Comparative Study of the Degree of Severity of Hepatitis B in Preicteric, Icteric and Posticteric Hbsag Seropositive Patients Using ALT, AST, Cu, Fe, Mn, Se, Mg, Zn and Albumin

Mathew Folaranmi OLANIYAN (Corresponding author)
School of Medical Laboratory Technology,Baptist Medical Centre
P.M. B. 43, Saki – Oyo State, Nigeria
Tel: 234-805-224-8019 E-mail: olaniyanmat@yahoo.com

Professor J.K. Oloke
Department of Pure and Applied Biology
Ladoke Akintola University of Technology
Ogbomoso, Oyo state, Nigeria
Tel: 234-38-721-342 E-mail: jkoloke@skannet.com/jkoloke@yahoo.co.uk

Abstract

Jaundice is the yellowish pigmentation of sclera of eye, skin and body fluids. It is associated with hepatitis B virus infection due to the destruction of the infected hepatocytes and intrahepatic-cholestasis caused by the scars of the healed hepatocytes. Hepatitis B patients that have never had jaundice are referred to as preicteric patients, while those that have recovered from jaundice are referred to as posticteric hepatitis patients. Those ones suffering from jaundice are referred to as icteric patients. This research work was designed to determine and compare the severity of hepatitis B using the serum levels of liver enzyme, albumin and micronutrients in HBsAg seropositive patients. One hundred and fifty HBsAg seropositive critically ill rural patients of the Atisbo/Saki-East/Saki-West federal constituency aged 5- 79 years; (75 females: 75males) classified into pre-icteric, icteric and posticteric patients were successfully monitored and investigated. One hundred and twelve HBsAg seronegative and HBV non-infected apparently healthy volunteers from the same community aged 4-80 years were studied as normal control volunteers. Forty three HBsAg seropositive patients were recruited from Ibadan as urban reference. The presence of hepatitis B surface antigen (HBsAg) and envelope antibodies to hepatitis B, (anti-HBe) were determined in the test and control subjects serologically (ELIZA). Similarly, the serum levels of liver enzymes Alanine-aminotransferase (ALT), and Aspartate-aminotransferase (AST) and micronutrients (Cu, Zn, Fe, Mn, Mg and Se) were biochemically (spectrophotometry and atomic absorption spectrophotometry) determined. Liver enzymes and albumin assays were routinely employed to recruit HBsAg seropositive patients in active liver destruction. Antibody to envelope antigen was used to detect patients that are infected but have been cleared of the antigens(HBsAg and HBeAg). The results obtained showed a significantly higher mean serum values of AST,ALT,Fe,Cu and Mn with a significantly lower serum albumin, Se,Mg,and Zn levels in patients compared with the results obtained from the controls during the 1st bleeding with $P<0.05$. There were significantly higher mean serum values of AST,ALT,Fe,Cu and Mn and significantly lower serum albumin, Se and Zn levels in patients compared with the results obtained from the control during the 2nd bleeding with $P<0.05$. A significantly higher mean serum levels of ALT, AST, Cu, Fe and Mn with a lower significantly mean serum levels of albumin, Se, and Zn was obtained in the patients than the control ($P<0.05$). Considering the significant biochemical alterations($P<0.05$) obtained from the HBsAg seropositive patients,hepatitis B was found to be more severe in HBsAg seropositive icteric patients compared with posticteric and preicteric HBsAg seropositive patients. The parameters studied could therefore be employed routinely to assess the severity of hepatitis B.

Keywords: Jaundice, Liver enzymes, Micronutrients, Hepatitis B surface antigen, Hepatitis B envelope antigen, Hepatitis B

1. Introduction

The causative agent of hepatitis B is a viral particle known as hepatitis B virus (HBV). (Ryan and Ray, 2004).

Hepatitis B virus is a hepatotropic virus that replicates in the liver and causes hepatic dysfunction (Logercio *et al.,* 1997; Lai *et al.,* 2003). During hepatitis B infection many virus particles are released from infected liver cells, resulting in large amount of viral antigen entering the blood. Hepatitis B surface antigen (HBsAg) is present in about 2 weeks before the onset of symptoms and persists throughout the course of the disease (Cheesbrough, 2002; Lok, 2002). At the recovery, it declines and is no longer detectable after 4 – 5 months. Persistence of HBsAg beyond six months indicates chronic infection or carrier state (Lok *et al.,* 2001; Cheesbrough 2002). Detectable antigens of hepatitis B virus also include; hepatitis B envelope antigen (HBeAg) a secreted product of the nucleocapsid gene of hepatitis B virus during chronic hepatitis B virus infection and its presence indicates that the virus is replicating and the infected individual has a high level of hepatitis B virus. The other type of the antigen is hepatitis B core antigen (HBcAg) which is secreted by inner nucleocapsid core that encases the genome (Lai *et al.,* 2003). Structural proteins such as pre-S1 and pre-S2 are regions involved in hepatitis B virus binding and entry into the hepatocytes. Hepatitis B envelope antibody (anti-HBe) is produced by immune system temporarily during acute hepatitis B virus infection or consistently during or after a burst of viral replication. It may be found in the convalescence stage and often in chronic hepatitis and the carrier state (Lai *et al.,* 2003). It is belived that the antibody response to viral envelope antigens contributes to clearance of the virus and that cytotoxic T cells mediate viral clearance by killing the infected cell. The virus does not kil cells directly but seems to activate cells in the immune system which cause inflammation and damage in the liver cell leading to increased serum level of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) **(**Kumar and Clark, 2002). Serum level of liver enzymes is a reflection of hepatocellular injury and tens to rise 1 – 2 weeks before the onset of jaundice (Lai, *et al.,* 2003). Hepatitis B virus is not directly cytophatic and the liver damage produced is by the cellular immune response of the host. Specific failure of T cells to recognize HBV antigen leads to viral persistence (Kumar and Clark, 2002).

