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Research Article

Comparative Study of Biochemical and Nutritional Status of Breast Cancer Patients on Chemotherapy/Radiotherapy in Ibadan

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Abstract

A comparative study of BCA patients on chemotherapy/radiotherapy and immune booster supplements (combination of essential vitamins and minerals) with apparently healthy controls was investigated by assessing the lipid profile, hepatic transaminases (plasma AST, ALT) total bilurubin, lipid peroxidation and enzymatic and non-enzymatic antioxidant-superoxide dismutase (SOD) activity and plasma vitamins C and E respectively. There was no significant difference ($p<0.05$) in total cholesterol, but we observed a significant increase in triglyceride level in BCA patients compared to control. Protein concentration decreased significantly ($p<0.05$) in BCA patients, compared to control. SOD and vitamin C levels were not significantly different while vitamin E decreased significantly ($p<0.05$) in BCA patients compared to control. Lipid peroxidation increased ($p<0.05$) in BCA patients compared to control. We observed an increase in AST, a decrease in ALT and total proteins in BCA patients that were significant and a non-significant decrease in the bilirubin level when compared with control.

The results support the fact that BCA patients on chemotherapy have altered dietary intake and the enhanced metabolism of lipids and proteins may increased oxidative stress.

Keywords: Breast cancer; supplementation; antioxidants; lipid profile; liver toxicity

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Introduction

Breast cancer (BCA) is the most common cancer type in women worldwide, a major cause of morbidity in women, with1.15million new cases and 410,000 deaths in 2002 [1]. In Nigeria, the prevalence of BCA is about 116 per 100,000 and about 27,840 new cases were expected to develop in 1999 [2]. The relative frequency of BCA among other female cancer types as recorded in cancer registries in Nigeria are 35.4% in Ibadan, 44.5% in Enugu, 37.5% in Lagos, 29.8% in Calabar and 20.5% in Zaria. This is relatively higher in Southwest (Ibadan) and Southeast (Enugu) regions compared to Northern (Zaria) and south-south (Calabar) regions of Nigeria. Many epidermiological risk factors have been identified and associated with BCA, and often the cause of individual BCA is unknown. However, BCA is considered to be the final outcome of multiple environmental, hormonal and genetic hereditary factors [3, 4]. Many epidemiological studies have suggested that dietary factors affect the risk of BCA, as the diet does not only provide nutrients but conveys other substances that may participate in promoting or inhibiting carcinogenesis.

The presence of cancer may alter dietary intakes and the metabolism of specific nutrients [5] as well as patients' treatment regime such as radiotherapy and chemotherapy which may have adverse effects on the biochemical and nutritional status of the patients. The relationship between lipids and breast cancer is not clear as conflicting results have been reported on the association between lipids and the risk of BCA [6]. Although there is a long established correlation between the incidence of BCA and dietary fats in populations [7] in lipids, the true relationship between fat intake and BCA, does not seem to be particularly consistent [8]. Various studies have linked dietary fat to tumor growth via an eicosanoids synthesis [9], consequently this may become an impediment on the effect of chemotherapy on treatment of the disease and the risk of BCA.

BCA under chemotherapy may exert its effect via generation of reactive oxygen species (ROS) which induces oxidative damage to DNA and proteins on the one hand, while on the other hand increase in ROS could lead to increased lipid peroxidation and neoplastic transformation [10, 1]. Formation of lipid peroxide is normally prevented or scavenged by antioxidants both enzymatic and non-enzymatic. Experimental evidence reveals that reactive oxygen species are involved in initiation, promotion and progression of carcinogenesis where reactive inactivation or loss of certain tumor suppressor genes is concerned. The extent of ROS induced oxidative damage can be exacerbated by a decreased efficiency of antioxidant defense mechanism. Critical to the effects of lipid peroxidation products are the enzymatic and non-enzymatic antioxidant defense that protects the cells from oxidative stress through reduction of the reactive intermediate molecules formed. Dietary antioxidants consumption may be associated with reduced BCA risk. Humans require external sources of vitamins E and C through the consumption of antioxidant rich vegetables and fruits, as the body is unable to produce nutrients. In general the association between dietary factors and BCA remain controversial in the epidemiology literature, but there is some support for the lipid peroxidation pathway and protective effect from in vitro studies [12]. According to the findings, attention to plasma antioxidant vitamins and lipid peroxidation is of great importance to promote the level of health in women suffering from breast cancer [13].

