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RESEARCH ARTICLE

Study of Some Hematological and Biochemical Parameters as a Bioindication in Breast Cancer Patients of Sindh, Pakistan

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ABSTRACT

Breast cancer is one the most dreadful and common health problem among females all over the world. A cross sectional survey was conducted on 150 randomly selected breast cancer patients at Nuclear Institute of Medicine & Radiotherapy (NIMRA) Jamshoro and Nuclear Medicine Oncology & Radiotherapy Institute (NORIN) Nawabshah. A questionnaire regarding patients' history and social status (age, BMI, marital status, ethnicity, literacy rate, duration of breast feeding and method of the treatment) was filled and the samples were subjected to the hematological (WBC, Platelets and Hemoglobin) and biochemical analysis (estrogen, progesterone, blood urea nitrogen and creatinine). The prominent rate of breast cancer was found in women exceeding age > 40 and < 60 years. The incidence of breast carcinoma was found out at the high point of age 40-50 years in "tribe 1" females along with increased body weight. The Results professed that the number of WBC, Platelets and the hemoglobin decrease in affected patients than normal range. After the test of CBC, HGB, at the time of diagnosis the patient's contents, WBC (7.57± 4.04X10^3/µL), platelets (283.41±317.87X10^3/µL) and the hemoglobin (9.68±2.02g/dl) levels were decreased in affected patients than normal range. The mean values of blood urea nitrogen (BUN) and creatinine level were 23.85±8.53 mg/ dl and 1.26±0.56 mg/ dl in affected patients. 44.5% cases were both ER and PR positive increasing with rising age. In conclusion, lower literacy rate, overweight and age 40-50 years are associated risk factors for breast carcinoma in female population of Sindh province and chemotherapy adversely affect the levels of creatinine and BUN vaguely exceeding the normal range. Further, study explained that superior concentration of ER and PR (+ve) is helpful biomarker for better diagnosis of breast cancer in female patients.

INTRODUCTION

Cancer is one of the malignancy diseases, in which the injured or abnormal cells divide unconditionally without any control which disturb and damage the neighbor tissues and slowly and sometime rapidly invade surrounding tissues (Lodish et al., 2000). There are several kinds of cancer disease, such as tumor, uterus, intestine, skin and breast cancer etc. Breast cancer in females the most severe problem in public-health nowadays that can lead to death (Bassili, 2000). Breast cancer becomes intricate disorder because of some environmental and genetically factors (Bener et al., 2010). The breast cancer is major cause of high mortality and morbidity rate throughout the world with almost 40% ratio in developed countries (Mishra et al., 2004). Among the Asian countries, rate of breast cancer is highest in Pakistan. The ratio of affected breast cancer patients of Pakistani women is 1:9 (Sohail and Alam, 2007) and mostly the youngest women affected by an advanced stage of breast cancer which has an adverse effect on diagnosis (Badar et al., 2005). The sign and symptoms of breast cancer include change in shape and consistency of breast, liquefied coming from the nipple, inflammation in the breast, cellulite or a red scaly patch of skin (Gayathri and Jyothi, 2020). Different factors have been accredited as a threat of breast cancer, such as socioeconomic

