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COMPARABLY STUDY OF DELTA BILIRUBIN IN Calcutta Medical Research Institute & MIDNAPUR MEDICAL COLLEGE AND HOSPITAL PATIENTS

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ABSTRACT-- Determination of serum bilirubin is one of the important liver function tests. Serum bilirubin has been differentially determined by diazo reagent as total bilirubin and direct bilirubin. Recently which involves a covalent bond to albumin has drawn attention because of clinical significance. There is a method to measure delta bilirubin levels in patients with acute liver disease to examine its clinical significance. For the measurement of delta bilirubin at first measure the total bilirubin, direct bilirubin from Vitros 250 Dry chemistry Method. So delta bilirubin correlated with total bilirubin and direct bilirubin. Hence we can conclude from this case study that delta bilirubin might have a significant role in the diagnosis of these three types of jaundice. Although it cannot be measured but can be calculated by Dry chemistry Auto analyzer, probably it may help in the differential diagnosis of different types of jaundice since its found to be highest delta bilirubin Percentage in Obstructive Jaundice.

Keywords- bilirubin, diazo reagent, delta bilirubin, Vitros 250, Obstructive Jaundice

1-INTRODUCTION

1.1-**BILIRUBIN**

Bilirubin is the breakdown product of the haem moiety of haemoglobin and other haemoproteins. A red blood cell has a lifetime of approximately 120 days in adults and 70 days in infants. The active component in a red blood cell is hemoglobin. Hemoglobin is a porphyrin ring with an iron atom in the center. It is able to bind and transport dioxygen to tissues and carbon dioxide to the lungs. When the red blood cell is degraded, iron is recovered but the heme porphyrin ring is degraded into the yellow-orange pigment of bilirubin. Because of internal hydrogen bonding, bilirubin is water-insoluble and requires enzyme-mediated glucuronidation in the liver for belier exc

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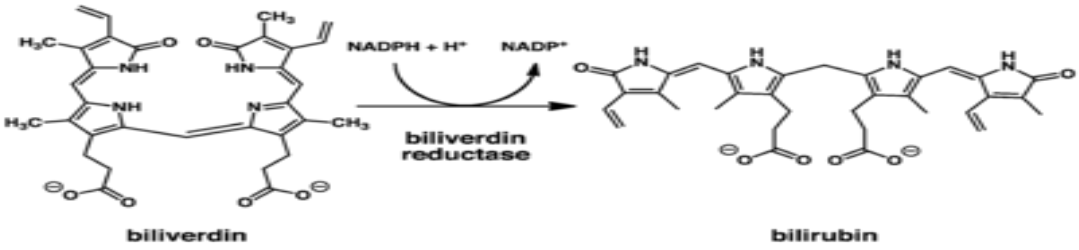

2-BILIRUBIN METABOLISM

2.1-BILIRUBIN SYNTHESIS

Old and damaged (senescent) erythrocytes remove from the circulation & Degradation begins inside macrophages of Reticuloendothelial (RE) system like Spleen . Heme is converted to biliverdin by the enzyme **heme oxygenase** (HOXG). NADPH is used as the reducing agent, molecular oxygen enters the reaction, carbon monoxide (CO) is produced and the iron is released from the molecule as the ferric ion (Fe^{3+}). CO acts as a cellular messenger and functions in vasodilation.

HMOX1/2
heme ----------> biliverdin + Fe^{3+}
/ \
H^+ + NADPH NADP+
O_2 CO

(In addition, heme degradation appears to be an evolutionary conserved response to oxidative stress. Briefly, when cells are exposed to free radicals, there is a rapid induction of the expression of the stress responsive heme oxygenase-1 (Hmox1) isoenzyme that catabolizes heme (see below). The reason why cells must increase exponentially their capability to degrade heme in response to oxidative stress remains unclear but this appears to be part of a cyto protective response that avoids the deleterious effects of free heme.)

