DISCUSSION
Chapter 4

DISCUSSION
4.1. Bilirubin and albumin

In the present study bilirubin level was found to be increased in NALC, ALC and HCC categories when compared to the control category. It is well known that the level of bilirubin increases in liver diseases. Huseyin et al., (2006) reported in their study, an increased level of total bilirubin in liver cirrhosis and hepatocellular carcinoma. The study of Alan et al., (1994) showed an increased level of total bilirubin in liver cirrhosis. Many other studies also showed increased level of bilirubin in cirrhosis and hepatocellular carcinoma, which is in accordance with our results.

Bilirubin is formed mainly in the spleen and is transported to liver for conjugation process. Conjugation step takes place in hepatic endoplasmic reticulum in the presence of enzyme bilirubin UDP-glucuronyltransferase. Conjugated bilirubin (mono or di glucuronates) is more soluble and easily gets eliminated through bile in normal case. The hepatocytes microtubular system, hepatic bile salt excretion and membrane carrier proteins, appear to facilitate the excretion of bilirubin glucuronates into bile (Berk and Noyer, 1994). They also reported that the process of biliary excretion, the rate-limiting step in bilirubin transport is susceptible to damage from a variety of acquired liver diseases, which leads to an increased concentration of conjugated bilirubin. In cirrhosis, the canaliculi are abnormal and the relationship between the liver cells and the canaliculi is destroyed. As a result, the liver is not able to eliminate toxic substances normally, and they can accumulate in the body (Bruix and Sherman 2005). So, there is regurgitation of conjugated bilirubin from liver to the circulation. A small fraction of conjugated bilirubin undergo deconjugation, and another small fraction covalently bind with albumin to form delta bilirubin, which is very slowly cleared from the plasma, resulting in an increased plasma bilirubin in liver diseases (Bosma et al., 1994, 1995). Fuad (2003) reported conjugated hyperbilirubinemia in liver cirrhosis and in bile duct obstructing tumors. Acetaldehyde formed by the enzymatic oxidation of
alcohol in the liver is said to play a major role in the development of alcoholic liver cell injury (Jenkins, 1984). Among the diseased category, ALC and HCC categories showed a significant increase in bilirubin level when compared to NALC category, probably due to the toxic effect of ethanol in the liver in ALC category, and tumor growth in HCC category, that might have blocked the removal of bilirubin through bile in that category.

Albumin is the most commonly measured serum protein and is synthesized exclusively by the liver. Serum level of albumin is an indication of the synthetic capacity of the liver. The rate of synthesis varies depending upon the hormonal environment, nutritional status, age and other local factors. Prijatmoko et al., (1993) showed a decreased level of albumin with increasing severity of cirrhosis, with an increase in total body water, whereas total body protein decreased with a significant decrease in albumin. Albumin is found to be decreased in response to inflammation and cellular damage of liver (Baumann et al., 1983, 1984; Beaudet et al., 1982). Ramadori et al., (1985) and Dinarello (1988) reported that output of liver derived proteins, albumin and transferrin, diminishes following infection, inflammation and injury.

In liver diseases, hypoalbuminemia is frequently present because of decreased production and sinusoidal leakage of albumin in patients with portal hypertension. The patterns of plasma protein alterations seen in liver disease depend on the type, severity and duration of liver injury. Hypoalbuminaemia is reported in cirrhosis (Luo et al., 2002) and hepatocellular carcinoma (Dufour, 2000). In cirrhosis, hepatic synthesis of albumin is reduced. Loss of albumin into ascitic fluid also seems to be responsible for the decrease in albumin in many cases. In our study also there is decreased level of albumin in NALC, ALC and HCC categories, when compared to the control category. This may be due to the above multifactorial effects like sinusoidal leakage, reduced synthesis and cell necrosis in liver diseases.
Albumin is a ubiquitous protein synthesized only by hepatocytes. It has been reported that the expression of ALB gene is reduced in various liver diseases and the degree of reduction in the hepatic ALB mRNA level is generally correlated with the severity of the disease (Osaki et al., 1991). The study of Shi–Min et al., (2004) showed that hepatic albumin mRNA in tumor tissue was lower than non tumor tissue. Another study shows that in inflammatory conditions, albumin synthesis is decreased by direct inhibition of cytokines, which are released during acute phase response (Ryffel et al., 1994). It is reflected in our study that the level of albumin is low in HCC category when compared to NALC and ALC categories.