factors, exogenous hormone use including post-menopausal hormone replacement therapy and oral contraceptive pills, the family record of breast cancer in first-degree relative, reproductive behaviors such as parity, age at first child birth, early onset menarche, late menopause, consumption of alcohol, ionizing radiation exposure, obesity (Bhupathiraju et al., 2016). While at primary prevention strategies, these factors have helped and currently understanding the pathogenesis of breast cancers in relation to biochemical indicators for the diagnosis of the tumor of major concern. In breast malignancies various tumor markers occur singly or in combination (Ravelli et al., 2015). Research studies revealed the short and long duration adjacent effects of chemotherapy on physiology of organs and cellular stages of malignancy (Crawford et al., 2004). The blood chemistry panel (BCP) procedures the levels of organic waste products, enzymes and chemicals in the blood can serve as tumor biomarkers. Any malignancy direct impact on blood parameters therefore it is essential to study the biochemical and hematological parameters at regular intervals during the course of disease and even during treatment (chemotherapy). Important information on the subject of numbers and the kinds of cells in the blood is provided by blood tests (a complete blood count CBC) and biochemical parameters), especially white blood cells, red blood cells and platelets (Shimada et al., 2004). The patient may have any symptoms such as fatigue, bruising or weakness, then CBC is important tool and helps differentiate the problem at initial stages. The blood count is an indispensable examination entreated from all cancer patients before use of radiotherapy, chemotherapy or surgical interventions. Anemia or coronary artery disease, high number of WBC predict a worse diagnosis and patient is foreseen in high risk of breast cancer with heart failure (Grimm et al., 1985; Mozaffarian et al., 2003). In patients a high platelet count is associated with poor survival and tumor progression with esophageal carcinoma (Shimada et al., 2004). Kidney damages is indicated with rise of two chemical factors in blood *i.e.* urea and creatinine. A rough quantity of the glomerular filtration rate is provided by Blood Urea Nitrogen (BUN) and creatinine. Estrogen is needed for the progress of tissues, breast, reproduction and normal growth. It regulates the woman's menstrual cycles and also important for childbearing. Breast cancer is formed due to high exposure to estrogen. Mitogen and genotoxin and led the path in development of Breast Cancer (Miller, 2003). Present study reported the risk factors for the development of breast cancer and its associated alterations in the haematological and biochemical parameters among breast cancer patients of Sindh, Pakistan.

MATERIALS AND METHODS

Study area

A cross sectional study was conducted on 150 randomly selected breast cancer patient who attended the Cancer hospitals Nuclear Institute of Medicine & Radiotherapy (NIMRA) Jamshoro, and Nuclear Medicine oncology and Radiotherapy Institute (NORIN) Nawabshah, Sindh Pakistan. These institutes accommodate referred cancer patients from all districts of Sindh Province. After seeking written consent from the patient, the information regarding their personal and family history, the hospital where they went for treatment, about occupation, age at diagnosis of cancer, where cancer is (left or right breast), pain in breast and discharge from breast, etc. was recorded on designed questionnaire.

Sample collection

Blood and serum samples were collected from all in 150 female patients who attended at NORIN-Nawabshah and NIMIRA-Jamshoro cancer hospitals during 2019. Blood samples (10 ml) of every patient were collected from cubical vein of upper limbs and 2.5-3 ml blood was transferred to EDTA bottle coated tubes for hematology. The remaining blood was transferred to clean dry plain tubes and tends to clot for 15-30 minutes for serum collection (Bacchus et al., 1980). The clotted blood was centrifuged at (4000g) for 10 minutes for harvesting of serum. The blood cell was analyzed after few minutes of blood collection because of great chances of hemolysis.

Hematological and biochemical analysis

Serum samples for urea, creatinine were analyzed using commercially available kits on auto analyzer (cobas® 4000 analyzer series). ER and PR status were determined using techniques BioGenex monoclonal mouse IgG1 (Clone 1D5) and BioGenex monoclonal mouse IgG1 (Clone 1A6) respectively (Chamness and McGuire, 1979).

Data analysis

The data obtained was subjected to the statistical analysis using Statistical software SPSS.16. For basic data description the chi-square test and t-test was used for finding P-values as statistical significance and determining the common statistical parameters frequency and % are given**.**

RESULTS

Present Study included 150 breast cancer patients with mean age 45.7±5.00. Maximum 60% were post-menopausal during clinical inventions. Although during histological cataloguing of cancer, 40% were ductal adenocarcinomas at the age of above 40 years. It was declared from study results that in Sindh population breast cancer was common in married women 92.85%; while in unmarried women 7.15% was found.