In the second reaction, biliverdin is converted to bilirubin by **biliverdin reductase** (BVR):

BVR
biliverdin ----------> bilirubin
/ \
H^+ + NADPH NADP+

After its formation within the phagocytic cells (heme Bili), bilirubin is released into circulation and is reversibly bound to albumin. This form of bilirubin is called **UNCONJUGATED** (or indirect) bilirubin and is

1. Water insoluble
2. Toxic to cells (especially brain tissue)

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Bilirubin is transported into the liver by facilitated diffusion bound to albumin where it is conjugated with glucuronic acid . Before entering the liver cells, bilirubin is dissociated from albumin . The reaction is catalyzed by the enzyme **UDP-glucuronide transferase** (UDPGUTF).

UDPGUTF

bilirubin + 2 UDP-glucuronate ----------> bilirubin diglucuronide

2 UMP + 2 Pi

(Pamplona et al.2007)

This form of bilirubin is called **CONJUGATED** (or direct) bilirubin and is

1. Water soluble
2. Relatively non-toxic to cells.

3-CLASSIFICATION

3.1-Conjugated (direct) bilirubin:

- Bilirubin coupled with diglucuronide to enhance solubility—soluble in water

3.2-Unconjugated (indirect) bilirubin:

- Bilirubin which is transported in the bloodstream bound to albumin—insoluble in water

3.3- **Δ-bilirubin (b_δ)**:

- This bilirubin molecule is covalently linked to albumin through an amide bond between one of its two propionic acid side chains and an ε-amino group of a lysine residue on albumin

4-DEFINITION OF JAUNDICE

Jaundice (also known as **icterus**;["Definition of Icterus". MedicineNet.com. 2011.] from the Greek word attributive adjective: **icteric**) is a yellowish pigmentation of the skin, the conjunctival membranes over the sclera (whites of the eyes), and other mucous membranes caused by hyperbilirubinemia (increased levels of bilirubin in the blood).[R; Dahl-Smith. *et.al.* (2012)] This hyperbilirubinemia subsequently causes increased levels of bilirubin in the fluid. Concentration of bilirubin in blood plasma is normally below 1.2 mg/dL (<25µmol/L). A concentration higher than 2.5 mg/dL (>50µmol/L) leads to jaundice.[Despopoulos A. *et.al.* (2009)] the term jaundice comes from the French word *jaune*, meaning yellow.

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5-TYPE OF JAUNDICE

5.1-PREHEPATIC OR HEMOLYTIC JAUNDICE

This condition is associated with increased hemolysis of erythrocytes (e.g. incompatible blood transfusion,malaria,sickle cell anemia).This results in the over production of bilirubin beyond the ability of the liver to conjugate and excrete the same. It should, however be noted that liver possesses a large capacity to conjugate about 3.0g of bilirubin per day against the normal bilirubin production of 0.3g/day

5.2-HEPATIC JAUNDIC

The type of jaundice is caused by the dysfunction of the liver due to the damaged to the parenchyma cells. This may be attributed to viral infection (viral hepatitis) poisons and toxins (chloroform, carbon tetrachloride, phosphorus etc) cirrhosis of liver cardiac failure etc.Among these, viral hepatitis is most common.

5.3- OBSTRUCTIVE JAUNDICE

This is due to an obstruction in the bile duct that prevents the passage of bile into intestine. The obstruction may be caused by gall stones, tumors etc.

Due to the blockage in bile duct, the conjugated bilirubin from the enters the circulation.

6-WHAT ARE LIVER FUNCTION TESTS?

A liver function test (LFT) is a blood test that gives an indication of whether the liver is functioning properly. The test is also very useful to see if there is active damage in the liver (hepatitis) or sluggish bile flow (cholestasis).
Liver function tests measure the amount of particular chemicals in the blood. This gives a gauge of possible damage to liver cells - damage that can be caused by many things including HCV. So a more correct term for a liver test would actually be a liver dysfunction test.

7-AIMS &OBJECTIVES

- To determined the delta bilirubin level in three type of jaundice patient.
- To determined the clinical significance of delta bilirubin in three type of jaundice.