Northern hybridization studies of Annoni et al., (1990) showed that, despite the presence of inflammation and fibrosis, the ALB mRNA levels of alcoholics were similar to the controls. Alcohol actually increases ALB mRNA in alcoholics, but the inhibition of albumin synthesis by alcohol is also reported (Dufour et al., 2000). There are many studies showing that there is a reduction in the level of albumin in alcoholic liver disease. Sivagurunathan et al., (2006) observed in their work a significantly low level of albumin in 80 % of cirrhotic patients with chronic alcohol abuse. Bilirubin values of these patients are also reported to be increased significantly. All these studies support our findings that the albumin level of ALC category is very low than NALC category, probably due to the inhibition of albumin synthesis by alcohol.

**SERUM ENZYMES**

4.2. **Aminotransferases (Aspartate transaminase and alanine transaminase)**

In the present study, serum AsT and AIT values were significantly increased in NALC, ALC and HCC categories when compared to the control group. Injury to liver, whether acute or chronic, eventually results in an increase in serum
aminotransferases (Cohen and Kaplan, 1979). Both aminotransferases are highly concentrated in liver. AsT is also diffusely present in the heart, skeletal muscles, kidneys, brain and red blood cells and AIT has low concentrations in skeletal muscles and kidneys (Wroblewski, 1958). An increase in serum AIT levels is therefore more specific for liver damage. In our results, increased AIT in all diseased categories indicates that there was severe liver damage.

It is reported that the liver AIT is localized solely in the cellular cytoplasm, whereas AsT is both cytosolic and mitochondrial (Rej, 1989). Zone 3 of the hepatic acinus has a higher concentration of AsT and damage to this zone may result in greater alteration to AsT levels (Edoardo et al., 2005). Aminotransferase clearance is carried out within the liver by sinusoidal cells (Kamimoto et al., 1985). The half life in the circulation is about 47 hours for AIT, about 17 hours for AsT, and an average of 87 hours for mitochondrial AsT (Dufour et al., 2000). About 80% of total activity of AsT is found to be by mitochondrial fraction. In the present study, AsT increase is higher than that of AIT indicating that the damage of liver tissue leads to the release of both cytoplasmic and mitochondrial enzymes.

Many studies showed increased levels of aminotransferase in different diseases. In ischaemic or toxic liver injury and acute hepatic injury, the level is found to be greater than 75 times the upper reference limit (Dufour et al., 2000). Siagris et al., (2006) in their study showed an increased level of AsT and AIT in chronic hepatitis patients. Sivagurunathan (2006) reported a significant increase in liver marker enzymes in liver cirrhotic patients. Our results also revealed the same, where the increase in AsT was approximately nine times in ALC category, and approximately five times both in NALC and HCC categories when compared to the control category.
Casaril et al., (2000) observed that Fe\(^{2+}\) together with alcohol intensified the liver fibrosis due to the additional activating effect of ethanol on the acceleration of redox reactions within hepatocytes. These redox reactions lead to a cytosolic pH decrease with increased release of free Fe\(^{2+}\) ions from ferritin. These reactions may induce lipid peroxidations and a consequent damage to the organelle membrane. These cellular alterations may result in siderogenesis. The study of Fabris et al., (1993) showed that patients with acute hepatitis had significantly higher concentrations of lipid peroxide compared with patients with chronic liver disease. In hepatocellular carcinoma, they could not find evidence of lipid peroxide liberation greater than that found in mild form of liver disease. They also found that the highest lipid peroxide concentration in patients with acute hepatitis were due to drugs or alcohol. Tsukamoto (2001) reported that the primary factors involved in the development of alcoholic liver disease are acetaldehyde, oxidative stress, hypoxia, membrane changes and immune response. All these factors indicate that in alcoholic liver disease, the intensity of liver necrosis is very high. Alcohol also appears to induce the expression of mitochondrial AsT on the surface of hepatocytes (Zhou et al., 1998). It is reported that alcoholic hepatitis is associated with increased plasma activities of mitochondrial AsT and it is proposed that mitochondrial AsT is a marker of chronic alcoholism (Nalpas et al., 1986; Okuno et al., 1988). Alcohol also leads to the release of AsT from other tissues, as AsT is present in other tissues also. It is known that increased level of aminotransferases is an indication for liver necrosis. In the present study, among the diseased category, serum values of AsT and AlT are higher in ALC category when compared to NALC and HCC categories, which may be due to these effects.