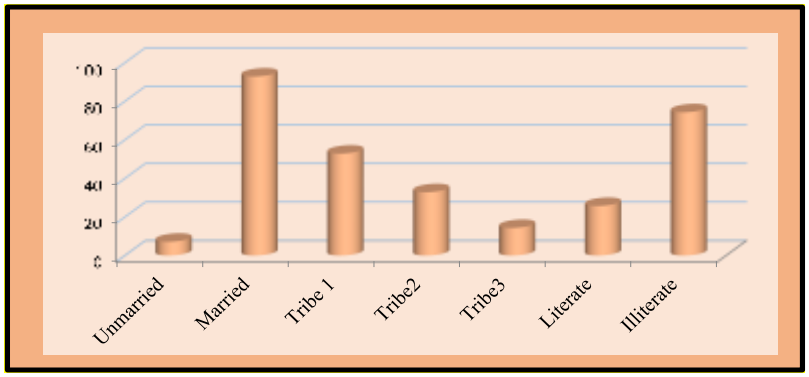

Fig. 1: Demographic data showing marital status, ethnicity & literacy rate of breast cancer patients

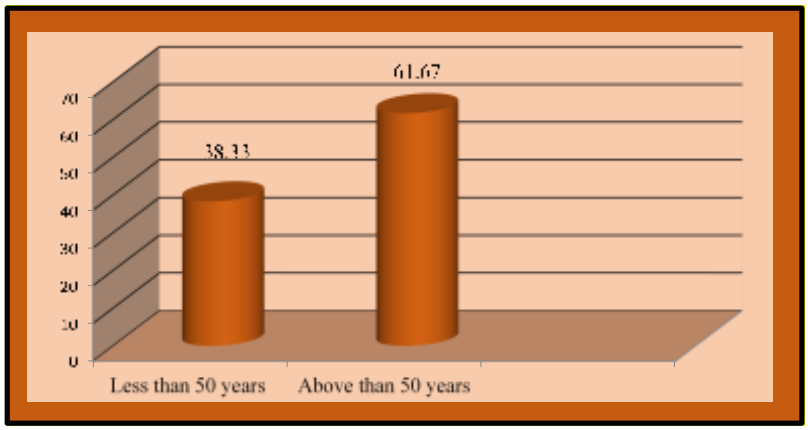

Fig. 2: Age of Breast Carcinoma Patients

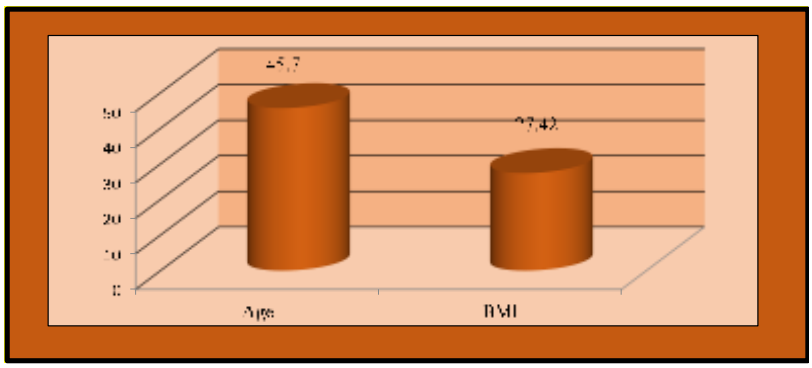

Fig. 3: Comparison of Body Mass Index and Age of Breast Carcinoma Patients

The maximum rate of breast carcinoma was found in tribe 1 women about 52.85% as compared to tribe 2 (32.85%) and tribe 3 (14.3%) breast cancer women (Fig. 1). The study results reveal that in Sindh population commonly the breast carcinoma was found in women of age exceeding 40 years. The percentage of breast carcinoma in women possessing age beyond 50 years was 61.67% while 38.33% were of age under 50 years (Fig. 2). Only few cases were detected with age below 35 years. Present study revealed that breast cancer generally was observed in overweight women but when we accumulated samples, it was noticed that weight reduced significantly due to use of chemotherapy and radiotherapy during the several phases of cure. The mean value of BMI of breast cancer patients was identified 27.42 % as shown in (Table 1). Many patients were illiterate 74.28%, only few patients were literate 25.72%. Organic constraints