Radiation therapy and chemotherapy with their visible side effects can also impact on the nutritional and biochemical status of BCA patients. Apart from physical impact of these treatment regimens, they also can interfere with digestion and metabolism of nutrients. In Nigeria, the incidence in BCA has been on the increase and the prevailing economic situation has greatly affected food intake, hence the comparism of age, body weight matched healthy control to BCA patients. To our knowledge, such study has been carried out on the biochemical and nutritional status of BCA patients in Nigeria with respect to these parameters. This gap informed our decision to investigate comparatively the impact of the ameliorative effects supplements given to BCA patients on radiotherapy or chemotherapy with normal controls. Also to investigate probable differences in their lipid profile, enzymatic and non-enzymatic antioxidant status, lipid peroxidation as index of oxidative stress and their liver function test as marker of hepatotoxicity. It is hoped that the study will improve our understanding of nutritional and biochemical status of BCA patients and probable relationship between nutrition, chemotherapy and pathogenesis of BCA.

Materials and Methods

Cholesterol, bilurubin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Triglyceride (TG) assay kits (Thermos Fischer) were bought from ABJ Chemicals Lagos, Nigeria. Adrenaline, Thiobarbituric acid (TBA), L-Ascorbic acid and bovine serum albumin (BSA) were purchased from Sigma Chemical (St Louis, MO, USA). All other chemicals were of the highest purity commercially available.

Study Location

Thirty BCA patients on either radiotherapy or chemotherapy were recruited from the Radiotherapy Department, University College Hospital (UCH) after obtaining their informed consent from both patients and controls. Furthermore, our protocol was approved by the College of Medicine ethical review committee of University of Ibadan. Patients in the middle income range and age- and -body weight matched healthy female volunteers in Ibadan North LGA. Exclusion criteria were presence of breast cancer, any other disease, mental instability and pregnancy. Thirty patients each and thirty controls, aged between 35 to 60 yrs were enrolled in study. Patient samples were collected in UCH and that of controls from Biochemistry Department, University of Ibadan. Laboratory analyses were carried out in the Clinical Biochemistry laboratory in Chemical Pathology Department, University college Hospital, Ibadan and in the nutritional Biochemistry laboratory, University of Ibadan.

Sample Collection

Eight mililitres of blood was obtained by vein-puncture from each subject and divided equally into ethylenediaminetetraacetic acid EDTA and lithium heparin anticoagulated bottles. Blood in lithium heparinised bottles was centrifuged at 300 rpm for 10 mins and the plasma was separated into clean tubes and stored at in the fridge until it was needed

Biochemical assays

Cholesterol was determined by cholesterol oxidase /peroxidase method using Randox kit by absorbance measurement at 490 nm when cholesterol reacts with FeS04 in glacial acetic acid

is treated with H2SO4. Triglycerides was estimated after enzymatic hydrolysis with lipases by measuring quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under catalytic influence of peroxidase as described by Rafai *et al*., 1999 [14]. HDL and LDL-cholesterol was determined using Human cholesterol liquiolor test kit [15]. The lipoproteins were assayed using an enzymatic colorimetric method for VLDL and LDL by precipitation using phosphotungistic acid and magnesium chloride. After centrifugation at 3000 g for 10min at 250C, the clear supernatant contained HDL fraction using HDL-cholesterol precipitant kit. The LDL-cholesterol (LDL-c) was calculated using the formulae of Friedwald *et al*. 1972 [16].

Lipid peroxidation was assayed using the method of Varshney and Kale 1990 [17] by estimating thiobarbituric acid reactive substances (TBARS) formation. SOD was determined using the method of Misra and Fridovich 1972 [18]. A simple colorimetric method, as described by Kway 1978 [19], was used for vitamin C, and vitamin E was determined using a slight modification of the method. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using assay kits as described by Reitman and Frankel and Schmidt and Schmidt [20, 21] respectively. Bilirubin was determined using assay kit by diazotizing plasma bilirubin to purple azobilirubin using sulphanilic acid (Ehrlich's reagent). Total bilirubin was determined in the presence of caffeine which releases albumin bound to bilirubin by the reaction of diazotized sulphanilic acid and the absorbance measurement was taken at 578 nm. Plasma protein was estimated by Biuret method using BSA as standard [22].