The level of AlT in the blood is increased in conditions where hepatocytes are damaged or dead. As cells are damaged, AlT leaks out into the blood stream. All types of hepatitis and cirrhosis have been reported to cause liver damage that can lead to elevations in the serum AlT activity (Cohen and Kaplan 1979). AlT is a
cytosolic enzyme of the liver, which can be increased in cases of liver cell death resulting also from other causes such as shock and drug toxicity. The level of ALT may also be correlated roughly with the degree of cell death or inflammation. In our study, it is noted that there is an increase in serum ALT activity in diseased categories approximately 4 times than that of the control category, and it was also noted that the rate of increase of ALT is less than that of AST. It has been found that in cirrhosis and malnutrition, there is greater decrease in cytoplasmic ALT than cytoplasmic AST (Ludwig and Kaplowitz, 1980). Malnutrition is common in cirrhosis and in carcinoma. Reuler (1985) reported that thiamine and pyridoxine deficiency is found to be common in chronic alcoholics. The causes of thiamine and pyridoxine deficiency in alcoholism include poor diet and reduced intestinal absorption. Pyridoxal deficiency is also related to ethanol metabolism in the liver because the production of acetaldehyde results in the displacement of pyridoxal phosphate from albumin, followed by urinary excretion of the unbound vitamin. Pyridoxal phosphate is very important for ALT activity. So, the deficiency of pyridoxine may be a reason for reduced activity of ALT than AST in alcoholic liver cirrhosis.

In our study, the AST/ALT ratio is found to be increased in all diseased categories than the control category. The study of Cohen and Kaplan (1979) and Correia et al., (1981) described that AST/ALT ratio of patients with alcoholic liver disease is above 2, and in patients with non alcoholic liver disease, the AST/ALT ratio was below 1. They also reported that there was a very low AST/ALT ratio in toxic and viral hepatitis, chronic active hepatitis and cholestasis jaundice.

Numerous studies have suggested that the serum AST/ALT ratio may help discriminate between alcoholic and non alcoholic liver disease. In the present study, the mean AST/ALT ratio of ALC category is 1.8 and it is highly significant when compared to that of NALC and HCC categories. Most of the cases in ALC category
showed the value above 2, while most of the NALC and HCC category showed the value below 1. The AsT/AIT ratio below 1 has been reported in patients with non alcoholic steatohepatitis (Diehl et al., 1988; Bacon et al., 1994). Chedid et al., (1991) and Cohen and Kaplan, (1979) reported that AsT/AIT ratio often exceeds 2 in patients with alcoholic liver disease. Our study also relates the work of Cohen and Kaplan (1979) and Correia et al., (1981), in which they showed that most patients with alcoholic liver disease have ratios >2, whereas, most patients with non alcoholic liver cirrhosis had a ratio of <1. Many studies showed that the AsT/AIT ratio is often greater than 1 in cirrhosis (Sharp 1995; Williams and Hoofnagle, 1988; Park et al., 2000; Giannini et al., 2003) which is in accordance with our results.

