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Evaluation of Lipid Profile In Liver Cancer

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Abstract
The lipid profile of ten liver cancer patients were compared with the lipid profile of ten normal subjects at the Chemical Pathology Department of the University Teaching Hospital Ado-Ekiti, Ekiti State Nigeria., with a view to establish whether liver cancer have significant effect on lipid profile. The parameters examined were Total Cholesterol (TC), Triglyceride (TG), High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C) and Atherogenic risk ratio (TC:HDL-C). The results obtained were subjected to statistical analysis to examine the level of Significance difference between lipid profile of liver cancer patients and those of normal subjects. The results of analysis of variance (ANOVA) under 0.05 (5%) probability with F-table value at degree of freedom 1, 18 being 4.41 showed that level of TC (F-calculated = 5.56) and LDL-C (F-calculated = 6.73) were significant while the level of HDL-C (F-calculated = 1.78), TG (F -calculated = 0.70) and TC:HDL-C (F-calculated 0.02) were not significant . The Duncan's multiple range test also showed that the difference between means of TC (DBM = 1.114) of the liver cancer patients and the normal subjects was significant when compared to 0.993 Least Significant Range (LSR). Similarly the difference between means of LDL-C (DBM = 0.946) of the liver cancer patients and the normal subjects was significant when compared to 0.766 LSR. However, there were no significant difference in the DBM and LSR of TG, HDL-C and TC: HDL-C since DBM was less than LSR in those cases. The results of Students T- test from the statistical analysis conformed to the results of the ANOVA and Duncan' Multiple Range Test. Thus, from the statistical analyses, it could be concluded that liver cancer have significant effect on the TC and the LDL-C with little or no effect on the TG, the HDL-C and the Atherogenic risk ratio of the subjects examined in this study. Since high level of LDL-C indicates high risk of Coronary Heart Disease (CHD), it was therefore recommended that lipid profile of liver cancer patients should be monitored to prevent CHD among liver cancer patients.

1.0 Introduction

Liver is one of the most important organs in energy metabolism. Most plasma apolipoproteins, endogenous lipids and lipoproteins are synthesized in the liver (Bell, 1979), which depends on the integrity of cellular functions of liver (Eisenberg, 1975). Under normal physiological conditions, liver ensures homeostasis of lipid and lipoprotein metabolism. Hepatic cellular damage and Hepatocellular Carcinoma (HCC) impairs these processes, leading to alterations in plasma lipid and lipoprotein patterns. Mortality due to liver cancer is the fifth common malignant tumor worldwide, and it is closely related to the infections of hepatitis B virus (HBV) (Buendia, 1992) and hepatitis C virus (HCV). HBV proteins, such as the hepatitis B X protein (HBx) that is large hepatitis B surface protein could regulate transcription of many candidate genes for liver carcinogenesis. As HBV and HCV infections are quite common in China and in other Southeast Asian countries, the mortality of HCC is 20,4/100,000 in the population of China, corresponding to about 18.8% of all fatal malignant tumors (Parkin *et al*., 2001). Hepatic diseases differ from most other causes of secondary dyslipidemia in that the circulating lipoproteins are not only present in abnormal amounts but they frequently also have abnormal composition, electrophoretic mobility and appearance. It has been demonstrated that patients suffering from acute hepatitis B could have lipid disorders, for instance, decreased plasma HDL (Colombo *et al.,* 1982). The aberrations of lipid metabolism are often seen in the chronic hepatitis B infection too. HDL and its major apolipoproteins, apoAI and apoAII, are frequently reduced in the patients suffering from cirrhosis or HCC. Decrease in the level of serum LDL cholesterol in patients with liver disease was significantly correlated to the increasing severity of the disease (Cooper *et al.,* 1996). Decreased serum levels of cholesterol and apoAI may indicate a poor prognosis.

The patients with HCC frequently have other liver diseases such as chronic hepatitis and cirrhosis. All these conditions (hepatitis and cirrhosis of the liver) are often associated with plasma lipid and lipoprotein aberrations. It has been demonstrated that plasma triglycerides (TG) decreased by 20–30% in the patients with HCC. In contrast, Alsabti (Alsabti, 1979) reported that serum TG in HCC patients were increased when compared to those with cirrhosis. Alsabti, 1979 reported that plasma TG levels in HCC patients were not significantly different compared with controls. These results emphasize the fact that changes of plasma lipid profile may not always imply the presence of HCC and one need to exercise caution in interpreting these results.