Statistical analysis

The results are expressed as means and standard deviation for the BCA patients and the controls. Statistical differences between group were assessed by the students t-test, P values less than ($p<0.050$) were considered significant.

Results

The anthropometric measurement data is shown on Table 1 and we only had a slight increase in the body weight of the control group compared with BCA patient. Table 2 shows the lipid profile of the BCA patients compared with the control group. The analysis for the lipid profile (total cholesterol, triglycerides, HDL Cholesterol and LDL cholesterol) showed that there was no significant difference ($p<0.05$) in the total cholesterol, HDL cholesterol and LDL cholesterol levels, but there was a significant increase ($p<0.05$) in the triglyceride level of BCA patient compared to control group.

Table 1 Anthropometric measurements of apparently healthy female adult (control group) and breast cancer (BCA) patient.

Parameters	BCA	Control
Age	42.37 ± 25.68	45.29 ± 20.77
weight (kg)	59.34 ± 23.78	74.51 ± 30.48*
height (cm)	168.31 ± 13.98	169.29 ± 11.22

Values are expressed as means ± S.D. *Values are statistically significant ($p<0.05$) between BCA patients and control subjects.

Table 2: Lipid profile, Antioxidant status (SOD, Vitamin C and E) in apparently healthy female

Parameters	Control	BCA Patients
Total cholesterol (mg/dL)	129.57 ± 35.68	128.83 ± 40.77
Triglyceride (mg/dL)	74.97 ± 40.78	108.83 ± 30.48*
HDL cholesterol (mg/dL	50.77 ± 23.98	46.17 ± 28.22
LDL cholesterol (mg/dL)	62.74 ± 26.30	61.58 ± 36.21
SOD (mmol/mg protein)	2.04 ± 1.01	1.71 ± 1.01
Vitamin C (mg /dL)	0.24 ± 0.12	0.24 ± 0.14
Vitamin E (mg /dL)	9.61 ± 1.46	8.01 ± 1.60*

Values are expressed as means ± S.D. *Values are statistically significant ($p<0.05$) between BCA patients and control subjects.

The antioxidant level, which is a marker of oxidative stress, as well as the total plasma protein of the BCA patient compared with the normal control group, is shown on Table 3. There was no significant difference ($p<0.05$) in the SOD and vitamin C level but there was a significant decrease ($p<0.05$) in the vitamin E level of the BCA patients compared with control. The oxidative stress index (MDA level) revealed a significant increase ($p<0.05$) in the lipid peroxidation level in BCA patient compared to control subjects. Total protein analysis showed a significant decrease ($p<0.05$) in protein in BCA patients compared with control with mean values of 62.13±6.28 and 76.28±7.56, respectively, thereby showing an 18.6% decrease.

Table 3 Plasma activity of Alanine amino transferase (ALT), Aspartate amino transferase (AST) and total bilirubin concentration in apparently healthy female adults (control) and Breast cancer (BCA) patients

Parameters	Control	BCA Patients
ALT (U/L)	8.60± 2.49	6.90 ± 2.87*
AST (U/L)	16.70 ± 3.52	19.83 ± 6.03*
Total Bilirubin (mg/dl)	0.52±0.16	0.49±0.17

Values are expressed as means ± S.D. *Values are statistically significant ($p<0.05$) between BCA patients and control subjects.

Table 4, shows the liver function test of the BCA patient compared with the control. There was a significant decrease ($p<0.05$) in ALT activity, a significant increase ($p<0.05$) in AST activity but there was no significant difference in the total bilirubin concentration of the breast cancer patients compared with control. However, the bilirubin level in the breast

cancer patients was also lower compared to control group 0.493±0.17 and 0.523±0.17, respectively.

Table 4 Lipid peroxidation and total protein levels in apparently healthy female adults and Breast cancer (BCA) patients.