Clearance of liver enzymes from plasma occurs at variable rates. The half life of AIT is 47 hours and the half life of cytosolic AsT is 17 hours which means more AsT is cleared from liver and the much longer half life of AIT leads to higher activities of AIT than AsT in most forms of hepatocellular injury. In many cases of liver inflammation AsT and AIT activities are elevated roughly in the ratio of 1:1. But in some conditions like alcoholic hepatitis and chronic hepatitis (infections), the serum AsT levels have been reported to be elevated higher than the serum AIT levels (Khokhar, 2003) that lead to the increase in AsT/AIT ratio to above 1.

4.3. Alkaline phosphatase (ALP)

In the present study, the level of ALP was found to be significantly increased in HCC category when compared to control, NALC and ALC categories. NALC and ALC categories showed only a slight elevation in ALP value than the control category. Increased synthesis of ALP in diseased human liver was reported by Moss (1994). Increased level of ALP has been reported in cirrhotic patients (Sivagurunathan et al., 2006).
Increased level of ALP is an indication of hepatobiliary disease. ALP is a membrane bound glycoprotein enzyme on the canalicular membrane of the hepatocytes (Ludwig, 1980). The fragments of hepatocytes membrane rich in ALP activity have been detected in plasma of patients with cholestasis. This is due to the result of the fragmentation by bile acids. Schlaeger et al., (1982) in their in vitro studies demonstrated the membrane fragmentation by bile acids. It has been found that fasting plasma bile acid concentration is elevated in hepatocellular carcinoma. Elevated levels of alkaline phosphatase are seen in primary or secondary liver cancer (Stigbrand and Wahren, 1992). Elevation of serum ALP with hepatic infiltration likely results from compression of small intrahepatic bile ducts. This implies that release of ALP by bile acid fragmentation of membrane and compression of small intrahepatic bile ducts may be the reasons for increased level of ALP in hepatocellular carcinoma.

4.4. Gamma glutamyl transferase (GGT)

In the present study, the serum GGT level was found to be increased in NALC, ALC and HCC categories when compared to the control category. McCullough (2002) and Edoardo et al., (2005) reported that the whole spectrum of liver diseases, regardless of cause may be responsible for altered GGT serum levels. GGT levels may be two to three times greater than the upper reference value in more than 50 % of patients with nonalcoholic fatty liver disease and above the upper reference values in about 30 % of patients with chronic hepatitis C infection. Tumor associated isoenzymes of gamma glutamyl transferase has been reported in HCC (Kojima et al., 1980; Kew et al., 1984). Further more, an increase in GGT levels in patients with chronic liver disease is associated with bile duct damage and fibrosis (Giannini et al., 1999).
GGT is a microsomal enzyme and its activity is found to be induced by several drugs (Rosalki et al., 1971). Edoardo et al., (2005) observed increased serum levels of GGT in alcoholic liver disease and they proposed that, it may be the result of enzyme induction and decreased clearance, and in these patients, GGT serum levels can be markedly altered (>10 times the upper reference values). Increased synthesis of GGT and decreased synthesis of total protein was observed in the HepG2 culture in the presence of alcohol (Moirand et al., 1990). Penn and Worthington (1983) also reported an increased level of hepatic microsomal GGT by the induction of alcohol. In the present study also, the serum level of GGT in ALC category is significantly higher than that of NALC and HCC categories, which implies the increased synthesis of GGT by alcohol.

