Liver Enzyme Parameters in Patients with Breast Cancer: Pre- and Post-Radiation therapy

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ABSTRACT

Breast cancer (BC) is one of the most lethal and life-threatening cancers and is the most common among women worldwide; in general, it is the second most common type of cancer. Radiotherapy (RT) remains an essential component of cancer treatment. RT's main purpose is to stop cancer cells from multiplying (cell division potential). RT used for cancer treatment depends on the production of reactive oxygen species (ROS). The main impact of RT happens indirectly through the ionization of water molecules to ROS, such as superoxide and hydroxyl radicles in the cytoplasm. This follow-up 1:1 study aimed to investigate some biochemical parameters in BC patients before and after RT treatment compared to the healthy as a control group. In this study, (60) healthy female subjects served as a control group and a (60) patient females who had been followed up before and after RT treatment. All The participant patients will get RT treatment after being diagnosed with malignant BC, undergo surgery (mastectomy or lumpectomy), and finish the chemotherapy cycles. Levels of Liver enzymes (alkaline phosphatase (ALP), alanine Transaminase (ALT) and aspartate transaminase (AST)) were all analyzed in patients and healthy controls. In the present study, the results will illustrate in three parts; Part one: a comparison between cancer patients before RT (G2) and healthy subjects as a control group (G1); the (ALP, ALT, and AST) concentrations levels were significantly higher in G2 compared to G1 ($p<0.05$). Part two: comparison between cancer patients after RT (G3) and healthy as a control group (G1); the (ALP, ALT, and AST) concentrations levels were significantly higher in G3 compared to G1 ($p<0.05$). Part three: comparison between cancer patients before (G2) and after (G3) RT; the (AST, ALT, and ALP) levels there was no difference between G3 and G2. In the present study, Patients with BC treatment methods are in a condition of high oxidative stress, necessitating antioxidant supplements.

Keywords: Radiotherapy, Liver enzymes, breast cancer, ALT, AST, ALP.

Introduction

Breast cancer (BC) or (C50) is a malignant tumour developed from breast cells. It occurs almost in women, but men can get it, too. It is highest among others in incidence and mortality in women worldwide. According to the World Health Organization (WHO) and International Agency for Research on Cancer (IARC), breast carcinoma is the most common malignant tumour in the world, comprising (11.7%) in 2020 of all female malignant cases (1). It is a heterogeneous disease that includes

many biologically different entities with distinct pathological features and clinical implications (2). BC occurs in a tumor when breast cells proliferate at an excessive rate (3). When proliferating cells invade neighboring tissues and organs and expand faster than surrounding cells, the tumor becomes malignant (4). Mammograms can be difficult to detect in the early phases of aberrant breast cell development (5)(6). Ionizing radiation is the name given to the type of radiation used because it produces ions (electrically charged particles) and deposits energy in the cells of the tissues it passes through. Tumor cells can be damaged, or genetic changes can be caused by this accumulated energy, resulting in a death of tumors. The DNA of cells is damaged by high-energy radiation, which prevents them from dividing and proliferating. The goal of RT is to increase the amount of radiation delivered to aberrant cancer cells while reducing the amount of radiation delivered to normal cells close to or in the path of the radiation, despite the fact that radiation damages both normal and cancer cells. A normal cell's repair process is usually faster and more efficient than that of a cancer cell. As a result, cancer cells are not as effective at repairing radiation damage as normal cells, resulting in differential cancer cell death (7)(8). As RT depends on ROS production and toxicity, it is supposed that cancer treatment protocols of chemotherapy and radiotherapy induce oxidation, free radicals' formation and at the same time reduce antioxidants. Antioxidants protect normal cells against radiation injury. In cancer patients there is disturbance in metabolism; when the patients are treated with chemotherapy then radiotherapy, they would experience another homeostasis disturbance which will affect the disease status. There is evidence to suggest that ROS may be involved in the onset, promotion, and progression of breast cancer stages. The cytotoxic effect of ROS production, which causes DNA damage and can kill tumor cells, can increase the oxidative stress produced by cancerous cells during adjuvant chemotherapy and radiotherapy. Comparatively high levels of lipid peroxide and protein carbonyl are associated with chemotherapy drugs. An increase in oxidant status has been found to be associated with these treatments. Cancerous and healthy cells alike can be killed by chemotherapy treatments, which use high doses of medication (9).