Parameters	Control	BCA patients
Lipid peroxidation (mmol/mg protein)	4.98±2.85	7.80±3.18*
Total protein (mg/g	76.28±7.56	62.13±6.28*

Values are expressed as mean ± S.D, *Values are statistically significant ($p<0.05$) between BCA patients and control subjects

Discussion

Most often cancer treatment involves combination of radiotherapy and/ or chemotherapy after surgery and these could further potentiate oxidative stress. ROS could lead to gene mutation, lipid peroxidation and possibly neoplastic transformation. Moreover, the presence of BCA may alter dietary intake and the metabolism of specific nutrients [10], on its own and during management with chemotherapy. There was no significant difference in total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) in this study and it might be due to dietary fat intake. However, the elevated level of plasma triglyceride in the breast cancer patients, when compared with control group is in line with the result obtained by Martyn *et al*. [23] and Shah [8] who reported significant higher triglyceride levels in BCA patient. This increase in the triglyceride level may be due to inhibition of adipose tissue lipoprotein lipases activity [23, 24]. However, in the present study we saw no evidence of increased triglyceride intake in the BCA patient compared with the control group. Increases in cholesterol in the body cells have been reported to activate cells and cause the production of oxygen free radicals, followed by oxidative stress and hypercholesterolemia [25].

Analysis of antioxidant level revealed that there was no significant difference in SOD and vitamin C levels, although there was a 16.2% and 2.8% decrease in their levels, respectively, in BCA compared to control. The decrease in SOD activity in BCA may be due in part to increased utilization of SOD [26] in the detoxification of endogenous and exogenous ROS generated by chemotherapy. Antioxidant vitamins have a number of biological activities such as immune stimulation and an alteration of metabolic activations of carcinogens. Vitamin C can readily scavenge ROS, thereby effectively protecting cellular biomolecules from oxidative damage. Since free radicals can lead to the development of cancer, there is reason to believe that antioxidants help reduce the risk of cancer incidence, and may help the body's immune system fight against BCA recurrence. In this study, there was no significant difference observed in plasma vitamin C concentration between the two groups (BCA patients and Control) which are in consonance with the findings of Avhgami [27]. Although earlier studies have observed

an increase in plasma vitamin C [28-30], which is different from our result. This finding may be due to the higher intake of vitamin C supplement by the BCA patient, especially as we used in -hospital patients on immune booster supplementation. The plasma vitamin E level was observed to be significantly lower in BCA patients compared with the control group in this study. Earlier studies [29-31], have reported decrease vitamin E concentration in BCA patient. The decrease in the plasma vitamin E may be due in part to increased turnover to prevent oxidative damage in these patients suggesting an increased defense against oxidant damage in BCA. This result indicates that in BCA cancer patients, the efficiency of Vitamin E in counteracting the damaging effects of free radicals may be significantly reduced. Tumor cells have been reported to sequester essential antioxidants to meet the demands of growing tumor [32]. The decrease in vitamin E in the BCA patient may be due to the continuous utilization of vitamin E, increased scavenging of lipid peroxides in response to increased lipid peroxidation observed in BCA patients and probably due to sequestration by tumor cells.

Plasma activities of AST and ALT are the most commonly used markers of hepatocellular injury [33, 34], this is because they are intracellular enzymes which are released into circulation after cellular damage. ALT is highly specific for the liver, whereas AST is also located in the heart, brain, kidney and skeletal muscles, making the enzyme less specific for liver injury. Their plasma activity increases as a result of cellular membrane damage and leakage [35]. The decrease in ALT activity in BCA patients may indicate recovery. The decrease in ALT activity may also confirm that there is no liver damage due to chemotherapeutic related toxicities, and metastasis of cancerous cells to the liver in the BCA patients. The elevated AST activity in the BCA patient may be due to inflammation or damage to other organs or metastasis of the cancer to other organs in the body. Bilirubin concentration in the BCA patient was lower but not significantly different ($p<0.05$) compared with the control. Total bilirubin concentration indicates the functional transport capacity of the liver [36] and it exhibits potent antioxidant properties. This may be due in part to reduced hepatocytes damage and no increase in the breakdown of hemoglobin in the BCA patient. Unconjugated bilirubin mediated reduction of BCA cell line proliferation in a dose dependent manner via the induction of apolipoprotein D, this may also explain the slight reduction of bilirubin seen in BCA patient in conjunction with high triglycerides levels in an attempt to reduce BCA cell growth.