conforming to tissue samples of the breast cancer patients incorporated in this study are exposed in (Table 1). A complete blood count performs a CBC and HGB test to observe the number of WBC, Platelets and hemoglobin, which decrease in affected patients compared with, than its normal range. At the time of diagnosis mean and standard deviation of WBC, Platelets and Hemoglobin (Hb) was observed 7.57± 4.04X10^3/µL, 283.41±317. 87X10^3/µL, 9.68±2.02g/dl, respectively (Table 2). As compared to normal range (7-20 mg/dl), the rank of blood urea nitrogen (BUN) was detected spare 23.85±8.53 during the chemotherapy courses as shown in (Table 2). The value of blood urea reduced during chemotherapy was regarded as a non - significant.

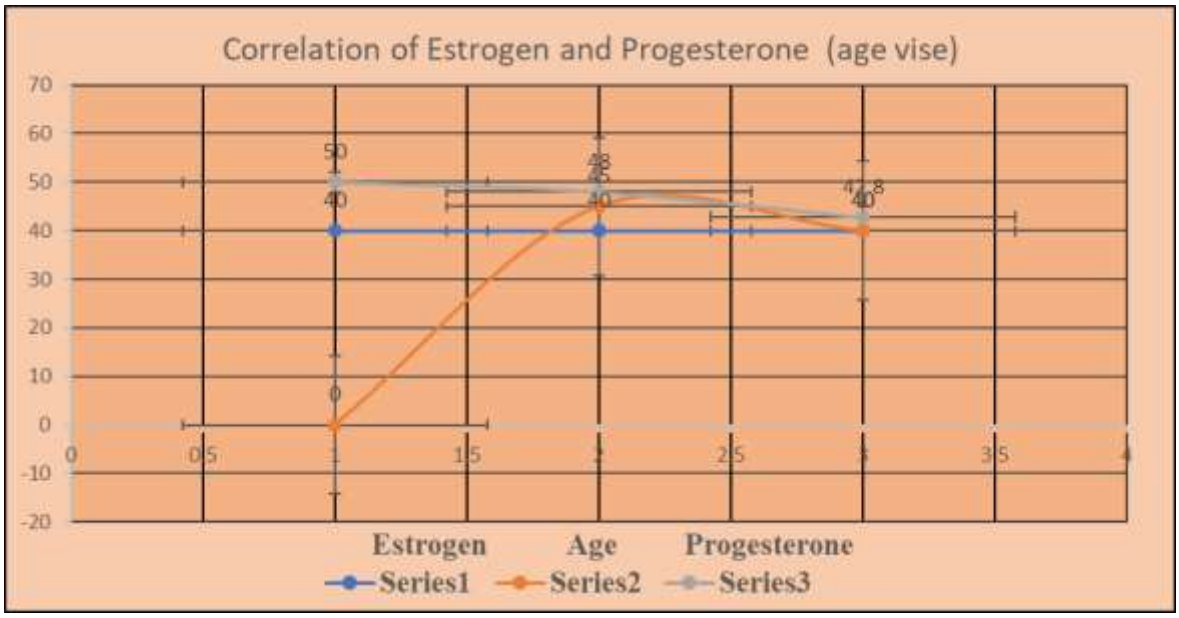

Figure 4: Correlation between the ER and PR with Age Vise (40-50 Years).

Table 1: Demographic Characteristics, the Body Mass Index and Age of Subject

Marital status of patients (female)		Ethnicity			Literacy rate		AGE ±Sd	BMI ±SD
Married (%)	Unmarried (%)	Tribe 1 (%)	Tribe .2 (%)	Tribe (%)	3 Literate (%)	Illiterate (%)		
92.85	7.15	52.85*	32.85*	14.3*	25.72	74.28	45.7±5.00	27.42±2.52

Table 2: Hematological Characteristics, Biochemical Parameters of Breast Carcinoma Patients