High blood levels of two common liver enzymes involved in amino acid breakdown (AST and ALT, also designated as "SGOT" and "SGPT") are a sign of acute liver cell injury. Such damage to cell integrity allows these chemicals to escape from the cells and is associated with viral hepatitis and the toxic effects of drugs and poisons (Baron *et al.,*., 1989).

Metabolism of micronutrients (Zn, Se, Mg, Cu, Fe), storage of Iron, Cu and synthesis of albumin are the functions of liver. Alanine transaminase (ALT/SGPT) and Aspartate transaminase (AST/SGOT) are liver enzymes though AST could be found in other tissues (Baron *et al.,* 1989).

The metabolism of various nutrients in the liver indicates the presence and severity of liver disorder, and can also reveal suitable nutritional treatment strategies. Trace elements play an essential role in maintaining vital functions, and excess or deficiency leads to metabolic disorders *(*Yasuyuki *et al.,* 2004).

Some micronutrients (e.g. selenium) are powerful antioxidants which prevent free radical cellular damage; stimulate regeneration of the damaged liver cells and improve the systems. They also trigger the body defences. Trace element status can affect the immune function not only in a direct way but also indirectly by modulating plasma levels of hormones that regulate the development and function of host defense cells (Yu *et al.,* 1997; Swati and Udipi, 2003; Ethan *et al.,* 2004).

Micronutrients may potentially influence some processes of nonspecific immunity by modulating inflammatory cell functions. Micronutrient deficiencies are of clinical and public health magnitude in developing countries and account for significant infectious morbidity. Some trace elements inhibit virus replication in the host cells, thus showing antiviral activity. Many trace elements act as antioxidants or help such functions that not only regulate immune responses of the host, but also may alter the genome of the viruses (Yu *et al.*, 1997; Swati and Udipi, 2003; Ethan *et al.,* 2004).

The immune system contributes to the maintenance of physiological integrity of the body mainly by eliminating foreign material and infectious microbes. This is mediated through several trace elements that are essential micronutrients and are required for various body functions and well being of the immune system. Trace elements and some of their compounds show antiviral activity by combining with cellular proteins and inactivating them. On the other hand, some trace elements enhance severity of various viral infections (Yu *et al.,* 1997; Swati and Udipi, 2003; Ethan *et al.,* 2004). Thus, trace elements may play an important role in diseases caused by viruses. For instance, Selenium is a trace element which is also essential for normal functioning of the immune system and it has been reported that; as severity of liver damage increases, the hepatic zinc concentration decreases (Yu *et al.,* 1997; Swati and Udipi, 2003; Ethan *et al.,* 2004; Ray and Ryan, 2004).

2. Materials and methods

2.1 Materials

2.1.1 Study Area / Volunteers

Two hundred and eleven critically ill HBsAg seropositive patients aged 5 – 80 years were initially recruited but only one hundred and fifty critically ill HBsAg seropositive patients aged 5 – 79 years were successfully monitored.The one hundred and fifty[150] critically ill HBsAg seropositive patients include ;50 Preicteric HBsAg seropositive test participants (patients without any episode of jaundice) aged 5 - 78years (25 females; 25 males), 50 Icteric HBsAg seropositive patients (patients manifesting the first episode of jaundice on recruitment) aged 7 - 79years (25 females; 25 males) and 50- Posticteric HBsAg seropositive patients/test aged 5 - 79years (that is patients that have had at least one previous episode of jaundice but were not jaundice on recruitment).

One hundred and twelve (112) HBsAg seronegative and HBV non-infected apparently healthy volunteers (56 females; 56 males) aged 4 – 80 years were recruited as normal control.

Test and control volunteers were recruited from Saki-West/Saki-East/ATISBO federal constituency of Oyo North Senatorial District. Volunteers that have been living in this area for not less than 5 years were studied. The major complaints of the critically ill Preicteric and Posticteric patients voulunteered to the clinician and the researcher were malaise, abdominal tenderness/pain, prolong headache, nausea/vomitting and loss of appetite. About 58% of the critically ill icteric patients gave these complaints in addition to the development of jaundice. However these complaints persist in about 65% of the patients investigated.