The liver function tests (LFTs) are a group of blood tests that commonly include bilirubin, albumin, total protein, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, and lactate dehydrogenase. These tests are routinely requested as a baseline for a number of clinical presentations and are connected with a variety of features of liver function, including cellular integrity, functioning, and biliary tract disorders. Numerous research are examining the use of LFT characteristics in predicting non-liver disease morbidity and death. Few studies have looked into LFTs and BC (10)(11)(12)(13)(14). Alkaline phosphatase (ALP) is a hydrolase enzyme that removes phosphate groups from molecules like nucleotides, proteins, and alkaloids. Dephosphorylation is the process of removing phosphate groups (15). ALP works best in an alkaline environment. It is also known as basic phosphatase (16). ALP is found in many tissues in humans, including bone, liver, intestine, kidney, and placenta (17). High ALP usually means that the bone or liver has been damaged (18). The normal range is 35-105 IU/L (14). Alanine transaminase (ALT) or glutamic pyruvic transaminase (GPT) is a transaminase enzyme. It is found in serum and various bodily tissues but is most associated with the liver. It catalyzes the two parts of the alanine cycle. High ALT levels often indicate other health issues like viral hepatitis, congestive heart failure, liver damage, bile duct issues, infectious mononucleosis, or myopathy (19). Reference range of ALT: <40 U/L (14). Aspartate transaminase (AST), also called glutamic oxaloacetic transaminase (GOT), it is an enzyme associated with liver parenchymal cells like alanine transaminase (ALT) (20). ALT is found in the liver, kidneys, heart, and skeletal muscle. So while both the ALT and AST can be elevated in diseases affecting the liver, the ALT is more specific for liver inflammation than the AST, which can be elevated in diseases affecting other organs such as the heart or muscles in

myocardial infarction (21). AST is commonly measured in clinical liver function tests to assess liver health. Reference ranges of AST: <40 IU/L (14).

Aims

The effects of RT on several systems have been studied (22)(23)(24)(25)(26)(27)(28)(29). So, this study's purpose was to examine how RT influenced liver function enzymes (Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), and Aspartate Transaminase (AST)) levels in Iraqi women with BC. To assess the impact of RT on biochemical markers, all patients were followed up before and after RT.

Patients And Methods

Radiotherapy Patients

Sixty Iraqi women were chosen as participants. (Age group: 26–62) They were in the ALAMAL NATIONAL HOSPITAL FOR CANCER MANAGEMENT, Medical City, in Baghdad, Iraq, from January to July 2021 to receive radiotherapy treatment. They were diagnosed with malignant breast cancer, which was treated with mastectomy or lumpectomy, and a breast tumor was identified in stages 1 to 4 of the disease. All four to nine chemotherapy treatments have been finished by the patients. Diabetes, hypertension, ischemic heart disease, thyroid diseases, thalassemia, inflammatory disease, hypoglycemia, gout, protein-energy malnutrition, osteoporosis, rheumatoid arthritis, liver diseases, and various cancers, and presence of other diseases known to be associated with elevated oxidative stress were all ruled out of the study. They'd never smoked or drank alcohol either. Each patient had followed up for two blood samples drawn. The first sample was taken before the start of radiotherapy (Radical treatment) or (Radiation), and the second was taken after finishing the course of radiation was completed (after three weeks from the first dose of radiotherapy, that was 2.67 Gray every day, five fractions per week, and the total dose was 40.05 Gray). The study protocol was approved by the Research Ethical Committee of the NCRRT according to Helsinki declaration and consent was obtained from each patient prior to inclusion in this study.