Variables	Breast cancer patients
Number of WBCs (X 10^3/µL)	7.57±4.04
Number of Platelets (X 10^3/µL)	283.41±317.87
Number of Hb (X g/dl)	9.68±2.02
Blood urea nitrogen (BUN) mg/dl	23.85±8.53
Creatinine mg/dl	1.26±0.56

**Values expressed as Mean ±SD, P value <0.05 was considered as significant.

Table 3: Estrogen and Progesterone (Age Vise) Status

Age (diagnosis year)	*NO. of case*	*ER+/PR+*	*ER+/PR-*	*ER-/PR+*	*ER- /PR-*
<40	15	6 (40%)	2 (13.3%)	1 (6.6%)	6(40%)
40-49	20	9 (45%)	2 (10%)	1 (5%)	8 (40%)
≥50	35	17(48.5%)	2 (5.7%)	1(2.8%)	15 (42.8%)

During the chemotherapy, a normal range (0.6-1.1 mg/dl) of creatinine was observed in breast cancer patients (Table 2). The signify rate of creatinine before the start of chemotherapy was noted to be 1.05± 0.59mg/dl. No specific modifications were analyzed during the various courses of chemotherapy in level of creatinine (Table 2). The signify amount of creatinine was 1.26±0.56, that was observed rather more than normal range (0.6-1.1 mg/dl) during chemotherapy. The creatinine was observed vaguely greater than normal range while level of blood urea nitrogen (BUN) was observed beyond normal range during chemotherapy courses (Table 2). Age vise percentile ratio of estrogen and progesterone in different number of patient cases were described; as in 32 (44.5%) ER and PR (+), 29 (40.9%) ER and PR (-); while 3 (4.8%) ER (-) and PR (+) and 6 cases were (9.7%) ER (+) and PR (-) respectively (Table 3). Progesterone and estrogen correlation was described the (+ve) in carcinoma patients' at the age of 40-50 years (Figure 4).

DISCUSSION

The chemical substances produced throughout the disintegration (metabolism) of certain substances or released from body tissues are measured by