Saki-West/Saki-East/ATISBO federal constituency is a rural community. It comprises three local government areas. The local governments include; Saki–West, Saki- East and ATISBO local government areas.It is located in the Northern part of Oyo–State and share boaders with Kwara-State and the Republic of Benin. It consists of 319 settlements.

2.1.2 Recruitment of Hepatitis B Patients

Hepatitis B surface antigen seropositive patients were recruited from 20 hospitals/clinics in Saki-West/Saki-East/ATISBO federal constituency after the ethical approval of the hospitals and the conscent of the patients of the children aged 5 – 15 years and that if the adult patiens have been obtained. The patients were further screened for active liver destruction using routine procedure such as the analysis of serum AST/SGOT, ALT/SGPT and albumin. Petients with increased ALT, AST and decreased serum albumin and are seropositive of serum HBsAg with signs and symptoms attributable to hepatitis B were studied.

2.1.3 Sample collection, separation, and preservation

Ten milliliters of blood was collected from each of the volunteers (tests and the controls) into a plain specimen bottles. The samples were allowed to clot and the serum was extracted. Serum extracted from each of the volunteers was used for the estimation of the micronutrients, albumin, and liver enzymes and for the determination of HBsAg, and anti-HBe.

Volunteers were all pre and post test counseled.

Blood samples were collected from each of the patient's initially on recruitment and also after at least six months after the initial bleeding and investigations.

The sample collection was carried out after the approval of reseach/ethical committee of the hospital and due consent of the patients in the hospitals / clinics where there is no research and ethical committee.

2.2 Methods

a. Patients were pre and post - test counseled to be able to meet the psychological needs of the test and control volunteers before and after the test.

b. Hepatitis B surface antigen (HBsAg) test was carried out to recruit the test and normal control volunteers by using a one step enzyme immunoassay technique of the sandwich type for the detection of HBsAg in human serum or plasma using the reagent kit of BIO –RAD Raymond Poincare, Marnes La Coquette.

Principle: MONOLISA AgHBs PLUS is a one enzyme technique of the sandwich type using three monoclonal antibodies selected for their ability to bind themselves to the various subtypes of HBsAg now recognized by World Health Organisation.

The solid phase is made up 0f 12 strips of 8 polystyrene wells coated with the first monoclonal antibody.the two other monoclonal antibodies are bound to the peroxidase.

c. **Anti HBe:** HBeAg and anti-HBe tests were carried out on the test and control volunteers by enzyme immunoassay for the determination of Hepatitis B e antigen and antibody in human plasma and sera using the reagent kit of: DIA.PRO

Diagnostic Bioprobes Srl

Via Columella, Milano – Italy

Principle of the test:

i. HBeAg; HBeAg if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation.

In the 2nd incubation, after washing, a tracer, composed of a mixture of two specific anti-HBeAg monoclonal antibodies,labeled with peroxidase,is added to the microplate and binds to the captured HBeAg.The concentration of the bound enzyme on the solid phase is proportional to the amount of HBeAg in the sample and its activity is detected by adding the chromogen / substrate in the 3rd incubation. The presence of HBeAg in the sample is determined by means of a cut – off value that allows for the semiquantitative detection of antigen.

ii. Anti – HBeAg ;anti – Hbe if present in the sample compete with recombinant HBeAg preparation for a fixed amount of an anti- HBeAg antibody, coated on the microplate wells. The competitive assay is carried out in two incubations, the first with the sample and recombinant HBeAg, and the second with atracer, composed of two antiHBeAg monoclonal antibodies, labeled with peroxidase.

The concentration of HBeAg specific antibodies in the sample is determined by means of a cut – off value that allows for the semiquatitative detection of anti – Hbe antibodies.

d. **Serum albumin estimation** was carried out on all the volunteers [test and control] by BromoCresolGreen method.

Principle of the test: Bromocresol green is an indicator which is yellow between P^H 3.5 – 4.2. When it binds to albumin the colour of the indicator changes from yellow to blue – green. The absorbance of the colour produced is measured in a colorimeter using an orange filter in a spectrophotometer at 632 nm wavelengths.

e. Determination of serum levels of Zn, Fe, Mn, Mg, Se & Cu.: Serum levels of these elements were determined with flame atomic absorption spectrophotometer (AAS) using a direct method described by Kaneko, (1999).

Principle of the test; the method is based on the principle that atoms of the element when aspirated into AAS vaporized and absorbed light of the same wavelength as emitted by the element when in the excited state.

f. Aspartate aminotransferase(AST/SGOT) was determined in all the sera of the volunteers spectrophotometrically by using the reagent kit of; Cromatest Linear Chemicals S.L, Joaquim Costa,18, 2ª Planta, 08390 Montgat–Barcelona(Spain). http://www.linear.es

g. Priciple of test: Aspirated aminotransferase (AST/SGOT) catalyses the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxaloacetate.The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to $NAD^{+,}$ proportional to the activity of AST present in the sample.