**SERUM ACUTE PHASE PROTEINS**

### 4.5. C-reactive protein

It has been found that the plasma half life of CRP is about 19 hours and is constant in all conditions of health and diseases. So, the sole determinant of circulating CRP concentration is the synthesis rate, which thus directly reflects the intensity of pathological processes stimulating CRP production (Vigushin et al., 1991). In the present study, the CRP level was found to be significantly increased in NALC, ALC and HCC categories when compared to the control group. It is known that serum CRP is elevated in various liver diseases such as acute hepatitis (Atono et al., 1989), cirrhosis (Lee et al., 1989) and hepatocellular carcinoma (Lee et al., 1989; Murakami et al 1989). However, those serum levels are not so high as in other inflammatory diseases (Lee et al., 1989). According to early *in vitro* studies, cultured hepatocellular carcinoma cells can produce CRP that is regulated in part by proinflammatory cytokines (Goldman and Liu 1997; Gabay et al., 1995). Patients with hepatocellular carcinoma and cirrhosis had higher CRP levels as reported by
Shiota et al., (1995). Our results also corroborate with this study. In the same study, Shiota et al., (1995) reported that serum hepatocytes growth factor levels were found to be increased in patients with liver disease and serum HGF showed a positive correlation with CRP. Interleukin–6 appears to be the principal regulator of most acute phase proteins (Baumann and Gauldie 1994; Taga and Koshimoto 1992) although other inflammation associated cytokines also contribute to this process.

Huseyin et al., (2006) showed an increase in IL–6 in hepatocellular carcinoma and liver cirrhosis than control, and a highest value was observed in hepatocellular carcinoma. IL–6 is found to be strongly inducing the expression of CRP in human hepatocytes (Streetz et al., 2001; Claire Arnaud et al., 2005). Other cytokines such as IL-1β can also induce CRP production (Pasceri et al., 2000). Cultured hepatoma cells can induce the production of CRP by monocyte factor (Neil and Teh, 1987). All these studies show that CRP is produced by the induction of inflammatory mediators like IL–6, IL–1β and monocyte factors. All these factors are produced in different milieu in cirrhosis and HCC leading to increased production of CRP than normal liver.

4.6. Transferrin

In the present study, serum transferrin level was found to be decreased significantly in all the three diseased categories (NALC, ALC, HCC) when compared to the control category. Transferrin, the main protein, in β fraction, is found to be decreased in liver diseases (Kawai, 1973). Ramadori et al., (1988) observed diminished output of liver derived proteins, albumin and transferrin, following inflammation and injury. Transferrin is a negative acute phase protein and its synthesis is found to be decreased by interleukins (Thompson et al., 1991). They also showed that insulin is able to inhibit the synthesis of transferrin, which is seen higher in liver diseases with insulin resistance. Hiramatsu et al., (1976) observed increased transferrin levels in chronic and inactive hepatitis but not in
cirrhosis and liver cancer. Shi-Min Luo et al., (2004) reported that the serum prealbumin and transferrin are lower in hepatocellular carcinoma patients with cirrhosis than normal control group. All these studies report that the serum transferrin level is decreasing in liver cirrhosis and liver cancer.

Potter et al., (1985) observed a significantly decreased mean transferrin concentration and decreased synthesis of transferrin in alcoholic liver cirrhotic patients. In the present study highest decrease in serum transferrin was observed in ALC category than HCC and NALC categories. Carbohydrate deficient transferrin is found to be increased in alcoholic hepatitis (Yamauchi et al., 1993). Transferrin is a glycoprotein and it exists in the form of glycosylated isoforms in serum. Specific alterations in the glycosylation of acute phase proteins occur in many pathophysiological states like acute and chronic inflammation and cancer (Hiron et al 1992; Feelders et al., 1993). It has been noted that the pattern of change in glycosylation is dependant on the particular state or the particular disease and to some extent on the nature of acute phase proteins. Glycosylated isoforms of transferrin is well studied (Feelders et al., 1993). The biological functions of glycosylated proteins is typically determined by the protein component and, carbohydrate can play a role in molecular stability, solubility, in vivo activity, serum half life and in particular can extend the serum half life of protein therapeutics (Elliott et al., 2003). N-glycosylated proteins with high sialic acid content were found to have reduced renal clearance and increased in vivo bioactivity (Creus et al., 2001; D’ Antonio et al., 1999).

