice and hyperbilirubinemia of the newborn are used for those infants whose primary problem is probably a deficiency or inactivity of bilirubin glucurony -transferase rather than an excessive load of bilirubin for excretion.

Normal value of bilirubin in newborns is probably 1.0mg/100ml (17µml/l) or more. In general, normal serum does not give a direct reaction suggesting that conjugated bilirubin is present in very low concentration, up to 0.3mg/100ml. In jaundice the total bilirubin concentration increases and the conjugated bilirubin may be present.

Solutions of bilirubin do not show a characteristic spectrum, therefore, reactions with diazonium salt is required to form azobilirubin in order to measure serum bilirubin optically. The azobilirubin is red-purple in acid and intense blue-purple in alkaline solution.

Principle and Procedure:

It consist of taking 0.01 ml of blood and diluting it with buffer saline to 1 ml. Then 0.2 ml serum of the blood was diluted with 3.8 ml distil water and 5 ml methyl alcohol, an amount insufficient to precipitate proteins and sufficient to ensure that all the bilirubin reacts with diazo reagent, was used. The bilirubin that measured was the water soluble conjugated bilirubin, direct bilirubin and the total bilirubin.

Solutions to measure direct and total bilirubin were prepared following the Van der Bergh reaction which is summarized in the table below and the diazo reagent was freshly prepared before use by adding 0.3ml solution B to 10ml solution A.

Solution A was prepared by dissolving 1g sulphanilic acid in 15ml concentrated hydrochloric acid and make up to one liter with distil water.

Solution B was prepared by dissolving 0.5g sodium nitrate in distils water and make up to 100ml.

Table 1

Standard	Blank	Direct Bilirubin	Total Bilirubin	Solution
3.8	3.8	3.8	3.8	Distil Water
-	0.2	0.2	0.2	Serum
0.2	-	-	-	Standarrd
-	1	-	-	Blank
-	-	1	1	Diazo
1	-	-	5	Methyl Alcohol
-	5	5	-	Distil water

Mix all solutions well and leave them for 20 minutes in a dark place and then read at 540 nm.

Calculation:
Indirect Bilirubin = Total Bilirubin – Direct Bilirubin

$$TSB = \frac{T-B}{S-B} \times C$$ = **multiply the result by 10**

T: is the reading of the total bilirubin solution.
B: is the reading of the blank solution.
S: is the reading of the standard solution.
C: is the concentration of the standard bilirubin.

Result

This study talked about an important scientific topic which is the measurement of bilirubin through a new method using very little amount of blood only(0.01ml)
according to Van der berg procedure to measure the direct and total serum bilirubin in new born infants opticallylook table (1).

We took fifty samples of blood (0.01 ml of blood for each) from fifty new borns infants and then dilute each one using buffer saline from 0.01ml to 1 ml with out any hemolysis.

The final result was multiplied by ten to reach the exact reading because we dilute the samples from 0.01 ml to 1 ml.

All the reading with their control are listed in table (2)with no significant
differences in the readings.

Statistically, to compare between the usual Van der berg method and our method look table (3)

(Buffer saline in high dilution to measure the concentration of conjugated and total bilirubin in new born) we used T-test through the well- known statistical computer program (SPSS) and after entering the data of both methods to know if there is any significance difference between them ,we look for the calculation of any normative
error and after we press on the (OK) key T- value (0.824)appeared ,so this mean there is no significance difference between the two mean values in comparison withcharted T-value (48,0.01)=2.75.the stastical estimation and values was listed in table 3 , 4 and 5 .

Discussion

Jaundice in observed during the first week of life in approximately 60% of term infants and 80% of preterm infants.

The significance of hyperbilirubinemia lies in the high incidence of kernicterus associated with serum bilirubin levels over 20 mg\dl (342 mmol\L) in term infants and lower level in preterm infants, the jaundice intensity bears no clinically dependable relation to the degree of hyperbiliurbinemia therefore, bilirubin determinations should be done on all jaundiced infants and better to done the estimation of bilirubin level by a method not hazardous to the newborn and carrying an advantage of preventing exposing the newborns to the risks of blood aspiration.

In this study, fifty newborns were studied, their ages between 2-7 days by taking very small amount of blood about (0.01 ml) of blood that can be used to measure the concentration conjugated and total serum bilirubin by using (Van der berg method) this amount of blood nearly one drop(10 micro liter) which adequate to measure the concentration of bilirubin while other methods needs more amounts of blood and needs venous puncture and some time from danger areas like neck veins may carry more hazard to the newborns babies, so this method looks superior to the usual method.

In this method we use buffer saline dilute the small amount of blood

which taking from those newborns in order to measure the concentration of conjugated and total bilirubin optically.

The result of paired sample test show no any difference with the control procedure.

In this study the calculation agree with the procedure of Van der berg method but our end result must multiplied by (10) to reach the final result because of the dilution by buffer saline from (o.o1ml) of blood to 1 ml.