Healthy Control Subjects

As a control group, there were (60) healthy female volunteers ranging in age from 26 to 61 years old. They had only ever had blood extracted from them once. The healthy controls were also subjected to the same exclusion criteria as the patients.

Treatment

All patients underwent a computed tomography (CT) scan to enable the creation of a 3-dimensional conformal radiation treatment. The radiotherapy regimen was carried out postoperatively and included irradiating the whole breast, including the axillary region, as well as the submandibular and subclavian lymph nodes, with opposite tangent fields of 6-MV or 10-MV photons. The overall dosage of irradiation was 40.05 Gray, which was raised by 10 Gray in the primary illness area (tumor bed). Daily dosages of 2.67 Gray in 5 days were used to give the specified radiation doses for a week.

Sample Collection and Preparation

Blood samples were collected from all patients twice (before starting the first session of and immediately after the end of last session of RT), and once from the control group. All the participants were fasting at least 8 hours from the last meal. Blood samples were centrifuged for 15 minutes at 3000 rpm, and serum samples were kept at -20°C in aliquots until analysis.

Measurement of ALT

UV-assay in accordance with the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) standards without activation with pyridoxal phosphate. The reversible transamination of L-alanine and a-oxoglutarate to pyruvate and L-glutamate is catalyzed by alanine aminotransferase. The pyruvate is subsequently reduced to lactate in the presence of lactate dehydrogenase (LDH), while the reduced ß-nicotinamide adenine dinucleotide (NADH) is simultaneously oxidized to ß-nicotinamide adenine dinucleotide (NAD^+). The change in absorbance (at 340 nm) is proportional to the amount of ALT present in the sample.

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightleftharpoons{ALT} \text{L-glutamate} + \text{pyruvate}$$

$$\text{pyruvate} + NADH + H^+ \xrightleftharpoons{LDH} \text{L-lactate} + NAD^+ + H_2O$$

Measurement of AST

UV-assay according to IFCC without pyridoxal phosphate activation. The AST catalyzes the reversible transamination of L-aspartate and -oxoglutarate to oxaloacetate and L-glutamate in the test reaction. Oxaloacetate is subsequently reduced to malate in the presence of malate dehydrogenase, followed by the oxidation of NADH to NAD+. The pace at which NADH degrades photometrically is exactly related to the rate of oxaloacetate production and consequently to the AST activity at 340 nm.

$$\text{L-aspartate} + \alpha\text{-oxoglutarate} \xrightleftharpoons{AST} \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightleftharpoons{MDH} \text{L-malate} + NAD^+ + H_2O$$

Measurement of ALP

(IFCC) modified method. By the action of ALP and magnesium ions, p-Nitrophenyl phosphate is catalyzed to p-Nitrophenol, and the absorbency increase is directly proportional to the activity of ALP.

$$\text{p-Nitrophenyl phosphate} + H_2O \xrightleftharpoons[Mg^{+2}]{ALP} \text{p-Nitrophenol} + Pi$$

Statistical Analysis

Analyses of the basic characteristics of the subjects were compared using the student t-test. The results were expressed as the mean ± standard deviation. The different groups were compared using the independent-t-test, and one-way ANOVA. Comparisons were performed before and after treatment using paired-t-test. Statistical significance was determined by P val ues less than 0.05. The SPSS statistical program version 20.0 was used for all statistical analyse s (IBM).

Results

Table (1) shows that positive estrogen receptors present in 76.67% and progesterone receptors is present in 66.67% of patients. Histological type IDC is present in 86.67% of patients, Grade II is the most presented in the cells of the tumors (76.67%), and clinical stage II and III is the most presented stages in (63.33%) of patients. Table 2 shows the clinical characteristics and the treatment protocols which clarifies that modified radical mastectomy is performed in 100% of the patients. Surgery+ chemotherapy+ radiotherapy is the major regime of treatment in the patients. The treatment regime may start by chemotherapy for 4 cycles then surgical intervention, followed by chemotherapy then radiotherapy.

