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## Screening of Liver Diseases in Urban Areas with Special Reference to Bilirubin and Iron



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### ABSTRACT

Mostly a disease is suitable for screening if it is common, if the target population can be identified and reached if a good screening test and an effective therapy are available. The present study was conducted on patients suffering from liver diseases especially from long periods they were belongs to urban area of Rewa district. The screening was done on the basis of liver function test, physically observation, subject's history and other associated complications. Most of the patients were found abnormal with special reference to bilirubin and iron contents. The investigation suggested the urbanization affected by the liver disease mostly repetition of jaundices is highly common whereas the jaundices and hepatitis were integrated, the major causes concern dietary involvement of iron and their different outward appearances.



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## INTRODUCTION

Bilirubin is an endogenous compound that can be toxic especially in neonates. It has recently been recognized that unconjugated bilirubin (UCB) exerts a strong anti-oxidant activity, and that mild hyperbilirubinaemia might have positive health effects. Bilirubin is the ultimate breakdown product of haemoglobin and serves as a diagnostic marker of liver and blood disorders<sup>1</sup>. It has a complex metabolism, which is important in relation to several processes involved in drug metabolism.

Liver and concern chronic disease is much more common than acute conditions. Mostly the rates of chronic liver disease for men are two times higher than for women<sup>2</sup>. It is categorized on both, the causes and directly shown the impacts on liver and their functions. Causes may include infection, injury, exposure to drugs or toxic compounds on autoimmune process, or a genetic defect that lead to the deposition and build-up of damaging substances such as iron or copper, certain physical and chemical changes. Impacts may include inflammation scarring, obstructions, clotting abnormalities, and liver failure etc. Screening of concern organs and diseases can be defined as analysis and examination of asymptomatic people to classify them as likely or unlikely to have the disease of interest<sup>3</sup>. Investigations are usually administered to individuals who do not have current symptoms but who may be at high risk for certain adverse health outcomes. Sensitivity and cost-effectiveness of disease screening enhance the screening specifically addresses populations known to be at higher risk of abnormality<sup>4</sup>. The medical screening to detect chronic liver disease usually involves a measures how well these organs are functioning, rather than testing for toxic substance itself. While there are numerous motives for this approach, the foremost cause is substances which cause chronic disease on liver are difficult to detect in body system.

Mostly Jaundice may be first manifestation of liver disease, defined as yellowish discolouration of skin due to excess amount of bilirubin present in blood<sup>5,6</sup>. Various biochemical parameters like serum calcium and iron investigated for screening and diagnosis of jaundice as well as to determine the changes which occurs in metabolic process associated with liver disease. Clinical finding of jaundice is not specific and may indicate a variety of disease, initial evaluation of jaundiced patient should focus upon whether the hyper bilirubinemia arises from predominance in serum of unconjugated form of bilirubin<sup>7</sup>.

The aim of this manuscript was to scrutinize the evidence for using iron and bilirubin testing as a primary screening parameter for jaundice.

### **Screening of patients**

Present study based on the screening of patients belongs to the urban part of the Rewa city. Mostly investigation included the concentration of serum bilirubin and iron, if any correlations exist between iron and other parameters; establish the relationship in different age and sex group wise. Significantly investigation of iron further compared with other known prognostic markers for completely detection and confirmation of other associated complication. Bilirubin in blood volume exceeds 1mg/dl is called hyperbilirubinemia and when it reaches 2.2 to 5mg/dl concentration ranges, it easily diffuses into the tissue which further appeared on skin and sclera yellowish due to deposition of bilirubin in tissues. This clinical condition is called jaundice which classified into three forms, hemolytic or prehepatic, hepatocellular or hepatic and obstructive or posthepatic. There is increased breakdown of hemoglobin to bilirubin at a rate in excess of ability of liver cell to conjugate and excrete it. Excess hemolysis occurs due to sickle hemoglobin, deficiency of glucose 6-phosphate dehydrogenase and Incompatible blood transfusion. In hemolytic Jaundice, more than normal amounts of bilirubin are excreted into the intestine, resulting in an increased amount of urobilinogen in feces and urine<sup>8,9</sup>.