8-MATERIALS AND METHODOLOGY

8.1-MATERIALS

- Alchohol ,Cotton,Needle,Syringe,Red tube,Vacutainer holder,Rubber gloves,Tube raker Needle disposal box,REMI Centrifuge,Vitros 250(BuBc, T BIL, D BBIL, ALP, AST, ALT, GGT, TOTAL PROTEIN, ALBUMIN, GLOBULIN SLIDES.)

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8.2-METHODS

- **Sample collection**

Blood sample were collected from patients at my project Institute Calcutta medical Research Institute&Midnapur college and hospital in a red tube.

- **Then the sample stay for 60 min.**
- **CENTRIFUGATION**

Then the sample centrifuged for 5-10 min at 2000 rpm

- **VITROS TEST**

Then the sample tubes enter into the sample tray of vitros 250. After that in control unit introduced the program which can be perform, in this case LFT was done. Wait for 5 min. Result was taken up which seen on the programming monitor.

9-RESULT

Comparing(CMRI&MIDNAPUR HOSPITAL) of percentage

A)FOR OBSTRUCTIVE=50 PATIENTS ,B)FOR HEPATIC= 50 PATIENTS, C)FOR HEMOLYTIC=50 PATIENTS ,D)FOR NORMAL=50 NORMAL PATIENT

%Means Value of Parameter(cmri)	Ostructive	Hepatic	Hemolytic	Normal
BC(mg/dl)	23.51%	15.72%	11.58%	6.82%
BU(mg/dl)	37.01%	49.25%	84.16%	72.82%
DELTA(mg/dl)	43.50%	35.05%	28.03%	20.29%

%Means Value of Parameter(midnapur)	NORMAL	Ostructive	Hepatic	Hemolytic
BC	4.82%	22.51%	15.60%	9.68%
BU	73.66%	36.68%	45.79%	82.44%
DELTA	21.00%	44.65%	35.00%	26.87%

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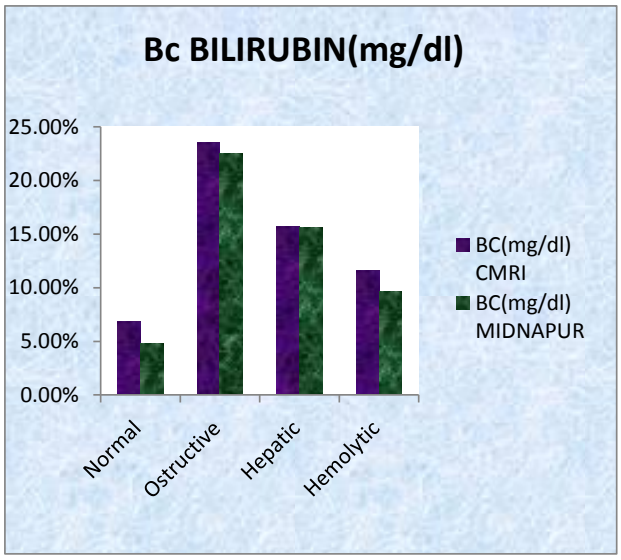
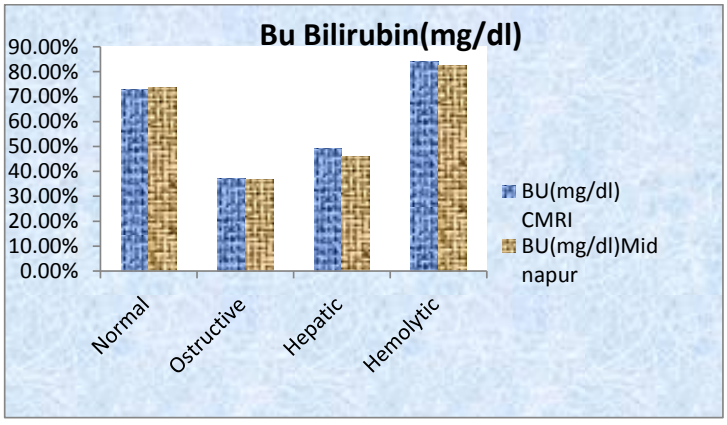
Percentage of conjugated bilirubin