TABLE (1): Breast Cancer in this Study: Characteristics and Investigations

Characteristics	**N**	**%**
Number of patients	60	-
Age (mean)	43.5	-
Rang age	26-62	-
Family history		
Present	40	66.67
Absent	20	33.33
Menopausal status		
Premenopausal	46	76.67
Postmenopausal	14	23.33
Cancer Site		

Right breast	28	46.66
Left breast	32	53.34
Both breast	0	0
Surgery		
Mastectomy	60	100
Lumpectomy	0	0
Grade		
Grade I	0	0
Grade II	46	76.67
Grade III	14	23.33
Clinical stage		
Early	22	36.67
Advanced	38	63.33

TABLE (2): Study Patients' Clinical Characteristics.

TNM classification:	**N**	**%**
Tumor size		
T_1	6	10
T_2	40	66.67
T_3	10	16.67
Lymph node involvement		
N_0	6	10
N_1	18	30
N_2	22	36.67
N_3	14	23.33
M_0	58	97.67
M_1	2	3.33
M_x	0	0
Molecular diagnosis:		
HER 2 enriched	16	26.67
Luminal	48	80
Triple negative	8	13.3
Surgery:		
BCS (Breast conservingsurgery)	2	3.33
MRM (Modified radical mastectomy)	58	96.67
No	0	0
Chemotherapy:		
AC× 4	2	3.33
AC× 3 then T×3	2	3.33
AC× 4 then T×4	52	86.67

AC× 4 then T×5	4	6.66
Radiotherapy:		
Surgery+ chemotherapy+ radiotherapy	60	100
Chemo+ radiotherapy	0	0
Hormonal:		
+ve hormonal treatment	52	86.67
No hormonal treatment	8	13.33
AC: regimen received Adriamycin (A), Cytoxan (C). Repeat cycle every 3 weeks for 4 or 3 cycles. Followed by paclitaxel (T). Repeat cycle every 3 weeks for 3 or 4 or 5 cycles.		

Radiotherapy was administrated in all patients (100%), and hormonal therapy was administrated to 86.67% of patients; Trastuzumab was administrated to 13.33% of the patients with positive HER-2.

Level of AST level in control group (G1) is 17.26± 4.80 U/L which is significantly lower than that in breast cancer group before radiotherapy (G2) (22.45± 9.27) U/L in table (1), and after radiotherapy (G3) (21.54± 5.29) U/L, in table (2). As regards ALT level in control group (G1) is 7.7± 2.13 U/L which is significantly lower than that in breast cancer group before radiotherapy (G2) (13.28± 7.87) U/L, and after radiotherapy (G3) (13.150± 8.02) U/L. ALP is lowest in control group (57.40±15.62) and is significantly higher in breast cancer group before radiotherapy (70.87±28.52) and after radiotherapy (74.90±27.03). In Table (3) shows that the AST, ALT, and ALP levels for the same patients before and after radiotherapy.

TABLE (3): Before radiotherapy, the results for the Control Group (G1) and the Patients Group (G2).

Groups	Test Name	Mean	SD	P-Value
G1	**AST**	17.268	4.8050	**0.000****
G2		22.450	9.2700	
G1	**ALT**	7.700	2.1370	**0.000****
G2		13.280	7.8700	
G1	**ALP**	57.400	15.6200	**0.002****
G2		70.870	28.5200	

TABLE (4): After radiotherapy, the results of the Control Group (G1) and the Patients Group (G3).

Groups	Test Name	Mean	SD	P-Value
G1	**AST**	17.268	4.8050	**0.000****
G3		21.543	5.2970	
G1	**ALT**	7.700	2.1370	**0.000****
G3		13.150	8.0200	
G1	**ALP**	57.400	15.6200	

G3		74.900	27.0300	**0.000****

TABLE (5): Patients Before Radiotherapy (G2) and Patients After Radiotherapy (G3) results.