In hepatocellular jaundice, there is some disorder of liver cells. Hepatic parenchymal cell damage impairs uptake and conjugation of bilirubin and results in unconjugated hyperbilirubinemia. Liver damage is usually caused by: Infection (viral) toxic chemical (such as alcohol chloroform), carbon tetrachloride, drugs etc. Patients with jaundice due to hepatocellular damage commonly have obstruction of the liver biliary that lead to increased plasma level of conjugated jaundice are: increased plasma concentration of conjugated and unconjugated bilirubin, decreased amount of urobilinogen in urine and feces, presence of bilirubin in urine, raised level of alanine transaminase<sup>10,11</sup>.

Posthepatic or obstructive jaundice occurs via an obstruction on common bile duct which prevents the passage of conjugated bilirubin from liver cells to intestine. Obstruction may be due to blockage of bile duct by gallstone, increased plasma of conjugated bilirubin, absence of urobilinogen in feces and urine, presence of bilirubin and bile salts in urine, raised level of

plasma alkaline phosphates enzyme, which is normally excreted via bile obstruction to flow of bile causes regurgitation of enzyme into the blood resulting in increased serum concentration.<sup>13</sup> Neonatal or physiologic jaundice occur in first few days after birth, an increased hemolysis and liver of new born is deficient in enzyme UDP-gucoronyl transferase that is necessary for conjugation. Deficiency of enzyme is more in premature infants, since the increased bilirubin is unconjugated; it is capable of penetrating the blood-brain barrier where their status in plasma exceeds 20 to 25 mg/dl<sup>14</sup>.

Bilirubin: Serum bilirubin elevated level of bile stream is a defect produced by liver and excreted in bile. Catabolism of Hb is outlined in graphic on left RBSs are incessantly undergoing a hemolysis process. Red blood cells disintegrate; the Hb is broken into globin, iron and heme. Heme initially breaks apart into biliverdin rapidly and reduced to bilirubin in reticulo-endothelial cells of liver<sup>15</sup>. Bilirubin is conjugated in hepatocytic microsomes in an ester linkage with sugar moieties donated by uridine diphosphate (UDP) sugars. Bilirubin is then further transported to liver where it reacts with a solubilizing form of uridine diphosphate (UDP) sugars<sup>16</sup>.

This more soluble form of bilirubin is excreted into the bile which goes through the gall bladder into intestine where the bilirubin is changed into stereobilinogen pigment which excreted in feces and urobilinogen. It is reabsorbed back into the blood. The blood transports the urobilinogen. This is reabsorbed back into the blood. The blood transports the urobilinogen back to liver where it is either re-excreted into blood for transport to the kidney. urobilinogen is finally excreted as a normal component of the urine<sup>17, 18</sup>.

### **Direct Bilirubin (Conjugated Bilirubin)**

The diagnosis is narrowed down further by looking at the levels of direct bilirubin. If direct (i.e. conjugated) bilirubin is normal, then the problem is an excess of unconjugated bilirubin and the location of the problem is upstream of bilirubin excretion. Hemolysis viral hepatitis or cirrhosis can be suspected. If direct bilirubin is elevated, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile duct obstruction by gallstone or cancer should be suspected<sup>19, 20</sup>.

### **IRON**

Iron is the most important essential trace metals. Numbers of human disease are related to iron deficiency or disorders of iron metabolism. Total iron content of the body is ~3-3.5g of this

amount -2.5g is in Hb, virtually all of which is contained within erythrocytes or their precursor in the bone marrow. However numerous cellular enzymes and coenzyme require iron notably peroxides and cytochrome that are also heme protein. Plasma contains only~2.5 mg of iron<sup>21,22</sup>.

## **MATERIALS AND METHODS**

### **Place of work**

The present work was done in department of Biochemistry A.P.S. University Rewa in cooperation with Dept. of Biochemistry, Shyam Shah Medical College, Rewa M.P., India.

### **Source of Data**

All the laboratory investigation was performed in patients. The study group compared of 111 subjects from 5-90 years of age in screening programme of liver disease, Patients was screened in central pathology lab of Biochemistry section of Biochemistry Dept. S.S. Medical College Rewa (M.P.) assigned for this study. Subjects' history was recorded for all the groups. Alcohol users, smokers, suffering from other diseases except liver diseases, HIV patients were excluded, women's used contraceptive tablets and iron tablets were not incorporated in study.