It is known that lipids and lipoprotein metabolism could be regulated by cytokines. For instance, interleukin-6 (IL-6), tumor necrosis factor (TNF-α), IL-1 may inhibit TG synthesis. Tumor cells are known to produce large amounts of pro-inflammatory cytokines that, in turn, may suppress plasma TG levels. Cooper, et al., 1996 reported that IL-1 profoundly affects lipid metabolism by delaying intestinal absorption and decreasing tissue uptake. IL-2 could induce severe hypocholesterolemia that is mediated by the inhibition of lecithin:cholesteryl acyltransferase (LCAT) activity. IL-1 and IL-6 significantly decreased microsomal triglyceride transfer protein (MTP) mRNA levels in HepG2 cells. It is believed that MTP is related to the synthesis of very low density lipoprotein (VLDL). In addition, these cytokines could also decrease lipolysis *in vivo*. Similar results have been reported in other types of cancer.

About 80% endogenous cholesterols are synthesized in the hepatocellular microsomes that contain cholesterol synthesis enzymes. In HCC and chronic liver diseases the synthesis and metabolism of cholesterol are impaired. It leads to a decrease in plasma cholesterol levels (Cooper *et al.,* 1996). Motta *et al.,* 2003 reported in their publication that the relationship between serum cholesterol and occurrence of cancers in 9021 employees aged from 35 to 64 years old. No evidence of association of serum cholesterol level with total cancer mortality was seen by Log-rank trend test. But there was a significant negative correlation between serum cholesterol level and HCC ($P < 0.05$). This negative correlation also existed between serum cholesterol level and chronic hepatitis and liver cirrhosis. Similar results were described in patients with gastrointestinal cancer, however, lower cholesterol levels are not related to the cancer stages. Decreased serum cholesterol concentrations were also found in other cancers, which are probably related to the increased consumption of cholesterol by the tumor cells, as cholesterol levels in the hepatoma tissues were doubled compared to the control tissues. In addition, it has been reported that synthesis of cholesterol is reduced under cancers and moderate increases of serum cholesterol levels and increased body mass index (BMI) may have a protective effect on cancer mortality. It was observed that the plasma HDL-cholesterol was inversely correlated to the cholesterol levels in the tumor tissues in cancer patients.

2.0 Materials and Method

In all the subjects, venous blood samples were collected after overnight fasting into tubes containing Lithium heparine for lipid profile assay. The samples were centrifuged to separate the serum from the blood plasma. The serums obtained were stored by refrigeration until the analyses were carried out.

Randox compact kits were employed for measurement of TC, TG, CHD-L and LDL-C. The instructions provided by the manufacturer were strictly observed to minimize variation.

Procedure for total cholesterol assay

The serum was mixed well and incubated at 37℃ for 5 minutes after which it was removed from the water bath and cooled to room temperature. The spectrophotometer was set to zero by using double distilled was as blank at 540nm wavelength. The absorbance of the standard and those of the serum samples were similarly obtained at 540nm using 1ml of reagent and sample in a cuvette of 1cm pathlenght.

Procedure for triglyceride assay

The serum was well mixed and incubated at 37℃ for 5 minutes. Using fresh double distilled water as blank; the spectrophotometer was set to zero. The absorbances of the standard and sample were similarly obtained using $1000\mu l$ of reagent with $10\mu l$ of standard and $10\mu l$ of sample respectively.

Procedure for HDL-C assay

Direct method of analysis was adopted for measuring of HDL-C concentration in serum sample without sample pretreatment.200ml of serum was well mixed with 500ml of precipitant. The mixture was allowed to sit for 10 minutes at room temperature and the centrifuged. The cleared supernatant was separated after 2hours and the cholesterol content of the supernatant was determined by CHOD PAP Method. After calibration of the spectrophotometer, a mixture of $100\mu l$ of standard and sample were separately added to $1000\mu l$ of reagent to obtain reading at 540nm

Determination of concentration LDL-C

LDL-C = TC - TG/2.2 - HDL-C

3.0 Results and Discussion

The results of the laboratory analysis of serum sample from liver cancer patients and normal subjects for total cholesterol (TC), triglycrides (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-C) concentration in mmol/l is presented in Table 3.0 while the result of atherogenic risk ratio (index) is presented in Table 3.1.

Table 3.1: The concentrations of total cholesterol (TC), triglycrides (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-C), measured in mmol/l.