Biochemistry profiles of blood. Important information about liver, kidney and other organs is provided by the analysis of blood chemistry. Current status of breast cancer in Sindh was revealed that most often those women were found with breast carcinoma who have age beyond 40 years, while present study indicates that 38.33% of females were of age underneath 50 years which is the lowest ratio as compare to age of above than 50 years (61.67%) (Fig 2). If we compare these results with worldwide almost same ratio was observed in the United States with about 78% of new breast cancer cases were found in 2011 in women of age above 50 years). A pervious study indicated that breast carcinoma threats were developed in women possessing age more than 50 years (DeSantis et al., 2011). According to thinking of Binder Foucard overage causes increase risk of breast cancer in women. The females with age more than 70 years, as compared to these women, breast cancer was found more (50%) in females of age > 60 years (Binder-Foucard et al., 2012; Hunter and Mcneil, 1997). The median age while analyzing breast cancer was 62 during year 2012-2016 (Howlader et al., 2020). This points out that half of women possessing breast cancer were younger at the time of diagnosis or were of age 62 years. Current study clarifies that mostly married women of Sindh population were diagnosed with breast cancer. The similar results were observed in previous studies and conclude that recommended factor for survival with breast cancer is a marital status (Osborne et al., 2005; Martínez et al., 2017). Here our study is contradicting with (Hunter, 1997), according to him as compare to married women, a hazard of breast cancer is high in unmarried women at the equal phase of age and also in married nulliparous women. One of the major reason is that in developing countries the greater part of women avoid to breast feed their kids. The women who ever had breastfed their infants were considered to be defensive as compared to women who never had breastfed their kids or breastfed to their infants for short lifetime. In General, if a woman breastfed to her kid every year then chances of developing breast carcinoma risk in that women lessen by 4.3%. (Berrington et al., 2002; Shema et al., 2007). Study proves that breast cancer patients had body mass index (BMI) 27.42 kg, higher than normal weight women. The women who were overweight, older or have had the disease were found with more breast cancer in contrast to obese women. This is because the average number of birth per woman declines while body mass index (BMI) raises (Prentice et al., 2006). (Cherry et al., 2002) clarified menopausal status and the obesity/overweight is risk predictor for breast cancer recurrence and death in reproductive study. The study explicates that blood parameters are directly affected by breast carcinoma and cure of breast cancer and the level of blood parameters (platelets, Hb and WBC) considerably shortened in the patients of breast cancer (Mwer et al., 1988) and (Beresford et al., 2006) approved that as compared to normal women the amount of WBC is significantly shortened in breast carcinoma patients. They stated that level of blood counts decrease in many malignancies because patients suffered from chemotherapy and thrombocytopenia. Patients suffer from lymphomas (Balducci, 2019; Nakamura et al., 2018). (Taucher et al., 2003; Sierko and Wojtukiewicz, 2004) explained that cancer prognosis proceeds poorly with increase in the number of platelets (thrombocytosis) in blood of breast cancer women because the major role of platelets is in pathogenesis of disease. As the creatinine serum and Blood Urea Nitrogen (BUN) is important biomarkers for identifying Cancer disease, The results of present study were in agreement with (Gross et al., 2005) who stated that for assessment of kidney functions using urea and creatinine play an important role in diagnosis and management of cancer disorder. Similar finding were described by other researchers, who affirmed that kidney disfunction assesses in patients as the side effect of chemotherapy due to increasing in level of creatinine serum and blood urea nitrogen (BUN) (Amos et al., 2011). The value of blood urea decreases during the chemotherapy was considered as non-significant. The findings of current study proved that ER and PR +ve had relatively better prognosis due to high concentration. Similar results were reported earlier that; in order to manage malignancy estrogen and progesterone receptors (ER, PR) play a leading role (Rampaul et al., 2001; Noda et al., 2002; Gown, 2008). The tumor tissue responds well to hormone therapy and chemotherapy in presence of receptors progesterone and estrogen (PR and ER) that participate vital part in assessment of breast cancer development. The overall breast cancer incidence enhances 75-80% due to estrogen receptor (ER)-positive breast cancer subtype (Althuis et al., 2005, Li et al., 2003, Glass and Hoover, 1990) assumed that hormonal risk factors rather than BRCA1/BRCA2 gene status cause increase incidence of ER +ve breast cancer such as early menarche, late menopause, HRT and the combined oral contraceptive pill (OCP).

Conclusion

Present study concluded that breast cancer in women was observed with highest incidence in married women who were 40-50 years of age and had overweight. Illiteracy and lack of awareness among rural women was the biggest reason for the occurrence of breast cancer in Sindh Pakistan. The chances of breast cancer reduce in females in pre and post-menopausal stage who breastfed their children. Breast cancer and treatment of breast cancer have adverse effect on blood parameters. Creatinine and BUN was observed vaguely

exceeding the normal range in breast cancer patients. Further, the current study explains that superior concentration of ER and PR (+ve) is helpful for better diagnosis of breast cancer in females.

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Authors' contributions

FG conducted the research work. **SP** provided guidance and helped in writing. **AA.** helped in literature review and editing. **DAS** helped in field survey and laboratory examinations. All authors read and approved the final manuscript.

REFERENCES

Althuis MD, JM Dozier, WF Anderson, SS Devesa and LA Brinton, 2005. Global trends in breast cancer incidence and mortality 1973–1997. International Journal of Epidemiology, 34: 405-412.

Amos SM, CP Duong, JA Westwood, DS Ritchie, RP Junghans, PK Darcy and MH Kershaw, 2011. Autoimmunity associated with immunotherapy of cancer. Blood, The Journal of the American Society of Hematology, 118: 499-509.

Bacchus R, B Kilshaw, M Madkour, S Bassam and B Farhan, 1980. Preliminary studies on a reference range for Saudi Arabian males: 1. Serum uric acid. Saudi Medical Journal, 1: 160-163.