- in Normal bilirubn (CMRI)is 6.82%i.e>(Midnapur) Normal bilirubn 4.82.
- in hemolytic jaundice(CMRI&Midnapur hospital) is 11.58%&9.68 i.e >normal,
- in obstructive(cmri& & Midnapur hospital is23.51%&22.45 %) hepatic jaundice(cmri& & Midnapur hospital is15.72%&15.60 %) is >normal.
- In obstructive is higher (23.51%&22.45 %) than hepatic (15.72%&15.60 %) hemolytic





Percentage of unconjugated bilirubin

- In Normal bilirubn (CMRI)is72.82% i.e>(Midnapur) Normal bilirubn 72.66%.
- In hemolytic jaundice(CMRI&Midnapur hospital) it is 84.16%&82.44 i.e>normal,
- in obstructive (CMRI-37.01%&Midnapur hospital-36.68%) & hepatic jaundice(CMRI-49.25%&Midnapur hospital-45.79%) is <normal.
- In obstructive is lesser(CMRI-37.01%&Midnapur hospital-36.68%) than hepatic(CMRI-49.25%&Midnapur hospital-45.79%) .

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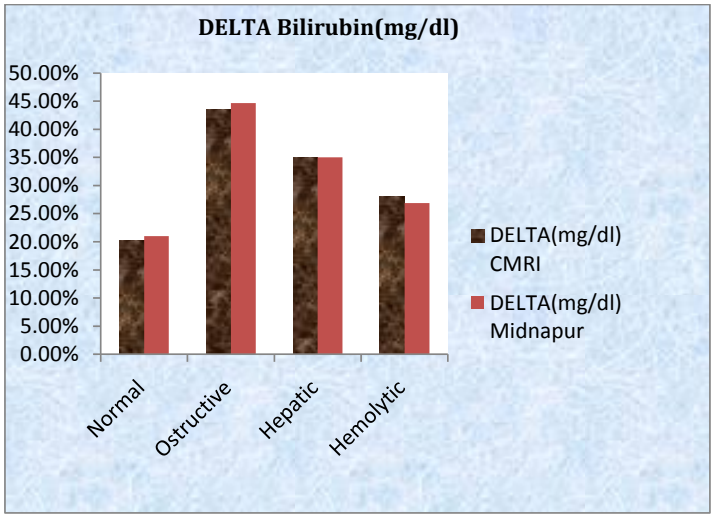

Percentage of delta bilirubin

- In Normal bilirubn (CMRI)is20.29% i.e<(Midnapur) Normal bilirubn 21.00%.
- In hemolytic jaundice(CMRI&Midnapur hospital) is 28.03%&26.87% i.e. >normal, and also
- In obstructive(CMRI-43.51&Midnapur hospital-44.65%) & hepatic jaundice(CMRI-35.05%&Midnapur hospital-35.00%) is >normal. In obstructive is higher (43.51%&44.65%) than hepatic (35.05%&35.00%) hemolytic

10-CONCLUSION:

Here we focused on the importance of measurement of Delta Billirubin in the various conditions of liver diseases. We got 20.29% delta bilirubin in normal patient, but in case of Obstructive jaundice(cmri) we got 43.50%&(Midinipurhospital)44.65%, in case of Hepatic Jaundice(cnri) it is 35.05%&(midini pur)35.00% and in Hemolytic Jaundice it is 28.03%&26.87%(cmri&midinipur) means in three cases we got higher percentage of delta bilirubin than normal cases. In Obstructive Jaundice the delta bilirubin Percentage is highest. Percentage of (cmri) obstructive jaundice-43.51%<(midnapur hospital)Hence we can conclude from this case study that delta bilirubin might have a significant role in the diagnosis of these three types of jaundice. Although it cannot be measured but can be calculated by Dry chemistry Auto analyzer,probably it may help in the differential diagnosis of different types of jaundice since its found to be highest delta bilirubin Percentage in Obstructive Jaundice.

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International Journal of Advanced Research in

Medical Science

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References

1. Ahlfors, C. E. (1981) Effect of serum dilution on apparent unbound bilirubin concentration as measured by the peroxidise method. *Clin. Chem.* **27,** 692–696.