	Unit of Measurement mmol/l							
S/NO	**TC_p**	**TC_n**	**TG_p**	**TG_n**	**$HDLC_p$**	**$HDLC_n$**	**$LDLC_p$**	**$LDLC_n$**
1	5.22	3.4	0.82	2.00	0.86	0.7	3.99	1.8
2	5.93	3.6	1.49	1.70	2.23	1.1	3.03	2.7
3	5.78	5.2	1.05	1.50	2.02	2.2	3.28	2.3
4	6.3	4.7	2.16	1.10	2.25	1.2	3.06	2.8
5	3.8	4.9	0.85	1.20	1.76	1.8	1.63	2.6
6	2.18	3.7	0.92	1.90	0.65	1.2	1.11	1.6
7	4.68	4.1	0.98	1.00	2.41	2.1	3.55	1.5
8	6.49	3.7	0.78	1.60	2.69	2.1	3.44	0.9
9	5.65	4.2	0.504	0.07	2.34	2.0	3.08	1.9
10	6.41	3.8	2.34	2.10	3.15	2.0	2.19	0.8

Table 3.2: Atherogenic risk ratio in serum of liver cancer patients and normal subjects.

	Atherogenic Risk Ratio	
S/NO	**$TC_p/HDLC_p$**	**$TC_n/HDLC_n$**
1	6.0698	4.8571
2	2.6592	3.2727
3	2.8614	2.3636
4	2.8000	3.9167
5	2.1591	2.7222
6	3.3538	3.0833
7	1.9419	1.9524
8	2.4126	1.7619
9	2.4145	2.1000
10	2.0349	1.9000

Table 3.3: ANOVA analysis of total cholesterol (TC) concentrations in serum of liver cancer patients and those of the normal subjects

S/NO	TC_p (mmol/l)	TC_p^2 $(mmol/l)^2$	TC_n (mmol/l)	TC_n^2 $(mmol/l)^2$
1	5.22	27.2484	3.4	11.56
2	5.93	35.1649	3.6	12.96
3	5.78	33.4084	5.2	27.04
4	6.3	39.69	4.7	22.09
5	3.8	14.44	4.9	24.01
6	2.18	4.7524	3.7	13.69
7	4.68	21.9024	4.1	16.81
8	6.49	42.1201	3.7	13.69
9	5.65	31.9225	4.2	17.64
10	6.41	41.0881	3.8	14.44
Total	52.44	291.7372	41.3	173.93
Mean	5.244		4.13	

Note: Subscript p implies HCC patient while n implies normal subject

Table 3.4: Summary of Statistical Analysis of The mean, ANOVA F-ratios and Duncan's multiple range tests of results of TC, TG, HDL-C, LDL-C and TC/HDL-C for both liver cancer patients and normal subjects

	MEAN		**ANOVA**			**Duncan's MRT**		
	M_p	M_n	F-cal	F-tab	Sig. of dif.	LSR	DBM	Sig. of dif.
TC	5.244	4.130	5.56	4.41	Sig.	0.993	1.114	Sig.
TG	1.189	1.417	0.07	4.41	Not Sig.	0.572	0.228	Not Sig.
HDL-C	2.036	1.640	1.78	4.41	Not Sig.	0.624	0.396	Not Sig.
LDL-C	2.836	1.890	6.73	4.41	Sig.	0.776	0.946	Sig.
TC/HDL-C	2.871	2.793	0.02	4.41	Not Sig.	1.040	0.078	Not Sig.

Sig. implies significance or significant and dif. Implies difference

Table 3.5: Summary of results of coefficient of Variation (CV%) and Student T-test for TG, HDL-C, LDL-C AND TC/HDL-C

	Mean		Standard deviation		variance		Coefficient of variation		Student t-test		
	M_p	M_n	σ_p	σ_n	σ_P^2	σ_n^2	CV_p%	CV_n%	T-cal	T-tab	Sig. dif.
TC	5.224	4.130	1.364	0.611	1.860	0.373	26	15	2.36	2.26	Sig.
TG	1.189	1.417	0.613	0.605	0.376	0.366	51	43	0.84	2.26	Not sig.
HDL-C	2.036	1.640	0.772	0.536	0.596	0.287	38	33	1.33	2.26	Not sig.
LDL-C	2.836	1.890	0.907	0.713	0.822	0.508	32	38	2.59	2.26	Sig.
TC/HDL-C	2.871	2.793	1.202	1.004	1.445	1.008	42	36	0.16	2.26	Not sig.