Pradeep and Lekshman (1997) in their animal studies showed marked decrease in the incorporation of labeled sugars into transferrin and a marked decrease in the enzymatic activities of glycosyl transferase and sialyl transferase occurred in rats, chronically administered with ethanol. Alcohol interferes with a number of glycoconjugation reactions, as a result of acetaldehyde inhibition of
hepatic glycotransferases. This inhibition leads to the formation of carbohydrate deficient isoforms of transferrin (Fleming et al., 2004; Golka and Weise, 2004; Wuyts and Delanghe, 2003). Consumption of large amount of alcohol is found to be causing decreased sialylation of transferrin (Vesterberg et al., 1984). It is also proposed that there is reduced activity of liver glycoprotein glycosyltransferase (Stibler and Borg, 1991; Ghosh et al., 1993) as well as increased hepatic and plasma activities of sialidase (Ghosh et al., 1993) associated with alcohol consumption. Stibler and Borg (1986) reported that chronic alcohol consumption reduces the number of carbohydrate moieties attached to serum transferrin and producing carbohydrate deficient transferrin. It has been found that the carbohydrate deficient transferrin occur in elevated levels in the blood of alcoholics (Stibler, 1991). The half life of carbohydrate deficient transferrin in serum is found to be very low. So, in ALC category the carbohydrate deficient transferrin is easily cleared from the serum leading to a decrease in transferrin than in NALC and HCC categories.

4.7. Ferritin

Isolated and non specific increase in serum ferritin levels are frequently found in the absence of iron over load and are associated with inflammation, liver necrosis and alcohol abuse (Bacon et al., 1994). In our study, serum ferritin was found to be increased significantly in HCC and ALC categories than in NALC and control categories. Bacon et al., (1994) also reported abnormal serum ferritin level in 58 % of patients with non alcoholic steatohepatitis. In our study also there is a slight increase in serum ferritin levels in non alcoholic liver cirrhosis, but that was not significantly different from the control category.

Many studies have shown that serum ferritin level is increased in liver cirrhosis and HCC. The study of Caroline et al., (2004) showed a significant increase in ferritin in liver cirrhosis than the control category. Rat liver and spleen ferritin synthesis is elevated 3–4 folds six hours after the onset of an experimentally
induced inflammatory response (Konijn et al., 1977; Campbell et al., 1989). Bell et al., (1994) found that serum ferritin is elevated in alcohol abusing patients with alcoholic liver disease than in patients with chronic liver disease. Serum ferritin and transferrin saturation can be increased in alcoholic liver disease (Brissot et al., 1981; Chapman et al., 1982). Many studies showed an elevated value of serum ferritin in alcoholic liver disease (Loreal et al., 1992; Bufler et al., 1995; Verhasselt et al., 1997; Sugawara 1998). In a HepG2 culture study, it was reported that there is an increased synthesis of ferritin and decreased total protein in the presence of alcohol (Moirand et al., 1990).

It has been found that IL–1β stimulates ferritin production by liver hepatoma cells (HepG2) because plasma iron level characteristically falls during acute phase response (Bazil et al., 1991). This reduction in iron level results from an increase in liver ferritin synthesis by IL-1β as demonstrated in a rat model by Konijn and Hershko (1981). Jack et al., (1990) reported that human hepatoma cells when stimulated with IL-1β, do exhibit a marked increase in the steady state level of ferritin shells and which can be measured by protein staining on non denaturing gels. They also suggest that ferritin protein accumulate in these cells rather than being degraded. There is also evidence that increased rat liver ferritin synthesis is controlled in the level of transcription during the acute phase response (Konijn 1981; Campbell et al., 1989). IL–1β is high in HCC than in cirrhosis. Increased level of IL–1β may be enhancing the synthesis of ferritin in HCC. In alcoholic liver cirrhosis, alcohol induces the synthesis of ferritin and release of ferritin from hepatocytes due to tissue necrosis as well as the induction of IL-1β for the synthesis of ferritin in hepatocytes. The over all effects may be contributing to the increased level of serum ferritin in ALC category than HCC category in the present study.
4.8. Ceruloplasmin