Groups	Test Name	Mean	SD	P-Value
G2	AST	22.450	9.2700	0.528
G3		21.540	5.3000	
G2	ALT	13.280	7.8700	0.933
G3		13.150	8.0200	
G2	ALP	70.870	28.5200	0.423
G3		74.900	27.0300	

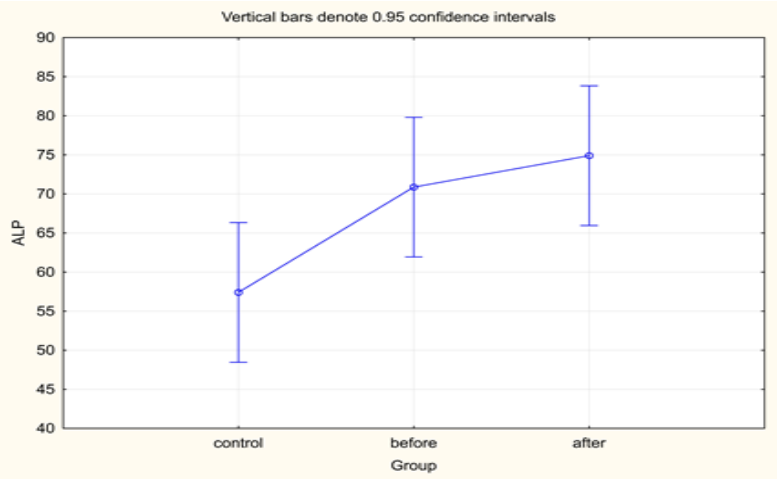

FIGURE (1): ALP Mean Concentration Levels (U/L), for Control Group, Patient Before RT, and Patient After RT.

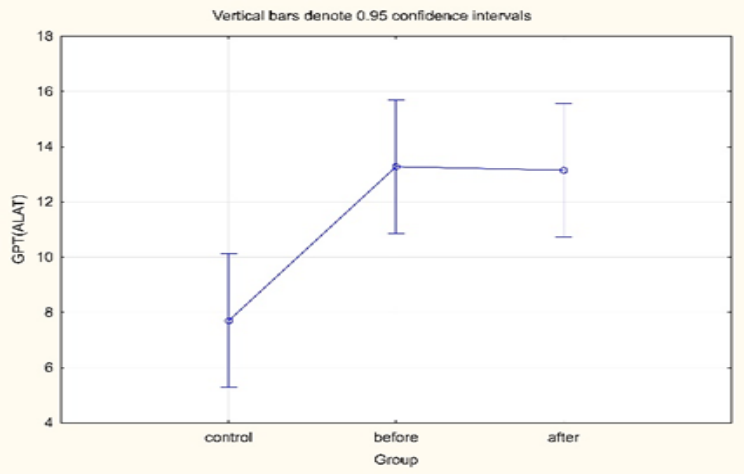

FIGURE (2): ALT Mean Concentration Levels (U/L), for Control Group, Patient Before RT, and Patient After RT.

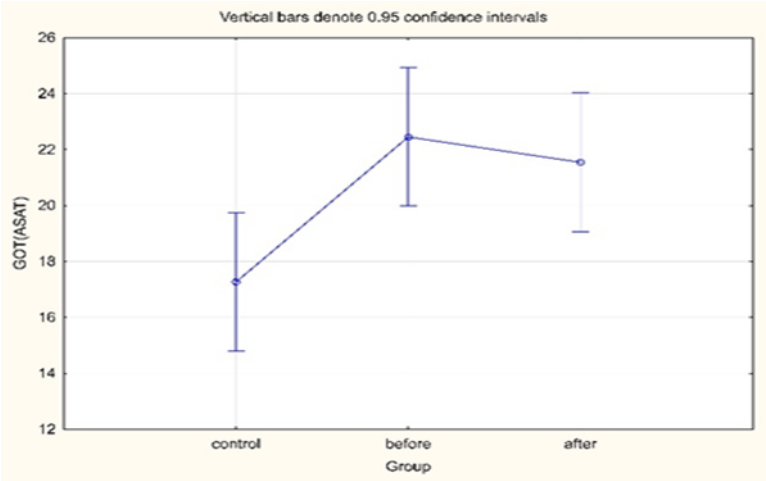

FIGURE (3): AST Mean Concentration Levels (U/L), for Control Group, Patient Before RT, and Patient After RT.