L-aspartate + 2-oxoglutarate AST/SGOT L-glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ MDH L- malate + NAD^+

The method follows optimized formulation of Berg Meyer *et al.,* (1978)

h. Alanine aminotransferase(ALT/SGPT) was determined in all the sera of the volunteers spectrophotometrically by using the reagent kit of; Cromatest Linear Chemicals S.L, Joaquim Costa,18, 2ª Planta, 08390 Montgat-Barcelona(Spain). http://www.linear.es

Priciple of test: Alanine aminotransferase (ALT/SGPT) reversibly transfers the amino group from alanine to alpha – ketoglutarate, forming pyruvate and glutarmate. The rate of formation of pyruvate is determined by coupling the ALT reaction with that of lactate dehydrogenase (LDH), which converts the pyruvate to lactate; the decrease in absorbance at 340nm is measured as NADH is oxidized to NAD^+.

Alanine + α-ketoglutarate ALT glutamate + pyruvate

Pyruvate + NADH + H^+ LD lactate NAD^+

The method follows optimized formulation of Berg meyer *et al.,* (1978)

Biotek elx 800 ELIZA readers and Biotek elx 50 ELIZA washers were the equipement used for Enzyme Immunoassay Techniques.

i. Data analysis. It was carried out using standard deviation,mean,standard error of mean and student 't' test as described by Norman,2004.

3. Results

The results obtained indicated significantly higher mean serum levels of AST, ALT, Fe, Cu and Mn and significantly lower mean serum values of Albumin, Se, Mg and Zn in the patients during the 1st bleeding compared with the results obtained in the same patients during the 2nd bleeding with $P<0.05$ (Table 1).

During the 1st bleeding, higher significant mean serum levels of Albumin, ALT and Se was found in posticteric patients compared with the preicteric patients with a significantly lower mean serum levels of AST, Mg and Cu in the posticteric patients compared with the Preicteric patients with $P<0.05$.No significant difference was observed in the mean serum values of Fe, Mn and Zn with $P>0.05$ (Table 2)

There were significantly lower mean serum levels of Albumin, ALT, Zn and Se and significantly higher mean serum levels of AST, Fe, Mn and Cu were found in the icteric patients than the posticteric patients with $P<0.05$. There was no significant mean serum level of Mg in icteric compared with the posticteric patients during the 1st bleeding with $P>0.05$ (Table3)

Significantly lower Zn and Mg levels with a higher significant mean serum levels of AST, ALT and Fe were found in icteric patients than the results obtained from the preicteric patients during the 1st bleeding with $P<0.05$.There was no significant difference in the mean serum levels of Mn, Se, Albumin and Cu in the icteric patients compared with the preicteric patients during the 1st bleeding with $P>0.05$ (Table 4)

The result obtained showed significantly higher mean serum levels of Zn, Se and Albumin with lower mean serum level of Fe were found in Preicteric patients compared with the posticteric during the 2nd bleeding with $P<0.05$.No significant difference was observed in the serum values of Mn, Cu, Mg, AST and ALT during the 2nd bleeding with $P>0.05$ (Table 5)

Higher significant mean serum levels of Zn, Se, Mg and Albumin with a significantly lower serum levels of Fe, Cu, and AST were found in preicteric patients compared with the icteric patients during the 2nd bleeding with $P<0.05$. There was no significant difference in the serum levels of Mn and ALT WITH p>0.0.5 during the 2nd bleeding. (Table 6)

Significantly higher mean serum Cu level and significantly lower mean serum level of Mg were found in the icteric patients compared with the posticteric patients during the 2nd bleeding with $P<0.05$.There was no significant difference in the mean serum level of Zn, Fe, Se, Mn, ALT, Albumin and AST in icteric patients compared with the posticteric patients during the 2nd bleeding with $P>0.05$ (Table 7).

Significantly lower mean serum levels of Zn, Se, Mg, Albumin and significantly higher mean serum levels of Fe, Cu, Mn, AST and ALT were found in the preicteric patients during 1st bleeding compared with the results obtained from the same patients during the 2nd bleeding with $P<0.05$ (Table 8).

Significantly lower mean serum levels of Zn, Se and Albumin and significantly higher mean serum levels of Fe, Cu, Mn, AST and ALT were found in icteric patients during the 1st bleeding compared with the results obtained from these patients during the 2nd bleeding with $P<0.05$. There was no significant difference in the serum level of Mg in the icteric patients during the 1st bleeding compared with the results obtained during the 2nd bleeding with $P>0.05$ (Table 9).

There were significantly higher mean serum levels of Mn and ALT and significantly lower mean serum levels of AST and Mg were found in the posticteric patients during the 1st bleeding compared with the results obtained from the same patients during the 2nd bleeding with $P<0.05$. There was no significant difference in the mean serum levels of Fe, Cu, Se, Albumin and Zn with $P>0.05$ (Table 10).