In our study, the ceruloplasmin level was found to be increased significantly in HCC category when compared to control as well as NALC and ALC categories. Ceruloplasmin is a member of acute phase protein family and its level is found to be increased during inflammation as well as in various malignancies (Gitlin, 1988; Ramadori et al., 1988). Diehl (1999) reported an increased ceruloplasmin concentration in the serum of hepatocellular carcinoma patients when compared to chronic hepatitis patients. Fey et al., (1994) reported that the elevation of ceruloplasmin during acute phase response is usually less than two fold. In the present study also there is a significantly increased level, but less than two fold increase of ceruloplasmin in HCC category when compared to control. Inflammatory cytokines, including interleukins locally produced in the liver cells are inducers of ceruloplasmin synthesis in cirrhotic patients (Ramadori et al., 1988). Hepatocellular hypoxia was demonstrated in experimental cirrhosis (Corpechot et al., 2002), which could induce an increased synthesis of ceruloplasmin mRNA (Mukhopadhyay et al., 2000). In the present study also some cases in NALC and ALC categories showed a slight increase in ceruloplasmin level than the control category, though there was no statistical significance.

Earlier studies reported a modest increase in ceruloplasmin concentration in the serum of hepatocellular carcinoma patients in comparison with chronic hepatitis patients (Casaril et al., 1989). Our studies reveal that the serum ceruloplasmin concentration of HCC category showed a significant increase when compared to NALC and ALC categories. The study of Dominique et al., (2001) showed that ceruloplasmin accumulated to extremely high levels in the serum of the transgenic mice developing hepatocellular carcinoma and they proposed that the accumulation of ceruloplasmin in the serum is due to the increased expression of ceruloplasmin genes as well as an increase in the mRNA stability. Ross, (1995) revealed that the half life of ceruloplasmin mRNA is increased in the transgenic mice. It has been
proposed that a longer mRNA half life may increase the rate of translation, leading to this elevated condition (Ross, 1995).

It is also noted that the amount of plasma ceruloplasmin is 10 times higher in humans than in mice, and the variation in the normal ceruloplasmin values within the human population is large (250 – 630 mg/L) (Ross, 1995). The results in our study also showed the same pattern. The mean value of ceruloplasmin is near to the upper reference limit, and about 11 out of 38 cases showed ceruloplasmin values above the upper reference limit. It is reported that moderately increased levels of ceruloplasmin is observed in human hepatocellular carcinoma, and is a prognostic value for elevated plasma ceruloplasmin concentration corresponding to more rapidly progressing tumor (Casaril et al., 1989; Knekt et al., 1992; Senra et al., 1997). Dominique et al., (2001) also reported that ceruloplasmin concentration increases with tumor mass. This indicates that the increased level of ceruloplasmin, above the reference limit, in some cases in HCC category, in our study, may be due to the increased tumor growth.

Ceruloplasmin is a glycoprotein and its half life in the plasma is determined by the extent of the sialylation of its N-glycan chains (Morell et al., 1971). It has been found that transgenic mice express in their liver much higher amount of the galactoside and sialyl transferase that transfer sialic acid specifically to N-glycans (Pousset et al., 1997). N-linked glycosylation can increase in vivo potency of molecule through elevating its half life (Angus et al., 2005). All these studies showed that the level of ceruloplasmin is increased in HCC probably due to increased mRNA synthesis, increased mRNA half life and increased half life of ceruloplasmin.