Total cholesterol (TC)

The total cholesterol of most of the liver cancer patients was higher than those of the normal subjects. However, three liver cancer patients were found to have total cholesterol value above 6.21mmol/l. These individuals were at higher risk of coronary heart diseases (CHD) according to National Cholesterol Education Program (NCEP) Adult Treatment Panel II, 2001 recommendation which stated that TC value higher than 6.21mmol/l indicates high

risk of CHD. Also three cancer patients had TC values less than 5.17mmol/l which is the desirable recommended level by NCEP ATP II, 2001 National Institution of Heart (NIH), 1995. The remaining four patients were within borderline 5.17-6.18mmol/l as recommended by NCEP ATP II, 2001.

All the normal subjects used as control had TC value less than 5.17mmol/l except one with 5.2mmol/l. the result of the Analysis of Variance (ANOVA) from Table 3.4 showed that the difference between the TC level of liver cancer patients and normal subjects was significant as the F-calculated value (5.56) was higher than F-table value (4.41) obtained under probability of 0.05 (5%). Similarly, the Duncan's multiple range test (DMRT) showed that the difference between means (DBM= 1.114) TC of liver cancer patients and normal subjects was significant when compared to least significant range (LSR= 0.993). Also, the student t-test result showed that under 0.05 probability, the calculated t-value (2.357) was significant being higher than t-table value (2.26). From the coefficient of variation (CV%), it was revealed that result of TC of the normal subjects were more uniform than those of the liver cancer patients.

Thus, it could be concluded from this study that liver cancer have significant effect TC or could alter TC level. The result from this study was similar to those reported by Alsabti *et al.,* 1979, Cooper *et al.,* 1996, who observed varying degree of difference in TC of patients suffered from hepatocellular carcinoma (liver cancer).

Triglyceride (TG)

The triglyceride concentrations of both liver cancer patients and the normal subjects were low and within acceptable range. The TG value of the liver cancer patients range from 0.78-2.16mmol/l which was far lower than the 2.8mmol/l recommended by NIH, 1993 and NCEP ATP II, 2001. Similarly, the range of TG in normal subjects range from 0.07-2.1mmol/l. This might be an indication that liver cancer have little significance on TG.

From table 3.4, the ANOVA result for TG showed no significant effect as the F-cal. Value (0.07) was very low compared to F-tab (4.41). The Duncan's multiple range test showed that DBM of means of TG of normal subject and liver cancer patients (0.228) for TG was not significant when compared to LSR (0.572).

From Table 3.5, the student t-test also showed that there was no significant difference between the means of TG of the liver cancer patients and the normal subjects as the Tcal. (0.84) was less than T-tab (2.26). CV% showed a relative average uniformity in both the normal subjects and the liver cancer patients. The result obtained for TG were similar to those reported by Alsabti *et al.,* 1979, who reported that there was no observable change in TG of liver cancer patient compare to normal subjects.

High density lipoprotein cholesterol (HDL-C)

High density lipoprotein cholesterol was higher in live cancer patients compared to those of the normal subjects. However, two liver cancer patients and one normal subject have HDL-C value lower than the recommended value of 1.03mmol/l by NCEP ATP II, 2001. This is a strong indication of coronary heart disease (CHD) as low level of HDL-C may increase the risk of CHD.

The ANOVA result of HDL-C showed that F-cal (1.78) was less than F-tab. (4.41), thus, the difference in the HDL-C of liver cancer patients and of normal subjects was not significant. The result of Duncan's multiple range test showed the DBM (0.396) of HDL-C of both liver cancer patients and normal subject was not significant being less than LSR (0.624). The student t-test result also buttress the fact that there was no significant difference between the HDL-C means of the liver cancer patients and the normal subjects. CV% showed fairness in uniformity of HDL-C in both the normal subjects and the liver cancer patients. The result of the analysis showed that the effect of liver cancer was minimal on HDL-C level in the subjects examined in this study. This was in accordance with report of Motta *et al.,* who reported that there was no significant difference between HDL-C of normal and hepatocellular carcinoma patients (Motta *et al.,* 2003).