The results obtained showed a significantly higher mean serum values of AST,ALT,Fe,Cu and Mn with a significantly lower serum albumin, Se and Zn levels in patients compared with the results obtained from the controls during the 1st bleeding with $P<0.05$.(Table 11)

There were significantly higher mean serum values of AST,ALT,Fe,Cu and Mn and significantly lower serum albumin, Se and Zn levels in patients compared with the results obtained from the controls during the 2nd

bleeding with P<0.05.There was no significant difference in the serum level of Mg in the patients during the 2nd bleeding compared with the control with P>0.05.(Table 12)

4. Discussion

Higher degree of biochemical alterations as evidenced by the serum level of the parameters studied found in the test than the control could be attributed to the fact that liver is the organ of metabolism of the substances and upon destruction these functions will be affected.(Baron et al.,1989)

Significantly higher mean serum albumin, ALT, Se with a lower significant mean serum AST, Mg and Cu were found in posticteric patients compared with the preicteric patients during the 1st bleeding. These biochemical alterations with the exception of serumALT and Mg levels, indicate that the infection is more severe in the patients at the preicteric phase compared with the patients at their posticteric phase as most of the posticteric patients could be at their convalescence state but during the 2nd bleeding; significantly higher mean serum Zn, Se and albumin with lower mean serum level of Fe were found in the preicteric patients compared with the post icteric patients. The above may also indicate convalescence in more of sthe preicteric patients than the posticteric patients and persistence of liver destruction in posticteric patients during the second bleeding (Ray and Ryan, 2004).

Significantly lower mean serum levels of Albumin ALT, Zn and Se and a significantly higher mean serum level of AST, Fe, Mn and Cu were found in the icteric patients than the posticteric patients during the 1st bleeding. During the second bleeding a significantly higher mean serum Cu with a lower significant mean serum Mg were found in the icteric patients compared with the posticteric patients (Tables 3 and 7).These findings could be attributed to the fact that in icteric patients the manifestation of jaundice follows active liver destruction/healing and in posticterics the level of liver destruction might be reduced compared with the icteric patients as many of the posicterics might be a carrier or probably at convalescence. This could be attributed to the high nutritional demand during infection and healing in icteric patients (Kumar and Clark, 2002). However, there was an improvement during the second bleeding especially in the icteric patients considering the changes in the micronutrients and liver enzyme serum levels.

There were significantly lower mean serum Zn, and Mg with higher significant mean serum levels of AST, ALT, and Fe in the icteric patients than the results obtained from the preicteric patients during the 1st bleeding (Table 4). However higher significant mean serum levels of Zn, Se, Mg, and Albumin with a significantly lower serum levels of Fe, Cu, and AST were found in the pre-iceteric patients compared with the icteric patients during the 2nd bleeding (Table 6). This could be ascribed to the fact that patients at the icteric phase have just experienced liver destruction and the nutritional demands for the healing of the damaged cells and other physiological activities of the patients will be higher than those at the preicteric phase because some of the patients at the preicteric phase might be a carrier or at their convalescence. The presence of jaundice in these patients could also alter the trace element serum level. In addition to the above facts, the findings of this work could also be associated with the fact that; transition to the icteric phase is marked by the disappearance of preicteric signs/symptoms in young children but the exacerbation of these signs/symptoms in older children adolescents and adults that constitute the majority of the patients studied (Kumar and Clark, 2002).

The significantly lower mean serum levels of Zn, Se, Mg, albumin with a significant higher mean serum level of Fe, Cu, Mn, AST and ALT found in the preicteric patients and also in the icteric patients (excluding Mg level) during the 1st bleeding compared with the results obtained in these patients during the 2nd bleeding (Tables 4and 6), could be as a result of clinical interventions after the 1st bleeding. Furthermore it may also be associated with the fact that some of the preicteric and the icteric patients might have been cleared of the virus and are either career or at convalescence by the 2nd bleeding (Lau and Wright, 1993).

However, significantly higher mean serum Mn and ALT with lower significant mean serum levels of AST and Mg were found in the posticteric patients during the 1st bleeding compared with the result obtained from these same patients during the 2nd bleeding (Table 10). The increased serum AST level in the patients during 2nd bleeding may be as a result of reoccurrences of liver destruction and the reactivation of the virus in some of the patients which could be associated to factors such as coinfection with other microbes e.g HIV and HCV (Ryan and Ray, 2004)

5. Conclusion

The biochemical alterations of the parameters studied in the patients was however more in the icteric patients than the pre and postictric HBsAg seropositive patients and in the test than the control. Considering the fact that Hepatitis B is associated with liver destruction which will also alter the storage and the metabolic functions of

the liver, it can therefore be deduced from this work that hepatits B is more severe in icteric HBsAg seropositive patients than the pre and post icteric patients.

References

Baron D. N., Whicher J. T. and Lee K. E. (1989). *A new short textbook of Chemicalpathology*. 5th edition. Printed and bound in Great Britain by Butler and Tanner Ltd., Frome and London.

Cheesbrough. (2002). District Laboratory practice in Tropical Countries Part 2. Cambridge Low Price edition; 252 – 264.

Ethaan Basch and Catherine Ulbrilit, Tracee Abrams, Cindy Quanch and Nancy Tannous. (2004). Evidence – based systemic review of selenium. *J.A.N.A*;7,2.

