

Bilirubin is a Grander Biomarker for Diagnosis and Treatment of Various Liver Diseases

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RESEARCH ARTICLE

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Abstract: Bilirubin is the orange-yellow waste pigment derived from the body that breaks down old red blood cells that are extracted and biotransformed in the liver. It is a by-product of the breakdown of hemoglobin that is a tetrapyrrolic compound originating from heme catabolism and is responsible for jaundice. It has a number of physiological functions, both beneficial and harmful. It is screened and monitored for liver, and gall bladder disorders, such as jaundice, cirrhosis, etc. and determining a blocked bile duct that allows bile to pass from the liver to the small intestine. Total bilirubin and conjugated bilirubin tell about the conjugatory and excretory functions of hepatocytes. Bilirubin is highly soluble in all cell membranes and excess of it is very toxic, and therefore, its excretion in the bile is one of the very important functions of liver.

Keywords: Total bilirubin, jaundice, liver disease

1. Introduction

Bilirubin is a substance available in bile that is made when our body breaks down old red blood cells, and it is a normal process. It is a part of the bile that our liver makes to digest the food and is insoluble in water (Gazzin et al., 2016). Red blood cells contain hemoglobin that is broken down into heme and globin. The heme is converted to bilirubin, which is then carried by albumin in the blood to the liver. Bilirubin is then removed from the body through mononuclear phagocytes of the spleen, bone marrow, and liver (Zahir et al., 2015).

In 1847, German physician, anthropologist, pathologist, prehistorian, biologist, writer, editor, and politician Rudolf Virchow (1821-1902) and his colleagues isolated bilirubin crystals from hematomas and conjectured that it was derived from blood. In the 1930s, significant advances in bilirubin isolation and synthesis were described by German organic Chemist Hans Fisher (1881-1945) and his colleagues (Watson, 1977).

The term “biliverdin” was coined by Swedish chemist Jöns Jacob Berzelius (1779-1848) in 1840 and the term “bilirubin” was developed later (Lightner, 2013). There are two forms of bilirubin: i) indirect (unconjugated) bilirubin that is poorly soluble in water, and is bound to albumin, and ii) direct (conjugated) bilirubin that is soluble in water, and travels from the liver into the small intestine; and the sum of these two is total bilirubin (Gwaltney-Brant, 2016). A small amount of bilirubin is normally present in the blood. Healthy adults make 250 to 350 mg (about 4mg/kg body weight) of bilirubin each day from heme-containing proteins of erythroid and non-erythroid sources. Bilirubin is a potent antioxidant but can be toxic at high concentrations (Hall et al., 2024).

2. Literature Review

A literature review is a written summary of journal articles, books, and other documents that describe the past and present state of information on the field of research (Creswell, 2016). It demonstrates the knowledge and understanding of the theoretical and

research issues related to the research question. It establishes the importance of the research area and its current state of development (Jharotia & Singh, 2015). Igino Rigato and his coauthors have critically reviewed the reported relationships of plasma bilirubin levels to the severity and/or incidence of various common non-hepatic diseases (Rigato et al., 2005). Xia Wang and her coworkers have shown that bilirubin is degraded in the intestine by bacteria into urobilinogens that are partly excreted in the urine. Increased production, reduced up take and low glucuronidation capacity can increase plasma unconjugated bilirubin levels (Wang et al., 2006).

Birkan Birben and his coauthors have tried to evaluate the efficacy of bilirubin values in predicting acute and complicated appendicitis in patients with leukocyte values in the normal reference range. They have stressed that high bilirubin levels may be useful in predicting the diagnosis of acute appendicitis (Birben et al., 2021). Shen-Dar Chen and his coauthors have shown that the neonatal jaundice is a common phenomenon caused by a combination of increased heme catabolism and physiologic immaturity of the liver in bilirubin conjugation and excretion. They have noticed that the most readily available methods for testing serum total bilirubin in neonates are the capillary tube-directed optics color method and serum biochemistry. Because inconsistency between these two methods may cause confusion in clinical practice, this study was designed to quantify their differences (Chen et al., 2009). Yan Wang and his coworkers have studied to summarize the recent epidemiological characteristics of bilirubin encephalopathy and assess the role of the total bilirubin-albumin ratio in the bilirubin encephalopathy (Wang et al., 2020).