Badar F, I Moid, F Waheed, A Zaidi, B Naqvi and S Yunus, 2005. Variables associated with recurrence in breast cancer patients-the Shaukat Khanum Memorial Experience. Asian Pacific Journal of Cancer Prevention, 6: 54-57.

Balducci L, 2019. Geriatric oncology, spirituality, and palliative care. Journal of Pain and Symptom Management, 57**:** 171-175.

Bassili A, 2000. Breast self-examination practice and its impact on breast cancer diagnosis in Alexandria, Egypt. Eastern Mediterranean Health Journal, 6: 34-40.

Bener A, HR El Ayoubi, AI Ali, A Al-Kubaisi and H Al-Sulaiti, 2010. Does consanguinity lead to decreased incidence of breast cancer? Cancer Epidemiology, 34**:** 413-418.

Beresford MJ, GD Wilson and A Makris, 2006. Measuring proliferation in breast cancer: practicalities and applications. Breast Cancer Research, 8: 1-11.

Berrington A, P Jha, J Peto and J Green, 2002. Oral contraceptives and cervical cancer. The Lancet, 360: 410.

Bhupathiraju SN, F Grodstein, MJ Stampfer, WC Willett, FB Hu and JE Manson, 2016. Exogenous hormone use: oral contraceptives, postmenopausal hormone therapy, and health outcomes in the Nurses' Health Study. American Journal of Public Health, 106: 1631-1637.

Binder-Foucard F, C Reitzer, J Jégu, B Schweitzer, F Koehl, J Kopferschmitt and M Velten, 2012. Use of psychotropic drugs, systemic antihistamines and medications for cough in 6-year-old children: a survey in the Bas-Rhin Region, France. Pharmacoepidemiology and Drug Safety, 21: 1112-1117.

Chamness G and W Mcguire, 1979. Steroid receptor assay methods in human breast cancer. Steroid Receptors and the Management of Cancer, 1: 3-30.

Cherry N, K Gilmour, P Hannaford, A Heagerty, MA Khan, H Kitchener, R Mcnamee, M Elstein, C Kay and M Seif, 2002. Oestrogen therapy for prevention of reinfarction in postmenopausal women: a randomised placebo controlled trial. Lancet , 360: 2001-2008.

Crawford J, D Dale and G Lyman, 2004. Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. Cancer, 100: 228-237.

Desantis C, N Howlader, KA Cronin and A Jemal, 2011. Breast cancer incidence rates in US women are no longer declining. Cancer Epidemiology and Prevention Biomarkers, 20: 733-739.

Gayathri G and BS Jyothi, 2020. Design and analysis of active contour model in breast cancer detection using conventional ultrasound and color doppler. Mukt Shabd Journal, 9: 1333-1342.

Glass AG and RN Hoover, 1990. Rising incidence of breast cancer: relationship to stage and receptor status. JNCI: Journal of the National Cancer Institute, 82: 693-696.

Gown AM, 2008. Current issues in ER and HER2 testing by IHC in breast cancer. Modern Pathology, 21**,** S8-S15.

Grimm RH, JD Neaton and W Ludwig, 1985. Prognostic importance of the white blood cell count for coronary, cancer, and all-cause mortality. Jama, 254: 1932-1937.

Gross JL, RN Younes, FJ Haddad, D Deheinzelin, CAL Pinto and MLV Costa, 2005. Soft-tissue sarcomas of the chest wall: prognostic factors. Chest, 127: 902-908.

Howlader N, G Forjaz, MJ Mooradian, R Meza, CY Kong, KA Cronin, AB Mariotto, DR Lowy and EJ Feuer, 2020. The effect of advances in lung-cancer treatment on population mortality. New England Journal of Medicine, 383: 640-649.

Hunter M and J Mcneil, 1997. Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. Ecology, 78: 977-986.

Hunter MD, 1997. When a picture taints a thousand words: true images of diet-induced diapause in a polyphagous insect herbivore (erratum). Ecology, 78**,** 2267.

Li CI, BO Anderson, JR Daling and RE Moe, 2003. Trends in incidence rates of invasive lobular and ductal breast carcinoma. JAMA, 289: 1421-1424.