Francis. J. Mahoney. (1999). Update in diagnosis, management and prevention of hepatitis B virus. *Clinical Microbiology Review*, 12(2); 351 – 366.

Kaneko J. J. (1999). Clinical Biochemistry of Animal.4th edition (Kaneko J.J,Editor) Academic Press Inc; New York.pp 932.

Kumar Preveen and Clark Michael. (2002). Clinical Medicine.Printed in U.K by Bath Press Ltd.Fifth edition pp: 21, 119 – 221, 335 – 405.

Lai C. L., Ratziu V., Yuen M. F., Poyard. (2003). Viral hepatitis B. *Lancet*, 362: (940).

Lau J. Y. N., Wright T. L. (1993). Molecular Virology and pathogenesis of hepatitis B.*Lancet*, 342:1335 – 1340.

Loguercio C., DeGirlamo V., Federico A., Feng S. L., Cataldi V., Del Vecchio, Blanco. C., Gialanel la. G. (1997). Trace elements and chronic liver diseases J. Trace Element Biological, 11(3); 158-161.

Lok. A. S., McMahon B. J. (2001). Chronic hepatitis B. *Hepatology,* 34, 1225-1241.

Norman. J.J. (1994). Statistical Methods in Biology. Cambridge University Press. Cambridge Low Price 3rd edition pp 1-3.

Ryan K. J., Ray C. G. (editors). (2004). Sherris Medical Microbiology, 4th ed., McGraw Hill, 247–9; ISBN 0838585299.

Swati.S and Udipi S. A. (2003). Role of nutrition in the management of HIV infection /AIDS.J.I.M.A.vol 100, No 6, 1 – 6.

Yasuyuki Arakawa, Mitsuhiko Moriyama and Yasuo Arakawa. (2004). Liver cirrhosis and metabolism (sugar, protein, fat and trace elements) *Hepatology Research* Volume 30, Supplement 1, December 2004, Pages 46-58.

Yu S., Zliu Y. J and Li W.G. (1997). Protective role of selenium against Hepatitis B virus and primary liver cancer in Qidong. *Biological Trace element Research,* 56 (1); 117 – 1124.

Table 1. Serum levels of micronutrients, liver enzymes and albumin in the patients during 1st and 2nd bleeding

	Mean values in patients during (n= 150)	Mean values in patients during Total (n = 150)
Serum micronutrients	$\overline{X}$ **± SE**	$\overline{X}$ **± SE**
Zn µmol/l	8.2 ± 0.22*	10.00 ± 0.31*
Mn µmol/l	18.94 ± 0.22*	14.18 ± 0.46*
Fe µmol/l	29.01 ± 0.48*	22.52 ± 0.75*
Cu µmol/l	26.89 ± 0.46*	22.29 ± 0.47*
Se µmol/l	0.57 ± 0.03*	0.821 ± 0.04*
Mg µmol/l	0.69 ± 0.01*	0.75 ± 0.01*
Serum Albumin /liver enzymes		
Alb g/l	2.25 ± 0.07*	2.99 ± 1.09*
AST U/L	50.31 ± 0.97*	34.16 ± 1.45*
ALT U/L	29.15 ± 0.76*	18.02 ± 1.03*

**** Significant at 0.05 level of probability***

Table 2. Serum levels of liver enzymes and albumin in preicteric and *posticteric* patients during 1st bleeding

	Preicteric patients (n= 50)	Posticteric patients (n = 50)
Serum micronutrients	**$\overline{X}$ ± SE**	**$\overline{X}$ ± SE**
Zn µmol/l	8.72 ± 0.40	9.0 ± 0.44
Mn µmol/l	19.0 ± 0.37	17.9 ± 0.48
Fe µmol/l	28.5 ± 0.75	27.0 ± 1.03
Cu µmol/l	28.82 ± 0.59*	22.2 ± 0.86*
Se µmol/l	0.49 ± 0.02*	0.79 ± 0.08*
Mg µmol/l	0.73 ± 0.01*	0.63 ± 0.03*
Serum Albumin /liver enzymes		
Alb g/l	2.15 ± 0.11*	2.64 ± 0.65*
AST U/L	52.2 ± 0.91*	25.64 ± 1.80*
ALT U/L	29.22 ± 0.99*	43.84 ± 2.47*

**** Significant at 0.05 level of probability***

Table 3. Serum levels of micronutrients, liver enzymes and albumin in icteric and *posticteric* patients during 1st bleeding

	Icteric patients (n= 50)	posticteric patients (n = 50)
Serum micronutrients	**$\overline{X}$ ± SE**	**$\overline{X}$ ± SE**
Zn µmol/l	6.87 ± 0.23*	9.0 ± 0.44*
Mn µmol/l	19.9 ± 0.199*	17.9 ± 0.48*
Fe µmol/l	31.8 ± 0.48*	27.0 ± 1.03*
Cu µmol/l	29.7 ± 0.38*	22.2 ± 0.86*
Se µmol/l	0.44 ± 0.07*	0.79 ± 0.08*
Mg µmol/l	0.67 ± 0.012*	0.63 ± 0.03*
Serum Albumin /liver enzymes		
Alb g/l	1.9 ± 0.044*	2.64 ± 0.17*
AST U/L	54.92 ± 0.48*	25.6 ± 1.80*
ALT U/L	32.6 ± 0.75*	43.84 ± 2.47*