Discussion

Due to the fact that radiation is dependent on ROS generation and toxicity, it is assumed that cancer treatment procedures involving chemotherapy and radiotherapy stimulate oxidation and the synthesis of free radicals while simultaneously depleting antioxidants. Antioxidants shield normal cells from radiation damage. There is a metabolic disturbance in cancer patients; when they get chemotherapy and later radiation, they will have another homeostasis disturbance, which will alter their illness state (28).

Hepatotoxicity is liver dysfunction cause d by an excess of hepatotoxins or hepatotoxica nts. Hepatotoxicity enhances necrosis, steatosis, fibrosis, cholestasis, and vascular injury. Liver functioning test (LFT) is mainly based on enzymatic levels of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) (12).

The ALT is a liver enzyme that is one of the most specific indicators of hepatocellular disease. The AST is less specific, as it can suggest cardiac, skeletal muscles, renal, and brain disease. Increasing AST and ALT values indicates cytolysis. ALP is linked to both liver and bone diseases. In the liver, a rise in ALP can indicate intra- or extrahepatic cholestasis. The toxicity of whole-liver radiotherapy for hepatocellular cancer and total body irradiation has been known for a long time (14).

In this study, there is significant increase in AST and ALT level in the breast cancer patients when comparing with healthy subjects. This was in disagreement with (Noha Mohamed Said,2019) study, but the significant increase in ALP level in the breast cancer patients when comparing with healthy subjects, was in agreement with (Noha Mohamed Said,2019) study (13) and (Pavithra V, *et.al*) study, who reported ALP levels are significantly higher in cases of breast cancer patients than healthy controls. ALP is a sensitive liver indicator. High levels imply bone or liver metastases. Hepatic metastases were seen in 55-75% of breast cancer deaths. Hepatic metastases are crucial for patient survival. Early detection of liver metastases improves prognosis. CT, MRI, and PET can identify breast cancer liver metastases. Acute hepatic symptoms such as jaundice, hepatomegaly, and ascites occur late in the disease process (11).

The study of (Sarah O. Nwozo, *et.al*) informed that 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 (10).

There was a non-significant change in the results of mean ±SD for (AST, ALT, and ALP) activity levels for the same patient before and after radiotherapy (between G2 and G3), this was in agreement with (D.C. Lauffer, et.al) follow-up study (14).

AST and ALT plasma activity are the most often utilized indicators of hepatocellular injury, as they are intracellular enzymes that are released into the blood following cellular damage. While ALT is highly specific for the liver, AST is found in the heart, brain, kidney, and skeletal muscles, making it less selective for liver damage. Their plasma activity rises as a result of damage to and leaking through the cellular membrane. In BC patients, a reduction in ALT activity may suggest recovery. The reduction in ALT activity may further demonstrate that BC patients do not have liver damage because of chemotherapeutic-related toxicities or spread of malignant cells to the liver. The increased AST activity in the BC patient might be a result of inflammation or injury to other organs, or it could be a result of cancer metastasizing to other organs in the body (10).

Conclusion: The current study assessed the serum biochemical profile of BC patients undergoing RT. RT may change the levels of biological components in the blood, influencing organ systems. Serum biochemical markers can assist evaluate disease progression, metastasis, and therapy options for BC. The liver enzymes in this study did not show any significant changes, which mean the radiotherapy does not affect liver.

Recommendations: It is recommended to expand the sample size in future studies to get clear correlations and to monitor biomarker changes over time.

Conflict of Interest: None declare.