Lodish H, A Berk, SL Zipursky, P Matsudaira, D Baltimore and J Darnell, 2000. Molecular analysis of photosystems. Molecular Cell Biology. 4th edition. WH Freeman, New York, USA.

Martínez ME, JT Unkart, L Tao, CH Kroenke, R Schwab, I Komenaka and SL Gomez, 2017. Prognostic significance of marital status in breast cancer survival: A population-based study. PLoS One, 12: e0175515.

Miller K, 2003. Estrogen and DNA damage: the silent source of breast cancer? Journal of the National Cancer Institute, 95: 100-102.

Mishra S, D Sharma and P Sharma, 2004. Studies of biochemical parameters in breast cancer with and without metastasis. Indian Journal of Clinical Biochemistry, 19**,** 71-75.

Mozaffarian D, R Nye and WC Levy, 2003. Anemia predicts mortality in severe heart failure: the prospective randomized amlodipine survival evaluation (PRAISE). Journal of the American College of Cardiology, 41: 1933-1939.

Mwer S, D Dykes and H Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids Research, 16**:** 1215.

Nakamura Y, W Okamoto, K Shitara, T Kojima, C Morizane, Y Naito, S Yuki, Y Kagawa, Y Narita and Y Nakashima, 2018. Large-scale analyses of tumor mutation burdens (TMBs) across various advanced gastrointestinal (GI) malignancies in the nationwide cancer genome screening project, SCRUM-Japan GI-Screen. Journal of Clinical Oncology, 36: 12094.

Noda K, Y Nishiwaki, M Kawahara, S Negoro, T Sugiura, A Yokoyama, M Fukuoka, K Mori, K Watanabe and T Tamura, 2002. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. New England Journal of Medicine, 346: 85-91.

Osborne C, GV Ostir, X Du, MK Peek and JS Goodwin, 2005. The influence of marital status on the stage at diagnosis, treatment, and survival of older women with breast cancer. Breast Cancer Research and Treatment, 93: 41-47.

Prentice RL, B Caan, RT Chlebowski, R Patterson, LH Kuller, JK Ockene, KL Margolis, MC Limacher, JE Manson and LM Parker, 2006. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA, 295: 629-642.

Rampaul RS, A Miremadi, SE Pinder, A Lee and IO Ellis, 2001. Pathological validation and significance of micrometastasis in sentinel nodes in primary breast cancer. Breast Cancer Research, 3: 1-4.

Ravelli A, JM Reuben, F Lanza, S Anfossi, MR Cappelletti, L Zanotti, A Gobbi, C Senti, P Brambilla and M Milani, 2015. Breast cancer circulating biomarkers: advantages, drawbacks, and new insights. Tumor Biology, 36: 6653-6665.

Shema L, L Ore, M Ben-Shachar, M Haj and S Linn, 2007. The association between breastfeeding and breast cancer occurrence among Israeli Jewish women: a case control study. Journal of Cancer Research and Clinical Oncology, 133: 539-546.

Shimada H, G Oohira, S-I Okazumi, H Matsubara, Y Nabeya, H Hayashi, A Takeda, Y Gunji and T Ochiai, 2004. Thrombocytosis associated with poor prognosis in patients with esophageal carcinoma. Journal of the American College of Surgeons, 198: 737-741.

Sierko E and MZ Wojtukiewicz, 2004. Platelets and angiogenesis in malignancy. Seminars in Thrombosis and Hemostasis, 30: 95-108.

Sohail S and SN Alam, 2007. Breast cancer in Pakistan-awareness and early detection. Journal of College of Physicians and Surgeons Pakistan, 17: 711-712.

Taucher S, A Salat, M Gnant, W Kwasny, B Mlineritsch, R-C Menzel, M Schmid, MG Smola, M Stierer and C Tausch, 2003. Impact of pretreatment thrombocytosis on survival in primary breast cancer. Thrombosis and haemostasis, 89: 1098-1106.