**** Significant at 0.05 level of probability***

Table 4. Serum levels of liver enzymes, albumin and micronutrients in icteric and *preicteric* patients during 1st bleeding

	Icteric patient's (n= 50)	Preicteric patients (n = 50)
Serum micronutrients	$\overline{X}$ ± SE	$\overline{X}$ ± SE
Zn µmol/l	6.87 ± 0.23*	8.72 ± 0.396*
Mn µmol/l	19.9 ± 0.199	19.0 ± 0.37
Fe µmol/l	31.8 ± 0.48*	28.5 ± 0.75*
Cu µmol/l	29.7 ± 0.38	28.5 ± 0.59
Se µmol/l	0.44 ± 0.07	0.49 ± 0.023
Mg µmol/l	0.67 ± 0.012*	0.73 ± 0.014*
Serum Albumin /liver enzymes		
Alb g/l	1.9 ± 0.044	2.15 ± 0.11
AST U/L	54.92 ± 0.48*	52.18 ± 0.91*
ALT U/L	32.6 ± 0.75*	29.22 ± 0.28*

**** Significant at 0.05 level of probability***

Table 5. Serum levels of micronutrients, liver enzymes and albumin in *preicteric* and posticteric during 2nd bleeding

	Preicteric patients (n= 50)	Posticteric patients (n = 50)
Serum micronutrients	$\overline{X}$ ± SE	$\overline{X}$ ± SE
Zn µmol/l	11.76 ± 0.51*	9.7 ± 0.55*
Mn µmol/l	13.5 ± 0.67	15 ± 0.88
Fe µmol/l	19.7 ± 1.22*	24 ± 1.41*
Cu µmol/l	21.46 ± 0.81	21.0 ± 0.76
Se µmol/l	1.074 ± 0.023*	0.7 ± 0.06*
Mg µmol/l	0.78 ± 0.02	0.8 ± 0.03
Serum Albumin /liver enzymes		
Alb g/l	3.43 ± 0.15*	2.79 ± 0.18*
AST U/L	30.19 ± 0.27	35.08 ± 2.57
ALT U/L	15.44 ± 1.75	20.33 ± 1.99

**** Significant at 0.05 level of probability***

Table 6. Serum levels of micronutrients, liver enzymes and albumin in *preicteric* and icteric during 2nd bleeding

	Preicteric patient's (n= 50)	Icteric patients (n = 50)
Serum micronutrients	**$\overline{X}$ ± SE**	**$\overline{X}$ ± SE**
Zn µmol/l	11.76 ± 0.51*	8.57 ± 0.46*
Mn µmol/l	13.5 ± 0.67*	13.94 ± 0.82*
Fe µmol/l	19.7 ± 1.22*	24.0 ± 1.202*
Cu µmol/l	21.46 ± 0.81*	24.0 ± 0.84*
Se µmol/l	1.074 ± 0.023*	0.662 ± 0.051*
Mg µmol/l	0.78 ± 0.02*	0.71 ± 0.298*
Serum Albumin /liver enzymes		
Alb g/l	3.43 ± 0.15*	2.74 ± 0.130*
AST U/L	30.19 ± 0.27*	37 ± 2.503*
ALT U/L	15.44 ± 1.75	18.32 ± 1.56

**** Significant at 0.05 level of probability***

Table 7. Serum levels of micronutrients, liver enzymes and albumin in icteric and *posticteric* during 2nd bleeding

	Icteric patients (n= 50)	Posticteric patients (n = 50)
Serum micronutrients	**$\overline{X}$ ± SE**	**$\overline{X}$ ± SE**
Zn µmol/l	8.57 ± 0.46	9.7 ± 0.55
Mn µmol/l	13.94 ± 0.82	15.0 ± 0.877
Fe µmol/l	24.0 ± 1.202	24.0 ± 1.41
Cu µmol/l	24.0 ± 0.84*	21.0 ± 0.76*
Se µmol/l	0.662 ± 0.051	0.7 ± 0.057
Mg µmol/l	0.71 ± 0.298*	0.8 ± 0.028*
Serum Albumin /liver enzymes		
Alb g/l	2.74 ± 0.130	2.79 ± 0.182
AST U/L	37 ± 2.503	35.08 ± 2.57
ALT U/L	18.32 ± 1.56	20.3 ± 1.99

**** Significant at 0.05 level of probability***

Table 8. Serum levels of liver enzymes, albumin and micronutrients in *preicteric* during 1st and 2nd bleeding