### **Collection of Samples**

Venous blood was collected from all subjects after 12 hour overnight fasting. 3ml of venous blood as sample was collected as routine diagnosis of the subjects and stored in a sterile vial. The blood was allowed to clot of room temperature. The clot was rimmed centrifuged; serum was separated by low-speed centrifugation and stored in a sterile vial. Serum Bilirubin, and iron were estimated. Hemolyzed and lipemic serum samples were rejected.

### **Biochemical analysis**

#### **1. Estimation of serum Bilirubin**

Serum bilirubin was estimated by colorimetric method. Determination of bilirubin in serum, any increases of bilirubin in blood is an indication of jaundice (derived from French word jaune meaning yellow) an estimation of bilirubin is a must in liver and biliary tract diseases. Bilirubin is derived from the destruction of red cells in reticulo-endothelial system excreted in urine as urobilinogen and in feces as stercobilinogen<sup>23</sup>.

## 2. Estimation of serum Iron

Serum iron was estimated by colorimetric method. Nearly two-third of body's iron (4-5g) is present in hemoglobin (338mg iron/100g) of which about 90 % is red cell hemoglobin, rest muscle hemoglobin. Greater part of remaining iron is stored, apparently combined with protein as ferritin in liver tissues. In addition, iron is present in most tissue in protein forms such as cytochrome and catalase, also contain heme. Iron is thus concerned with the transport of oxygen by blood and in the cellular activations systems. Blood iron is almost entirely present in red cell hemoglobin, but there is a small amount in plasma complex with a protein<sup>24</sup>.

### Statistical analysis

All the results were expressed as mean  $\pm$  SD. Student "t" test was used to assess statistical significance of the result within age and sex.

### Interpretation of data

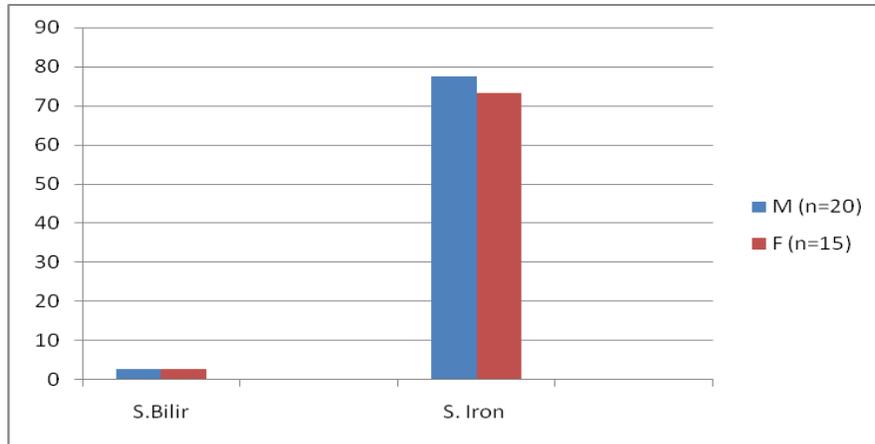
Normal range for serum has been variously given. There is general agreement that it is a little higher in men as compared to women. The following ranges for normal have been obtained, 80-175mg/100ml. Female, 60-60mg/100ml and although both higher and minimum limits have been suggested, most workers have give ranges hear to those above.

## RESULTS AND DISCUSSION

The present study was done with an aim to screen the subjects 5-90 yrs of age in Rewa city for liver diseases. The serum iron level obtained was then correlated with other parameters which were determined.

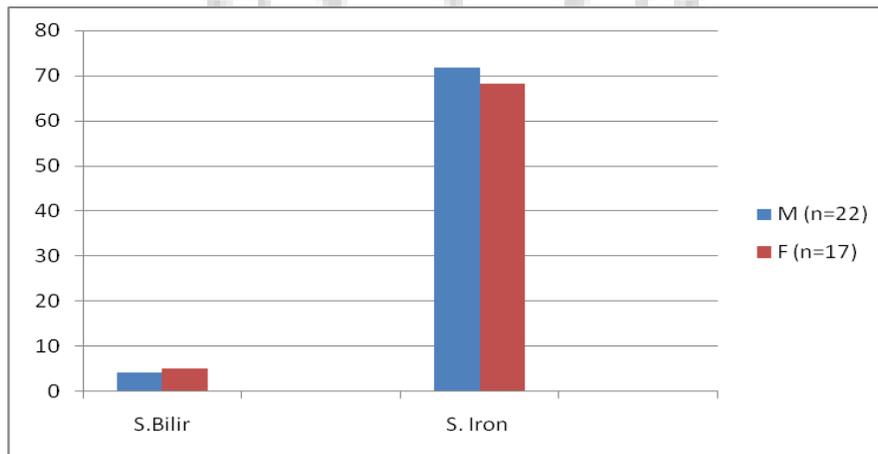
**Table 1. The level of bilirubin and iron in the age group of 0-30 yrs**