To get the level of indirect(unconjugated) bilirubin we subtracted the direct bilirubin from the total bilirubin.

Table(2) (TSB: total serum bilirubin, DSB: direct serum bilirubin)

Our method		Traditional Method			Our method		Traditional Method		
DSB	TSB	DSB	TSB		DSB	TSB	DSB	TSB	
0.4	8.0	0.4	8.0	26	0.4	8.3	0.5	8.3	1
0.5	10.	0.5	10.	27	0.9	13.	0.9	13.	2
1.0	8	1.0	8	28	0.4	8	0.4	9	3
1.0	16.	1.0	16.	29	0.6	7.0	0.6	7.0	4
0.6	1	0.6	1	30	0.5	12.	0.4	12.	5
0.6	14.	0.6	14.	31	0.7	5	0.7	5	6
0.6	6	0.6	7	32	0.4	8.8	0.5	8.8	7
0.4	9.9	0.5	9.9	33	1.1	10.	1.1	10.	8
0.8	10.	0.8	10.	34	0.3	5	0.3	5	9
0.7	0	0.7	0	35	0.3	10.	0.3	10.	10
0.5	10.	0.5	10.	36	0.5	0	0.5	0	11
0.5	5	0.5	5	37	0.5	17.	0.4	17.	12
0.4	11.	0.4	11.	38	1.1	1	1.1	2	13
0.4	0	0.4	0	39	0.3	7.5	0.3	7.5	14
0.5	14.	0.6	14.	40	0.7	7.8	0.7	7.9	15
0.6	1	0.6	0	41	0.5	10.	0.5	10.	16
0.4	13.	0.4	13.	42	0.6	8	0.6	8	17
0.4	5	0.4	5	43	1.2	9.3	1.2	9.3	18
0.5	13.	0.5	13.	44	0.4	18.	0.4	18.	19
0.5	3	0.5	3	45	0.5	0	0.3	0	20
0.4	8.0	0.4	8.0	46	0.4	7.0	0.4	7.0	21
0.4	7.5	0.4	7.5	47	0.7	11.	0.7	11.	22
0.9	9.0	0.9	9.0	48	0.7	7	0.6	5	23
0.6	9.6	0.6	9.6	49	0.6	10.	0.6	10.	24
0.5	12.	0.5	12.	50	0.9	9.	0.9	9	25

Table (3)

	a	B
1	8.3	8.3
2	13.9	13.8
3	7.0	7.0
4	12.5	12.5
5	8.8	8.8
6	10.5	10.5
7	10.0	10.0
8	17.2	17.1
9	7.5	7.5
10	7.9	7.8
11	10.8	10.8
12	9.3	9.3
13	18.0	18.0
14	7.0	7.0
15	11.5	11.7
16	10.9	10.9
17	12.1	12.1
18	20.0	20.0
19	8.5	8.5
20	6.8	6.8
21	10.6	10.5
22	12.2	12.2
23	11.5	11.5
24	9.6	9.6
25	14.5	14.5
26	8.0	8.0
27	10.8	10.8
28	16.1	16.1
29	14.7	14.6
30	9.9	9.9
31	10.0	10.0
32	10.5	10.5
33	11.0	11.0

34	14.0	14.1
35	13.5	13.5
36	13.3	13.3
37	8.0	8.0
38	7.5	7.5
39	9.0	9.0
40	9.6	9.6
41	12.1	12.1
42	8.7	8.6
43	10.3	10.4
44	7.5	7.5
45	7.8	7.8
46	7.4	7.4
47	6.9	6.9
48	13.6	13.5
49	7.9	7.9
50	8.8	8.8

T-Test

Table (4) paired Samples Statistics

		Mean	N	Std.Deviation	Std.Error Mean
Pair 1	A	.584	50	.2244	.0317
	B	.586	50	.2232	.0316

Paired Samples Correlations

	N	Correlation	Sig
Pair 1 A g B	50	.978	.000

Paired Samples Test

	Paired Differences					
				95%Confidence interval Of the Difference		
				Lower	Upper	
Pair 1 A - B	-0020	.0473	.0067	-.0155	.0115	-.299

Paired Samples Test

	df	Sig. (2-tailed)
Pair 1 A - B	49	.766

T-Test

Table (5) Paired Samples Statistics

		Mean	N	Std.Deviation	Std. Error Mean
Pair 1	a	10.676	50	3.0801	.4356
	b	10.670	50	3.0757	.4350

Paired Samples Correlations

	N	Correlation	Sig
Pair 1 a g b	50	1.000	.000

Paired Samples Test

	Paired Differences					
				95% Confidence interval 0f the Difference		
				Lower	Upper	
Pair 1 a - b	.0060	.0512	.0072	-.0085	.0205	.829

Paired Samples Test

	df	Sig. (2-tailed)
Pair 1 a - b	49	.411

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