The increasing global incidence of BCA emphasizes the need to understand its etiology, response to treatment, nutritive and biochemical well being of patients. Diet plays a critical role since it is not only a source of nutrients but also a vehicle for other substances that may participate in promoting or inhibiting the disease prognosis. We have shown from this study that BCA can lead to alteration in the metabolism of lipids and protein depletion, also a cumulative functional insufficiency of the non-enzymatic antioxidant system (i.e. Vitamin C and E) may play an essential role in the development of increased oxidative stress in BCA patient. The patients were on supplementation and this should be encouraged alongside ingestion of fruits and vegetables rich in antioxidants which is necessary for BCA patients to prevent oxidative damage, deterioration of tissues and treatment related toxicities. Oxidative stress resulting from an imbalance between ROS and

scavenging capacity of antioxidants induces damage which may progress to disease state and development of cancers including breast cancer [27]. In this study, we observed a marked increase in lipid peroxidation levels in the BCA patients. This may be attributed to ROS generation from treatment related toxicities, a deficiency of antioxidant defense and endogenous ROS in the BCA. Studies have shown lipid peroxidation increases in plasma in the case of solid tumor [29, 30]. The results of the present study are consistent with these findings [37-39] and in addition, Gonec *et al*., (2001) [38] also reported that a significant difference between plasma levels of Malonyldiaaldehyde (MDA) in BCA patients compared to control subjects. Total protein level in the BCA patient was significantly lower when compared with control. The marked decrease in protein concentration may be due to altered dietary intake, increased protein metabolism and increased demand of proteins by BCA cells. Treatment related toxicities in BCA patients due to nausea and lack of appetite may have contributed.

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References

1. Ruddon RW. Cancer Biology, characteristics of Human Cancers. 3rd ed. Oxford University Press; 1995:3-10
2. Shibuya K, Mathers C, Pinto C, Lopez A, and Murray C. Global and regional estimates of cancer mortality and incidence by site: result for the global burden of disease. BMC Cancer, 2000, 2:1-26
3. Adebamowo CA, Ajayi OO. Breast Cancer in Nigeria. West Afr. J. of Medicine. 2000, 19:179-191
4. Banjo AAF. Overview of Breast and Cervical Cancers in Nigeria: are there regional variations? Presentation at the International Workshop on New Trends in Management of breast and Cervical cancers, Lagos, Nigeria. 2004
5. Spratt JS, Donegan, WL. P.A. Greenberg Epidemiology and etiology In: Cancer of the Breast. Eds, Spratt JS, Donegan, WL and Sigdenstant CP, Sunders Inc. U.S.A. 1999. p116-141
6. McPherson K, Steel CM, Dixon JM. ABC of Breast diseases. Breast cancer epidermiology, risk factors and genetics, BMJ. 2000, 21:624-628
7. Clinton SK, Miller EC, Giovannicci ED. Nutrition in the Etiology and prevention of Cancer. Nut J. 2000, 5:45-49
8. Shah FD, Shukla SN, Shah PM, Patel HR, Patel PS. Significance of alteration in plasma lipid profile levels in breast cancer. Integrative Cancer Therapies. 2008, 7:33-41
9. Armstrong B and Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int. J of Cancer. 1975, 15:617-631
10. Smith-Warne SA, Spiegelman D, Adami HO, Beeson WL, *et al*. Types of dietary fat and breast cancer: a pooled analysis of cohort studies. Int. J. Cancer. 2001, 92:767-774