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Ethical Clearance: The patients gave verbal and analytical consent before obtaining the sample.

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References

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. Int J cancer [Internet]. 2021 Apr 5;149(4):778–89. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ijc.33588
2. Ali HR, Jackson HW, Zanotelli VRT, Danenberg E, Fischer JR, Bardwell H, et al. Imaging mass cytometry and multiplatform genomics define the phenogenomic landscape of breast cancer. Nat Cancer [Internet]. 2020 Feb 17;1(2):163–75. Available from: http://www.nature.com/articles/s43018-020-0026-6
3. Chakravarti B, Ravi J, Ganju RK. Cannabinoids as therapeutic agents in cancer: current status and future implications. Oncotarget [Internet]. 2014 Aug 15;5(15):5852–72. Available from: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.2233
4. Tarin D. Clinical and Biological Implications of the Tumor Microenvironment. Cancer Microenviron [Internet]. 2012 Aug 3;5(2):95–112. Available from: http://link.springer.com/10.1007/s12307-012-0099-6
5. Zhang B-N, Cao X-C, Chen JJ-Y, Chen JJ-Y, Fu L, Hu X-C, et al. Guidelines on the diagnosis and treatment of breast cancer (2011 edition). Gland Surg [Internet]. 2012 May;1(1):39–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25083426
6. Bhattarai S, Klimov S, Aleskandarany MA, Burrell H, Wormall A, Green AR, et al. Machine learning-based prediction of breast cancer growth rate in vivo. Br J Cancer [Internet]. 2019 Sep 10;121(6):497–504. Available from: http://www.nature.com/articles/s41416-019-0539-x
7. Beyzadeoglu M, Ozyigit G, Ebruli C. Basic Radiation Oncology [Internet]. Basic Radiation Oncology. Berlin, Heidelberg:

Springer Berlin Heidelberg; 2010. 1–575 p. Available from: http://link.springer.com/10.1007/978-3-642-11666-7