Constanza P. Soto Conti has wanted to review the processes whereby bilirubin causes cell damage and determine its beneficial antioxidant effects (Soto Conti, 2021). Xin Bai and her coworkers have investigated the connection between two kinds of bilirubin; total bilirubin and direct bilirubin, and

lipid levels in local male patients undergoing health checkups (Bai et al., 2023). Libor Vitek and his coauthors have discussed the beneficial effects of increasing plasma bilirubin that incorporates emerging areas of bilirubin biology and provides critical concepts to advance the field (Vitek et al., 2023). Maziar Nikouei and his coauthors have wanted to investigate the association between plasma bilirubin levels and the incidence of metabolic syndrome and diabetes mellitus across all populations. In the study, they have found that the elevated levels of bilirubin may have a significant protective effect against the development of diabetes mellitus and metabolic syndrome (Nikouei et al., 2024).

3. Research Methodology of the Study

Research is a scientific and systematic search for pertinent information on a specific topic. A researcher needs to understand the assumptions underlying various techniques of successful research (Redman & Mory, 1933). Methodology is the systematic and theoretical analysis of the methods applied to a field of study. It comprises the theoretical analysis of the body of methods and principles associated with a branch of knowledge (Irny & Rose, 2005). Research methodology aims to describe and analyze methods, throw light on their limitations and resources, clarify their limitations and resources, develop their presuppositions and consequences, relate their potentialities to the twilight zone at the frontiers of knowledge (Pavan & Kulkarni, 2014).

4. Objective of the Study

Bilirubin is a waste product derived from the heme moiety of the hemoglobin released from damaged erythrocytes that are destroyed in the reticuloendothelial cells. The heme portion from hemoglobin metabolized to bilirubin, and transported as a complex with serum albumin to the liver (Kapitulnik, 2004). Other sources of bilirubin are the breakdown of hemoproteins, such as

myoglobin, catalase, peroxidase, tryptophan pyrrolase, and cytochromes; and the catabolism of immature red cells in the bone marrow (Fujiwara et al., 2018). About 80% of the daily bilirubin production (250 to 400 mg) is derived from hemoglobin; the remaining 20% are from other hemoproteins. Total bilirubin is the sum of the conjugated and unconjugated bilirubin. It is water-insoluble and requires enzyme-mediated glucuronidation in the liver for biliary excretion (Wang et al., 2006). Main objective of this article is to discuss the basic concept of bilirubin that is a biomarker of liver damage (Mohajan, 2024g-m). Other minor objectives of the study are as follows:

- to focus on general properties of bilirubin,
- to highlight on types of bilirubin, and
- to discuss the effects of jaundice.

5. Types of Bilirubin

Bilirubin circulates in the bloodstream in two forms: indirect (unconjugated) bilirubin, and direct (conjugated) bilirubin. Usually, about 90% of total bilirubin is unconjugated and the rest 10% is conjugated (Gazzin et al., 2016).

Direct bilirubin: Direct bilirubin is determined in absence of caffeine by the direct reaction with diazotized sulphanilic acid to form red-colored azobilirubin, with the color intensity measured at 546 nm is proportional to the concentration of the direct bilirubin in the sample (Cohen et al., 2011). It dissolves in water and is made by the liver from indirect bilirubin, and loosely bound to albumin. It is non-toxic, and presents in low concentration in the blood, filters through renal glomeruli, and excretes in urine (Nikouei et al., 2024).

Sulfanilic acid + NaNO₂ \xrightarrow{HCl} Diazotized sulfanilic acid.

Bilirubin + Diazotized sulfanilic acid $\xrightarrow{pH\ 1.4}$ Azobilirubi.

Conjugated hyperbilirubinemia is associated to a decreased excretion of bile due to liver diseases or extrahepatic cholestasis (Mohajan, 2024a-f).

Indirect bilirubin: Indirect bilirubin is a toxic substance and insoluble in water, and travels through the bloodstream to the liver, where it is changed into a water-soluble direct bilirubin. It is tightly complex to albumin and the chief form of direct bilirubin. It is not filtered through renal glomeruli, and is not excreted in urine (Roche & Kobos, 2004). High levels of unconjugated bilirubin indicate that too much hemoglobin is being destroyed or that the liver is not actively treating the hemoglobin it is receiving. Unconjugated hyperbilirubinemia is seen in newborns due to increased red cell destruction. About 200 mg per day of unconjugated bilirubin are transported to the liver (Bonnett et al., 1976).