Low density lipoprotein cholesterol (LDL-C)

The level of low density lipoprotein cholesterol in the liver cancer patients ranged from 1.11 to 3.99mmol/l was higher than that in the normal subjects in this study, which ranged from 0.8 to 2.8mmol/l. Although the level of LDL-C was higher, the values were with the borderline of 3.64-4.11mmol/l reported by NCEP ATP II, 2001. This was indicative that majority of the liver cancer patients were at risk of developing CHD

The ANOVA result of LDL-C showed that F-cal (6.73) was higher than F-tab. (4.41), thus, the difference in the LDL-C of liver cancer patients and of normal subjects was significant. The result of Duncan's multiple range test showed the DBM (0.946) of HDL-C of both liver cancer patients and normal subject was significant being higher than LSR (0.766). The student t-test result also underscred the fact that there was significant difference between the LDL-C means of the liver cancer patients and the normal subjects. CV% showed fairness in uniformity of LDL-C in

both the normal subjects (CV% = 38) and the liver cancer (CV% = 32) patients. The result of the analysis showed that the effect of liver cancer was of high significance on LDL-C level in the subjects examined in this study. The report of Motta *et al.,* 2003 supported the findings of this study because they reported that hepatocellular carcinoma in liver cancer patients significantly affected LDL-C level when compared to that of normal subjects

Atherogenic risk ratio (TC/HDL-C)

The atherogenic risk ratio calculated as the ratio of TC to HDL-C is an indicator for coronary heart disease (CHD). According to Cooper *et al.,* 1996, atherogenic ratio greater than 6.0 indicates high risk of CHD. The atherogenic risk ratio among the subjects examined was very low except a liver cancer patient with risk ratio 6.07.

From table 3.4, the ANOVA result for atherogenic risk ratio showed no significant effect as the F-cal. Value (0.02) was very low compared to F-tab (4.41). The Duncan's multiple range test showed that DBM (0.078) of means of atherogenic risk ratio of normal subject and liver cancer patients for atherogenic risk ratio was not significant when compared to LSR (1.040).

From Table 3.5, the student t-test also showed that there was no significant difference between the means of atherogenic risk ratio of the liver cancer patients and the normal subjects as the Tcal. (0.157) was less than T-tab (2.26). CV% showed a relative uniformity in both the normal subjects and the liver cancer patients. The result obtained for atherogenic risk ratio was similar to those reported by Buendia, 1992.

4.0 Conclusion

The statistical analysis showed that liver cancer significantly affect the concentration of TC and LDL-C but have minimal effect on the TG, HDL-C and Atherogenic risk ratio. This result showed that liver cancer had effect on some parameters of lipid profile and might have negative influence on the management of coronary heart disease (CHD). Thus liver cancer patients must monitor their lipid profile to avoid CHD.

References

Alsabti EA: Serum lipids in hepatoma. *Oncology* 1979, 36(1):11-1

Bell AW: Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. *Prog Lipid Res* 1979, 18(3):117-164.

Buendia MA: Mammalian hepatitis B viruses and primary liver cancer. *Semin Cancer Biol* 1992, 3(5):309-320.

Eisenberg S, Levy RI: Lipoprotein metabolism. *Adv Lipid Res* 1975, 13:1-89.

Colombo E, Mauri A, Scapaticci R, Bosoni AM, Bertella M, Nutta *orino)* 1982, 139(2):187 191. .G: [Clinical significance of HDL-cholesterol variations in viral hepatitis]. *Arch Sci Med (T*

Cooper ME, Akdeniz A, Hardy KJ: Effects of liver transplantation and resection on lipid parameters: a longitudinal study. *Aust N Z J Surg* 1996, 66(11):743-746Allen JK, Cotran

Miller, K.W. (1990) Surface-to-core and interparticle equilibrium distribution of triglyceride-rich lipoprotein lipids. *J. Biol. Chem.* 258, 13772-13784

Motta M, Giugno I, Ruello P, Pistone G, Di Fazio I, Malaguarnera M: Lipoprotein (a) behavior in patients with hepatocellular carcinoma. *Minerva Med* 2001, 92(5):301-305.

National Institutes of Health Consensus Conference. Triglyceride, high-density lipoprotein and coronary heart disease. JAMA 1993 Jan;269(4): 505-10.

National Institutes of Health Consensus Conference. Treatment of hypertriglyceridemia. JAMA 1995 Mar 2;251(9):1196-1200.

National Cholesterol Education Program. Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). Circulation 2001 Mar;89(3):1329-1445.

Parkin D.M, Bray F, Ferlay J, Pisani P: Estimating the world cancer burden: *Int J Cancer* 2001, 94(2):153-156.

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