8. Baskar R, Lee KA, Yeo R, Yeoh KW. Cancer and radiation therapy: Current advances and future directions [Internet]. Vol. 9, International Journal of Medical Sciences. 2012. p. 193–9. Available from: http://www.medsci.org/v09p0193.htm
9. Reitz LK, Baptista S de L, Santos E da S, Hinnig PF, Rockenbach G, Vieira FGK, et al. Diet quality is associated with serum antioxidant capacity in women with breast cancer: A cross sectional study. Nutrients [Internet]. 2021 Dec 30;13(1):1–14. Available from: https://www.mdpi.com/2072-6643/13/1/115
10. So N, Nwozo SO, Solomon O, Abimbola OO, Kikelomo DO. Comparative Study of Biochemical and Nutritional Status of Breast Cancer Patients on Chemotherapy / Radiotherapy in Ibadan [Internet]. Vol. 1, American Journals of Cancer Science. 2013. p. 1–10. Available from: http://ivyunion.org/index.php/ajcs/
11. V. P, G ST, Kasthuri1 K, Mallika DS, Amos SJ. ENZYMES AND ANTIOXIDANT AS BIOMARKERS IN BREAST CANCER.pdf. Int J Cur Res Rev. 2014;6(11):24–9.
12. Swapna VS, Sudhakar V, Javerappa D. Study of liver function tests in breast carcinoma patients before and after chemotherapy. Vol. 14, International Journal of Biotechnology and Biochemistry. 2018. p. 177–84.
13. Said NM. Three gold indicators for breast cancer prognosis: a case–control study with ROC analysis for novel ratios related to CBC with (ALP and LDH). Mol Biol Rep [Internet]. 2019 Apr;46(2):2013–27. Available from: http://link.springer.com/10.1007/s11033-019-04650-9
14. Lauffer DC, Miglierini P, Kuhn PA, Thalmann SU, Gutierres-Demierre N, Khomsi F, et al. Impact of adjuvant radiotherapy on biological and clinical parameters in right-sided breast cancer. Cancer/Radiothérapie [Internet]. 2021 Jul 8;25(5):469–75. Available from: http://link.springer.com/10.1007/s00066-019-01531-9
15. Kim EE, Wyckoff HW. Reaction mechanism of alkaline phosphatase based on crystal structures. J Mol Biol [Internet]. 1991 Mar;218(2):449–64. Available from: https://linkinghub.elsevier.com/retrieve/pii/002228369190724K
16. Tamás L, Huttová J, Mistrík I, Kogan G. Effect of carboxymethyl chitin-glucan on the activity of some hydrolytic enzymes in maize plants. Chem Pap. 2002;56(5):326–9.
17. AL-Mashhadani, Z. I., Mukhlis, A. J. A., & AL-Faraji AAR(. Estimation of ALP , GPT and GOT Activities in Iraqi Patients Female With Breast Cance r Abstract. Ibn Al-Haitham J Pure Appl Sci. 2012;25(1).
18. Posen S, Doherty E. The Measurement of Serum Alkaline Phosphatase in Clinical Medicine. In: Advances in Clinical Chemistry [Internet]. Elsevier; 1981. p. 163–245. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0065242308600487
19. Giboney PT. Mildly elevated liver transaminase levels in the asymptomatic patient. Vol. 71, American Family Physician. 2005. p. 1105–10.
20. Almo SC, Smith DL, Danishefsky AT, Ringe D. The structural basis for the altered substrate specificity of the r292d active site mutant of aspartate aminotransferase from E.coli. Protein Eng Des Sel [Internet]. 1994;7(3):405–12. Available from: https://academic.oup.com/peds/article-lookup/doi/10.1093/protein/7.3.405
21. Lott JA, Stang JM. Serum enzymes and isoenzymes in the diagnosis and differential diagnosis of myocardial ischemia and necrosis. Clin Chem [Internet]. 1980 Aug 1;26(9):1241–50. Available from: https://academic.oup.com/clinchem/article/26/9/1241/5664458
22. Veni GK, Rao DB, Kumar DM, Usha B,

Krishna VM, Rao TR. Clinical evaluation of oxidative stress in women with breast cancer. Recent Res Sci Technol. 2011;3(1):55–8.
23. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J [Internet]. 2013 Apr;21(2):143–52. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1319016412000357
24. Marín A, Martín M, Liñán O, Alvarenga F, López M, Fernández L, et al. Bystander effects and radiotherapy. Reports Pract Oncol Radiother. 2015;20(1):12–21.
25. Rangarajan N, Vijayalakshmi K, Rouia ARA, Dawsar KI, Nawfal AMN, Nada NA-S, et al. The effect of radiotherapy on oxidative stress , Biochemical and Hematological Parameters in women with Breast Cancer. AJPS. 2014;14(2):10–7.
26. Khalil Arjmandi M, Moslemi D, Sadati Zarrini A, Ebrahimnezhad Gorji M, Mosapour A, Haghhaghighi A, et al. Pre and post radiotherapy serum oxidant/antioxidant status in breast cancer patients: Impact of age, BMI and clinical stage of the disease. Reports Pract Oncol Radiother [Internet]. 2016 May;21(3):141–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1507136715001716
27. Mahmood AS. Biochemical Study and Immunohistochemistry of Breast Cancer after and before Radiotherapy. Indian J Public Heal Res Dev [Internet]. 2019;10(5):376–81. Available from: www.ijphrd.com
28. Youssef H, Salem H. Oxidative Stress Parameters in Patients with Breast Cancer before and after Radiotherapy. Egypt J Radiat Sci Appl [Internet]. 2019 Oct 21;32(2):0–0. Available from: https://ejrsa.journals.ekb.eg/article_54302.html
29. Mahmood AR, Abdullah MR, Khalaf HS. Estimation of some trace elements and antioxidant status in breast cancer patients undergoing radiotherapy. J Glob Pharma Technol. 2018;10(6):213–7.