2. Ahlfors, C. E., and DiBiasio-Erwin, D. (1986) Rate constants for dissociation of bilirubin from its binding sites in neonatal (cord) and adult sera. *J. Pediatr.* **108,** 295–298.

3. American Academy of Pediatrics. Provisional Committee for Quality Improvement and Subcommittee on Hyperbilirubinemia (1994) Practice parameter: Management of hyperbilirubinemia in the healthy term newborn. *Pediatrics* **94,** 558–565.
4. Brodersen, R. (1974) Competitive binding of bilirubin and drugs to human serum albumin studied by enzymatic oxidation. *J. Clin. Invest.* **54,** 1353–1364.

5. Brown, A. K., and Johnson, L. L. (1996) *in* The Yearbook of Neonatal and Perinatal Medicine (Fanaroff, A. A., and Klaus, M. H., Eds.), pp. xvii–xxviii, Mosby-Year Book, Chicago.
6. Chen TS, Chen PS (1984) *Understanding the Liver. A History*. Westport, CT: Greenwood Press, p. 99.
7. Chen, R. F. (1973) *in* Fluorescence Techniques in Cell Biology (Thaer, A. A., and Sernetz, M., Eds.), pp. 273–282, Springer- Verlag, New York.

8. Come SE, Shohet SB, Robinson SH (1974) Surface remodeling vs. whole-cell hemolysis of reticulocytes produced with erythroid stimulation or iron deficiency anemia. *Blood* 44, 817–830.

9. Diamond, I., and Schmid, R. (1966) Experimental bilirubin encephalopathy. The mode of entry of bilirubin-14C into the central nervous system. *J. Clin. Invest.* **45,** 678–689.

10. Faerch, T., and Jacobsen, J. (1975) Determination of association and dissociation rate constants for bilirubin–bovine serum albumin. *Arch. Biochem. Biophys.* **184,** 351–357.

11. Funato, M., Tamai, H., Shimada, S., and Nakamura, H. (1994) Vigintiphobia, unbound bilirubin, and auditory brainstem responses. *Pediatrics* **93,** 50–53.

12. Jacobsen, J., and Wennberg, R. P. (1974) Determination of unbound bilirubin in the serum of newborns. *Clin. Chem.* **20,** 783–789.

13. Knudsen, E., Pedersen, A. O., and Brodersen, R. (1986) Spectroscopic properties of bilirubin–human serum albumin complexes: A stoichiometric analysis. *Arch. Biochem. Biophys.* **244,** 273– 284.

ISRJournals and Publications

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International Journal of Advanced Research in

Medical Science

Volume: 1 Issue: 1 05-Mar-2015,ISSN_NO: xxxx-xxx

14. Kodak Ektachem test methodology: BuBc test methodology (1986) Health Sciences Marketing Division Publication MP2-40. Eastman Kodak Company, Rochester, New York.

15. Lauff, J. J., Kasper, M. E., Wu, T. W., and Ambrose, R. T. (1982) Isolation and preliminary characterization of a fraction of bilirubin in serum that is firmly bound to protein. *Clin. Chem.* **28,** 629–637.

16. London IM, West R, Shemin D *et al.* (1950) On the origin of bile pigment in normal man. *J Biol Chem* 184, 351–358.

17. Nakamura, H., Yonetani, M., Uetani, Y., Funato, M., and Lee, Y. (1992) Determination of serum unbound bilirubin for prediction of kernicterus in low birth weight infants. *Acta Paediatr. Jpn.* **34,** 642–647.

18. Odell, G. B. (1959) Studies in kernicterus. I. The protein binding of bilirubin. *J. Clin. Invest.* **38,** 823–833.

19. Polacek, K. (1966) Risk of kernicterus in newborn infants with a high level of conjugated bilirubin. *Acta Paediatr. Scand.* **55,**401–404.

20. Robinson SH (1977) Origins of the early-labeled peak. In: Berk PD, Berlin NI (eds) *Bile Pigments: Chemistry and Physiology*. Washington, DC: US Government Printing Office, pp. 175–188.

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