11. Rasha H, Dena S, XiaoYan Z, Rina D, Marti J. Control of the growth of human breast cancer cells in culture by manipulation of arachidonate metabolism. BMC Cancer. 2007, 7:138
12. Aruoma OI. Free Radicals, antioxidants and International Nutrition, Asia Pacific J Clin Nutr, 1999, 8:53-63
13. Ray G and Husain SA. Oxidants, antioxidants and carcinogenesis. Indian J Exp Biol, 2000, 40:1213-1232
14. Rafai N, Bachorik PS, Albers JJ. Lipids, lipoprotein and Apolipoproteins. In: Burtis CA, Ashwood ER editors. Tietz textbook of clinical chemistry 3rd ed. Philadelphia: WB Saunder Company; 1999, 809-861
15. Gordon T and Gordon M. Enzymatic method to determine the serum HDL-Cholesterol. Am. J. Med. 1977, 62:707-708
16. Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of LDL-cholesterol. Clin. Chem. 1972, 18:499-515
17. Varshney R and Kale RK. Effect of calmodulin antagonist on radiation induced lipid peroxidation in microsomes. Int. J. Rad. Biol. 1990, 58:733-743
18. Misra HP and Fridovich I. The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for the superoxide dismutase. J. Biol. Chem. 1992, 217:3170-3175
19. Kway A. A simple colorimetric method for Ascorbic acid determination in blood plasma. Clin. Chim. Acta. 1978, 86:153-157
20. Reitman S and Frankel S. Quantitative in vitro determination of alanine amino transferase and aspartate amino transferase determination in serum, Am. J. Clin. Path. 1957, 28, 57-63
21. Schmidt E and Schmidt FW. Determination of serum GOT and GPT activities. Enzyme. Biol. Clin. 1963, 3:1-5
22. Gormal AG, Bardawill JC, and David MM. Determination of serum proteins by means of Biuret reaction. J. Biol. Chem. 1949, 177:751-766
23. Martyn L, Knapp S, Al-shelbani P, Riches G. Alterations of serum lipids in breast cancer: effects of disease activity, treatment and hormonal factors. Clin. Chem. 1991, 37:2093-2101
24. Spiegel IRJ, Schaefer EJ, Mcgarth IT, Edward BK: Plasma lipid alterations in leukaemia and lymphoma. Am. J. Med. 1982, 72:775-782
25. Stuller I, Rausch P, and Miller C. Increased serum concentrations of cholesterol and triglycerides in the progression of breast cancer. J Cancer Res. Clin. Oncol. 1988, 114:514-518
26. Kok FJ, vanPoppel G, Melse, J., *et al*. Do antioxidants and polyunsaturated fatty acids have a combined association with coronary atherosclerosis? Artherosclerosis 1991, 86: 85-90
27. Aghvami T, Djalali M, Keshavarz A, Sadeghi MR, Zeraati H, *et al*., Plasma level of antioxidant vitamins and lipid peroxidation in breast cancer patients. Iranian J Publ Health. 2005; 35:42-47
28. Ramaswamy G, Krishnamoorthy L. Serum carotene, vitamin A and vitamin C levels in breast cancer and cancer of the uterine cervix. Nutr. Cancer. 1996, 25:173-77
29. Ray G and Husain SA. Role of lipids, lipoproteins and vitamins in women with breast cancer. Clin Biochem, 2001, 34:71-76
30. Kumaraguruparan R, Subapriya R, Kabalimoorthy J and Nagini S. Antioxidant profile in the circulation of patients with fibroadenoma and adenocarcinoma of the breast. Clinical Biochemistry. 2002, 35:275-279
31. Torum M, Yardim S, Gonenc A, *et al*. Serum B-carotene, vitamin C, vitamin E and malondialdehyde levels in several types of cancer. J Clin Pharm Ther. 1995, 20:259-263
32. Blokhina O, Virolainen E, and Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bit (Lond). 2003, 91:179-194
33. Henderson BE, Preston-Martin S, Edmondson HA, Peters PL, Pike MC. Hepatocellular carcinoma and oral contraceptives. Br. J. Cancer. 1983, 48:437-440
34. Friedman LS, Martin P, Munoz SJ. Liver function tests and the objectives evaluation of the patients

with liver disease. In Zakin D, Boyer TD. Eds Hepatology: A textbook of liver diseases, 3rd ed. 1996. p791-833

35. Kaplan MM. Laboratory tests In Schiff L, Schiff ER, eds Diseases of the liver 7th ed. JB Lipincott, 1993, p108-144
36. Dickson ER, Grambsch PM, Fleming TR, Fischer LD and Langworthy A. Prognosis in primary biliary cirrhosis: Model for decision making. Hepatology. 1989; 10:1-7
37. Kumar K, Thangaraju M, and Sachdanandam P. Changes observed in antioxidant system in the blood of postmenopausal women with breast cancer. Biochem. Int, 1991, 25:371-380
38. Gonenc A, Ozkan Y, Torun M, Simsek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. J. Clin. Pharm Ther. 2001, 26:141-144
39. Hristozov D, Gadjeva V, Vlaykova T, and Dimitrov G. Evaluation of oxidative stress in patients with cancer. Arch Physiol. Biochem. 2001, 109:331-336