Total bilirubin: The total bilirubin is the sum of the direct and indirect bilirubin (Mohajan, 2024n-z). The total bilirubin concentration is determined in the presence of caffeine by the reaction with diazotized sulphanilic acid to produce an intensely colored diazo dye (560-600 nm), with intensity of color proportional to the concentration of total bilirubin (Kapitulnik, 2004).

In obstructive jaundice, there is an increase in total bilirubin, with direct bilirubin being the primary cause of the increase. In hemolytic jaundice, the total bilirubin increase occurs primarily in the unconjugated fraction. In hepatitis, both fractions are increased, with the conjugated fraction becoming more prevalent over time (Vitek et al., 2023).

6. General Properties of Bilirubin

Bilirubin is a linear open chain of the final product of the breakdown of the cyclic tetrapyrrole (MW 585 Da) ring of haemoglobin, myoglobin, and the cytochromes. It is formed by oxidative cleavage of a porphyrin in heme that affords biliverdin and is reduced to bilirubin (Bonnett et al., 1976). It is then released into the plasma and transported to the liver bound by albumin which is called unconjugated

(Mohajan, 2024A-F). In the liver, unconjugated bilirubin is matched by the hepatocytes and then conjugated with glucuronic acid. In this stage, it is called conjugated bilirubin (Blumgart et al., 2007).

The normal level of bilirubin in the blood is total bilirubin is 0.3-1.0 mg/dl, with direct (conjugated) bilirubin 0.1-0.3 mg/dl, and indirect (unconjugated) bilirubin 0.2-0.7 mg/dl (Chew et al., 2011). The increased bilirubin of normal level is considered as jaundice that may indicate liver damage or certain types of anemia. Therefore, bilirubin measurements are performed in the diagnosis of liver diseases, in the detection of hemolytic anemia, and to evaluate degrees of jaundice (Roche & Kobos, 2004). The average level of the bilirubin produced in humans from different sources ranges between 250 to 300 mg/day; of which 80% is derived from the heme moiety of the hemoglobin released from senescent erythrocytes that are destroyed in the reticuloendothelial system (Gazzin et al., 2016). The remaining 20% is produced from erythrocytes destroyed in the bone marrow and from catabolism of other heme proteins, such as myoglobin, catalase, peroxidase, tryptophan pyrrolase, and cytochromes (Sedlack & Snyder, 2004).

Mild jaundice in newborns usually does not cause problems. But too much bilirubin can cause brain damage (kernicterus) and other serious problems due to inherited disorders of bilirubin glucuronidation (Chowdhury et al., 2001).

7. Effects of Jaundice

When the bilirubin is produced faster than the liver can metabolize it, the unconjugated bilirubin may exceed the conjugating capacity of the liver that can cause the levels of unconjugated bilirubin to increase in the circulation and is associated with jaundice. The jaundice itself is not a disease, but rather a sign of many possible diseases (Roche & Kobos, 2004).

Jaundice is the discoloration of the skin and sclera of the eye that happens when bilirubin stores greater than 2.5 mg/dl. Increased direct bilirubin in the urine

usually means that the biliary ducts obstruction, cirrhosis, Crigler-Najjar syndrome, Dubin-Johnson syndrome, and hepatitis (Chowdhury et al., 2001). Increased indirect or total bilirubin indicates Gilbert disease, transfusion reaction, hemolytic anemia, sickle cell anemia, pernicious anemia, erythroblastosis fetalis, and resolution of a large hematoma (Nikouei et al., 2024).

Destruction of liver cells will lead to a reduced conjugating capacity with a raised serum level of indirect bilirubin and a low level of direct bilirubin indicates hepatic jaundice (Cohen et al., 2011). If the bile duct is blocked or damaged to hepatocellular structure causes increases in the levels of both conjugated and unconjugated bilirubin in the circulation that indicates cholecystitis due to liver damage. It is associated with itchiness (Birben et al., 2021).

8. Conclusions

The bilirubin is the breakdown product of the heme moiety of haemoglobin and other haemoproteins. It is a greenish-yellow pigment excreted in bile, urine, and feces. It is a potent antioxidant that is present at a time of great exposure to oxidative damage. An increase in bilirubin concentration in the serum is called jaundice that occurs in toxic or infectious diseases of the liver, such as liver hepatitis, obstruction of the bile duct, and hyperbilirubinemia in newborns. The conjugated bilirubin is excreted in the urine, and renal failure may result in the highest serum bilirubin levels. Bilirubin estimation in serum is used for the diagnosis, differentiation, and treatment of jaundice related diseases.

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