	Preicteric patients (n= 50)	Preicteric patients (n = 50)
Serum micronutrients	$\overline{X}$ **± SE**	$\overline{X}$ **± SE**
Zn µmol/l	8.72 ± 0.40*	11.76 ± 0.502*
Mn µmol/l	19.0 ± 0.37*	13.5 ± 0.67*
Fe µmol/l	28.5 ± 0.75*	19.7 ± 1.22*
Cu µmol/l	28.82 ± 0.59*	21.46 ± 0.809*
Se µmol/l	0.49 ± 0.02*	1.074 ± 0.074*
Mg µmol/l	0.73 ± 0.01*	0.78 ± 0.016*
Serum Albumin /liver enzymes		
Alb g/l	2.15 ± 0.11*	3.43 ± 0.15*
AST U/L	52.2 ± 0.91*	30.19 ± 2.38*
ALT U/L	29.22 ± 0.99*	15.44 ± 1.75*

**** Significant at 0.05 level of probability***

Table 9. Serum levels of liver enzymes, albumin and micronutrients in *icteric* during 1st and 2nd bleeding

	Icteric patients (n= 50)	Icteric patients (n = 50)
Serum micronutrients	$\overline{X}$ **± SE**	$\overline{X}$ **± SE**
Zn µmol/l	6.87 ± 0.23*	8.57 ± 0.445*
Mn µmol/l	19.9 ± 0.199*	13.94 ± 0.82*
Fe µmol/l	31.8 ± 0.48*	24.0 ± 1.202*
Cu µmol/l	29.7 ± 0.38*	24.0 ± 0.84*
Se µmol/l	0.44 ± 0.07*	0.662 ± 0.0509*
Mg µmol/l	0.67 ± 0.012	0.71 ± 0.0296
Serum Albumin /liver enzymes		
Alb g/l	1.9 ± 0.044*	2.74 ± 0.13*
AST U/L	54.92 ± 0.48*	37.2 ± 2.503*
ALT U/L	32.6 ± 0.75*	18.32 ± 1.56*

**** Significant at 0.05 level of probability***

Table 10. Serum levels of liver enzymes, albumin and micronutrients in *posticteric* during 1st and 2nd bleeding

	Posticteric patients (n= 50)	Posticteric patients (n = 50)
Serum micronutrients	**$\overline{X}$ ± SE**	**$\overline{X}$ ± SE**
Zn µmol/l	9.0 ± 0.44	9.7 ± 0.55
Mn µmol/l	17.9 ± 0.48*	15.0 ± 0.88*
Fe µmol/l	27.0 ± 1.03	24.0 ± 1.41
Cu µmol/l	22.2 ± 0.86	21.0 ± 0.76
Se µmol/l	0.79 ± 0.08	0.7 ± 0.05
Mg µmol/l	0.63 ± 0.03*	0.8 ± 0.028*
Serum Albumin /liver enzymes		
Alb g/l	2.64 ± 0.17	2.79 ± 0.18
AST U/L	25.6 ± 1.80*	35.1 ± 2.57*
ALT U/L	43.84 ± 2.47*	20.3 ± 1.99*

**** Significant at 0.05 level of probability***

Table 11. Serum levels of micronutrients, liver enzymes and albumin in test and normal volunteers. (1st bleeding)

	Test / patients (n = 150)	Normal control (n =112)
Serum micronutrients	**$\overline{X}$ ± SE**	**$\overline{X}$ ± SE**
Zn µmol/l	8.2 ± 0.22*	13.0 ± 0.19*
Mn µmol/l	18.94 ± 0.22*	9.35 ± 0.2*
Fe µmol/l	29.01 ±0.49*	14.6 ± 0.42*
Cu µmol/l	26.89 ± 0.46*	16.0 ± 0.28*
Se µmol/l	0.57 ± 0.03*	1.1 ± 0.04*
Mg µmol/l	0.68 ± 0.01	0.78 ± 0.008
Serum Albumin /liver enzymes		
Alb g/l	2.25 ± 0.07*	4.1 ± 0.47*
AST U/L	50.31 ± 0.97*	15.0 ± 0.61*
ALT U/L	29.15 ± 0.76*	7.13 ± 0.02*

**** Significant at 0.05 level of probability***

Table 12. Serum levels of micronutrients, liver enzymes and albumin in test and control (2nd bleeding)

	Test / patients (n = 150)	Normal control (n =112)
Serum micronutrients	**$\overline{X}$ ± SE**	**$\overline{X}$ ± SE**
Zn µmol/l	10.00 ± 0.31*	13.0 ± 0.19*
Mn µmol/l	14.18 ± 0.46*	9.35 ± 0.7*
Fe µmol/l	22.52 ± 0.75*	14.6 ± 0.44*
Cu µmol/l	22.29 ± 0.47*	16.0 ± 0.28*
Se µmol/l	0.82 ± 0.03*	1.1 ± 0.04*
Mg µmol/l	0.75 ± 0.013	0.78 ± 0.01
Serum Albumin /liver enzymes		
Alb g/l	2.99 ± 0.09*	4.1 ± 0.05*
AST U/L	34.16 ± 1.5*	15.0 ± 0.61*
ALT U/L	18.02 ± 1.03*	7.13 ± 0.02*

**** Significant at 0.05 level of probability***