Variables	Male (n =20)	Female (n=15)
S. Bilirubin	2.44 $\pm$ 4.03	2.39 $\pm$ 2.83
S. Iron	77.52 $\pm$ 17.85	73.31 $\pm$ 15.60



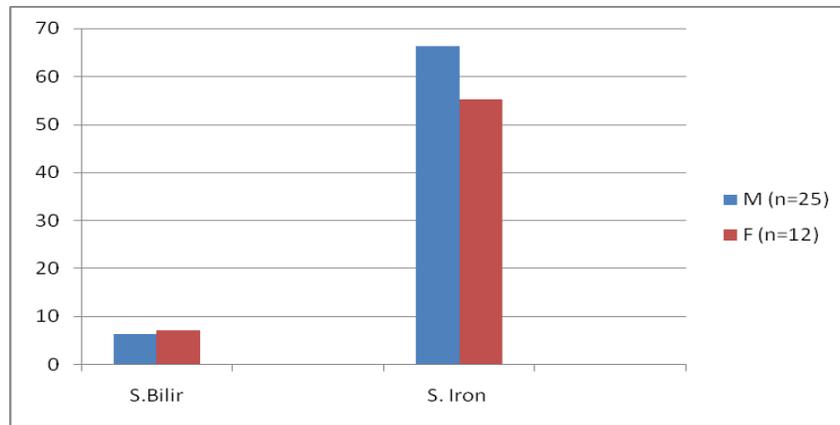
**Table2. The level of bilirubin and iron in the age group of 31-60 yrs**

Variables	Male (n=22)	Female (n=17)
S. Bilirubin	4.0± 2.61	4.82 ± 1.34
S. Iron	71.80 ± 25.40	68.21 ± 12.80



**Table3. The level of bilirubin and iron in the age group of 61-90 yrs**

Variables	Male (n=25)	Female (n=12)
S. Bilirubin	6.30±1.78	7.09 ± 2.5
S. Iron	66.27 ± 13.90	55.21 ± 17.74



### Correlation Coefficient and significance in the study group

Parameter	Correlation Coefficient	P. Value
Bilirubin & Serum Iron	-0.78	p<0.001

In this study we were able to examine the distribution of serum direct and conjugated bilirubin levels in a urban community. Our study did have some important limitations. We were unable to represent nutritional databases of subject. Liver is largest parenchymatous organ and central for intermediate metabolism<sup>25</sup>. Hepatocyte with different cellular structure of liver such as fenestrated sinusoidal, endothelial cells, Kuffer cells and canaliculi is important for performance of intermediate metabolism of nutrients. These cells are affected via abnormalities of bilirubin. The obtained data were suggested that the involvement of iron is a primitive factor which directly and indirectly associated in the bilirubin metabolism<sup>26</sup>. Hepatitis is more serious disease it accompanied by an increase in bilirubin levels. It has been observed that importance of drug induced liver injury in clinical trial as well as clinical practices. Some evidence shown that the statin drugs may be used safely in patient with chronic liver disease which safe to the liver tissues during coronary heart disease<sup>27</sup>.

The diagnostic criteria for establishing the presence of decrease iron concentration. Analysis of the iron composition and quality of bile is probably under used as a tool for pre-operative screening and early post operative monitoring of subjects at high risk of developing liver failure following major hepatobiliary procedures.

Results were very sensitive to changes in the baseline incidence of chronic bilirubin mainly hepatomegalies on enlarged firm liver function tests are available to function of liver. Test of liver enzymes in blood that are found normally most abundant in liver tissue, metabolites or product serum protein, serum albumin, globulin, A/G Ratio Alanine transaminase (ALT), Aspartate transaminase (AST), Prothrombin time (PT), Partial thromboplastin time (PTP), Platelet count (PC) and liver function test are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver<sup>28-30</sup>.

