

protein thiols and TEARS levels in the patients associated with elevated level AFP.

Lipid peroxidation is known to play a key role in carcinogenesis (5). Thiobarbituric acid reactive substance (TBARS) measurement is the method of choice for screening and monitoring lipid peroxidation, as they are major indicators of oxidative stress (6).

Sulfhydryl compounds protect normal and cancer cells from free radical damage (7). Plasma (protein) SH groups are susceptible to oxidative damage (8) and are often low in patients suffering from diseases such as coronary artery disease (9). An assay to measure protein thiols and TBARS levels in plasma during liver cancer is also discussed.

MATERIALS AND METHODS

Levels of serum AFP, copper, ceruloplasmin, protein thiols and TBARS were estimated in patients with liver cancer. Study was conducted on the blood samples of patients sent for investigation to the department of Clinical Biochemistry, Kasturba Medical College, Manipal.

Serum samples were obtained from 25 patients (Mean age: 60 ± 15 years) with clinically and histologically verified liver cancer. Normal healthy age and sex-matched volunteers were taken as controls. The AFP estimation in these samples was done using Elecsys based on the electrochemiluminescence principle. Copper in the samples was estimated using bathocuproin disulphonate method (10). Ceruloplasmin level was determined by diamine oxidase method (11) based on the

property of ceruloplasmin to catalyze the oxidation of colorless para-phenylene diamine to a blue violet complex, which can be estimated spectrophotometrically. Four molecules of thiobarbituric acid will react with two molecules of malonaldehyde to form a coloured compound, which is measured colorimetrically at 532 nm (12). All the chemicals and reagents were obtained from standard companies and were of analytical grade. The optical density of the reaction mixture was measured using GENESYS 10 UV (Thermospectronic). Statistical analysis was done using t-test for equality of means and one-sample test for TBARS. Significance was determined by Mann-Whitney U test, which was applied to compare the distribution of values in the test and control groups. $P < 0.01$, and $P < 0.001$ were taken as significant and highly significant, respectively.

RESULTS AND DISCUSSION

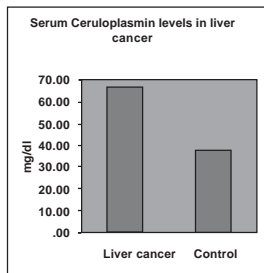
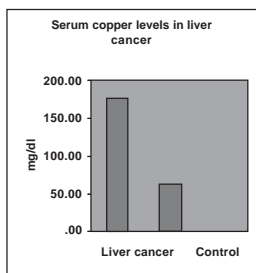
Table I depicts the serum levels of AFP, copper, ceruloplasmin, their ratio and TBARS levels in healthy volunteers and liver cancer patients. The serum AFP estimation is very useful in monitoring liver cancer patients, its early diagnosis and in defining the response to therapy and detecting an early recurrence of the disease. The AFP level was markedly elevated in patients with liver cancer. As reported by several workers we also noticed the decreased level of total protein and albumin when compared to control group. Rising trend in serum AFP will indicate appearance of early recurrence. Copper, ceruloplasmin and their ratio were significantly increased in liver cancer patients as compared to controls. Studies have reported that copper

TABLE I: Serum copper, ceruloplasmin, protein thiols and TBARS status in patients with liver cancer.

Groups	Copper	Ceruloplasmin	Cu: Ceruloplasmin	Protein	TBARS	AFP
Control	61.56±16.8	37.40±6.14	1.70±0.65	218.10±125.4	0.53±0.32	3.46±1.38
Liver Cancer	175.4±89.39**	66.52±17.59**	2.93±2.38**	116.9±60.01**	0.932±0.752*	13239±28129*

n = 25, *P<0.05, **P<0.001. Values are shown as mean ± SD.

The AFP values include an outlier (AFP = 34148) and three extreme values (AFP values = 59908, 59600 and 121000). When these four values were excluded from the analysis, mean and standard deviation of remaining 21 AFP values were 2682.45 and 3692.23 respectively. The values remained significantly higher compared to control group values.



Ceruloplasmin is a copper binding protein, which increases in several carcinomas. Lightman and Brandes (15) reported that decreased concentrations of zinc and the increased concentrations of copper in serum do not seem to result from a shift of zinc into or release of copper out of the malignant tumor tissue.

and ceruloplasmin levels were also significantly increased in prostate, colon and ovarian cancer (13). Our study showed a decrease in protein thiol without much variation in the TBARS level.

Sufficient evidence has been noted in the recurrence of the disease by rise in serum AFP concentration earlier than any clinical or radiological investigation. Therefore they suggest that patients with liver cancer should get serial estimation of serum AFP during the course of their treatment.

Cupric ions are reported to inhibit the production of singlet oxygen; this is of particular significance because of the latter's ability to cross the cell membrane and its high reactivity towards various biomolecules (14).

Lipid peroxidation is a well-established mechanism of cellular injury, which leads to production of lipid peroxides and their byproducts. Malonaldehyde peroxidation of polyunsaturated fatty acids and related esters provide a convenient index of lipid peroxidation. In the present study we have seen a significant increase in TBA reactivity. Plasma lipids are protected from peroxidation by naturally occurring antioxidants. The enhanced chromogen development with acetic acid of plasma and lipid standards (in which peroxidation has not been induced but which nevertheless contains small amount of peroxides) possibly reflects the acids solvent properties towards the less polar peroxidation products characteristic of the early stages of lipid peroxidation (16). Elevation of serum Copper, Ceruloplasmin and their ratios have

been reported to be useful in diagnosis and prognosis of other malignancies (17).

It has been noted that plasma SH groups are susceptible to oxidative damage and

often low in patients suffering from CHD and rheumatoid arthritis (18). Hence the estimation of copper, ceruloplasmin and protein thiols may serve as an adjuvant for the diagnosis of liver cancer.

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