Mixed hyperbilirubinaemia in alcoholic patients, with decreased biliary secretion also observed in patients suffered from overproduction of bilirubin due to haemolysis. The haemolysis can result from decreased glutathione content of the erythrocytes or from decreased red cell membrane fluidity owing to high triglycerides<sup>31</sup>. The serum iron moves little lower during menstruation than at other times. The concentration of serum iron at birth in most infant is in the range between 150-220mg/100ml and so is a good deal superior to the mother. However, it falls shortly within few hours to below 100 and does not reach adult value intended for three to seven years. There is a diurnal variation in serum iron which is uppermost on rising and fall during the day by standard of 16%. It has been found that the suboptimal biochemical responders had significantly higher baseline level of total serum bilirubin. However bile acids appear to show a significant improvement control clinical trials are needed before any of the bile acid can be recommended for this indication<sup>32</sup>.

Activity of conjugated enzyme was also influenced by a variety of post-translational conditions, such as age, gender and microsomal enzyme inducing agent. The enzyme activity slowly increases after birth<sup>33,34</sup>. Conjugation rate is rate limiting for the overall bilirubin elimination out of the body in normal situations, because bilirubin can only be disposed off efficiently following conjugation. As such, the maximal biliary secretion rate, a measure of hepatic elimination, was depend on conjugation rate, as documented under different experimental conditions. Bilirubin production rate was increased as is the case in haemolysis, the relationship between conjugation and elimination rate, and consequently the serum levels, remains identical but is situated at a higher status.

It has been shown that alcohol abuser is as major cause of abnormal liver function. Throughout the world while measurement of liver enzyme activities (ALT, AST) and bilirubin are important

screening tools for detecting liver disease, due to lack of ethanol specificity and inconsistencies regarding the definition of significant alcohol consumption, several other blood test are usually needed to exclude competing and co-existing causes of abnormal liver function information on the specific race of ethanol consumption behind hepatotoxicity may be obtained through measurement of blood ethanol and its specific metabolites ethyl glucuronide, phosphatidyl ethanol, protein-acetaldehyde condensates and associated autoimmune responses<sup>35</sup>.

In addition, appreciable random fluctuation appears to occur in normal person. So that result covering most of the normal range can be obtained in a single individual at different times. It is best to take specimen for determination of serum iron at the same time of day preferably between 9 and 10 am morning.

Additional disturbed bilirubin status depends on mild changes in serum aminotransferase levels and in bilirubin ranges are frequent in thyroid disorders and cardiac decompensation, but they often pass unnoticed. Clinical jaundice may be present with serum bilirubin levels, results also justify with concentration of 19 mg/dl<sup>36</sup>. Both mild and unconjugated hyperbilirubinaemia as well as cholestasis and conjugated hyperbilirubinaemia can be seen. In cardiac decompensation, mild unconjugated hyperbilirubinaemia may result from diminished uptake by the hepatocyte because of reduced flow, whereas a mild increase in conjugates can be present because of anoxic suppression of the biliary secretory mechanisms<sup>37,38</sup>. Recent studies have indicated that being overweight is another increasingly common cause of abnormal liver enzyme level may also increase the impact of ethanol consumption and liver pathology along with abnormality of bilirubin<sup>39</sup>.

Interestingly, increased liver enzyme level activities in circulation may reflected not only hepatic function but can also serve as indicator of general health and the status of oxidative stress in vivo. ALT and AST activities predict insulin resistance metabolic syndrome, mortality from coronary heart disease and even mortality from all causes. It was the upper reference limits for liver enzyme activities were defined based on the data obtained from normal weight abstainers the clinical value of liver enzyme measurements as screening tools and in patient follow-up could be significantly improved<sup>40</sup>.

## CONCLUSION

The present study concluded on patients at the acute phase of the disease. Decreased iron may lead to abnormal synthesis of bilirubin and other disease on the other hand prolonged deficiency of iron may well make possible abnormal construction of bilirubin. Bilirubin is an interesting molecule, its complete metabolism is frequently disturbed due to the lack of dietary awareness in the urban areas. Bilirubin and iron is the strong predictor for the occurrence of both for physical appearance and liver disease with severity or certain complications in this study subjects.

A well accepted fact were also find and concluded that the increasing incidence of disease with advancing age, gender two more possible explanation intended for the association of iron deficiency with the pathogenesis of several disorders. Long term follow up in a large number of patients would be necessary to confirm these results.

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