Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.793
*Biol. Clin. Sci. Res. J., Volume, **2024**: 793*
Original Research Article

CORRELATION OF TUMOR MARKERS HER2 NEU ER PR AND KI 67 IN BREAST CANCER PATIENTS

RASHID MN[1*], HUDA NU[2], RIAZ L[3], SHAHID RA[4], SIDDIQUI AM[2], BUTT F[5]

[1]*Department of Physiology/ Medical Education, Research and Ethics Department, Shaheed Mohtarma Benazir Bhutto Medical College, Dow University of Health Sciences, Karachi, Pakistan*
[2]*Department of Anatomy, Dow University of Health Sciences Karachi, Pakistan*
[3]*Department of Forensic Medicine, Dow University of Health Sciences Karachi, Pakistan*
[4]*Department of Physiology, DDC, Dow University of Health Sciences Karachi, Pakistan*
[5]*Department of Physiology, Dow University of Health Sciences, Karachi, Pakistan*
**Corresponding author's email address:* drnomanrashid@hotmail.com

(Received, 19th December 2023, Revised 09th March 2024, Published 6th April 2024)

Abstract: *Every year, more than a million women are diagnosed with breast cancer, and over 700.000 of them have positive for hormone receptors (FIR). The expression of key markers, such as ER and PP, and clinical circumstances show specific biological traits. Risk factors may have a role in the development of some hormone receptor-positive breast cancers.* ***Objective:*** *The objective of this study was to investigate the correlations between key tumor markers, including HER2, ER, PR, ALP, and KI67, in hormone receptor-positive breast cancers.* ***Methods:*** *Tumour tissue samples were collected for histopathology (10% neutral buffered Formalin-fixed paraffin-embedded tissue specimens) during Trucut biopsy or mastectomy at the oncology department of a tertiary care hospital in Karachi from October 2022 to July 2023. Tissues were analyzed under a light microscope for tumor hormone receptors at a tertiary care hospital in Karachi.* ***Results:*** *There were several significant correlations observed between HER 2 NUE, KI 67, ER, and PR. Specifically, a weak negative correlation was noted between HER 2 NUE and KI 67, while weak positive correlations were observed between HER 2 NUE and both ER and PR. Additionally, a weak positive correlation was found between ER and HER 2 NUE, PR, and KI 67, and a weak positive correlation existed between PR and ER, HER 2 NUE, and KI 67. Finally, KI 67 displayed a weak positive correlation with HER 2 NUE, PR, and ER.* ***Conclusions:*** *Her two nurses, ER, ALP, KI 67, and breastfeeding, are significantly and directly related. PR is not significantly related to other mentioned tumor markers.*

Keywords: Breast Cancer, Estrogen Receptor, HER2, KI67, Progesterone Receptor

Introduction

The most devastating illness to affect women worldwide is breast cancer. According to a reliable source, the National Cancer Institute, there will be 281,550 new cases of breast cancer diagnosed in the US in 2021 (1). The most common type of cancer among women is breast cancer. Action taken immediately to screen for breast cancer can significantly enhance patient care (2). The primary prerequisites for detecting breast cancer are the availability of diagnostic tools, appropriate technology, qualified practitioners, and expert analysis (3). Active chemicals known as tumour markers can reveal the presence and progression of a tumour. The overexpression of tumour markers can efficiently guide breast cancer diagnosis and treatment (4). The traditional techniques for detecting tumour markers have some pitfalls, such as insufficient sensitivity, costly equipment, and intricate operations. Compared with these methods, biosensors have the advantages of high sensitivity, simple operation, low equipment cost, and can quantifiably detect all kinds of tumour markers (5). According to statistics, more than one million women are diagnosed with breast cancer every year, and over 700.000 of them have four positive hormone receptors (HR). The hormone receptors are proteins expressed in the breast stroma and epithelium. Circulating hormones bind to receptors, which mediate their biological actions (6).

Progesterone receptors (PR) and estrogen receptors (ER) are well-known hormone receptors. ER is regarded as a superior marker to assess the presence of cancer, the effectiveness of cancer treatment, and the course of the disease, maybe in breast cancer. A hormone called estrogen is involved in cell growth, division, and proliferation. The presence of estrogen receptors in a moderate amount in a friendly environment is significant (7). A tissue's excessively high ER value could be a sign of cancer. ER is a favourable biologic marker for the response to treatment in breast cancer, in addition to prognostic reference. It has genetic functions and is a steroidogenic hormone. Estrogen 17p-estradiol regulates it (E2) (8). On various tissues, ER expresses itself as homodimers or heterodimers, primarily in the forms of ERp and ERq. Both receptors are localised on distinct chromosomes, with ERa being one of them. Human chromosome 6 is the location of ERa, while ERp is found on the chromosome. Serum ER concentrations are a reliable prognostic indicator. It could play a role in the development of hormone-resistant breast cancer. ER is beneficial for both invasive and metastatic breast cancer detection (9). ER serum levels may impact the treatment plan. Estrogen serum concentration recommendations for hormonal therapy, such as tamoxifen and raloxifene, for peri-menopausal and postmenopausal women. Due to low anaplasty and a more significant scale of differentiation, ER expression is comparatively linked to a better prognosis (10).

The breast cancer tumour marker known as progesterone receptors (PR) helps to predict the prognosis and outcome

[Citation: Rashid, M.N., Huda, N.U., Riaz, L., Shahid, R.A., Siddiqui, A.M., Butt, F. (2024). Correlation of tumor markers HER2 NEU ER PR and KI 67 in breast cancer patients. *Biol. Clin. Sci. Res. J.*, **2024***: 793.* doi: https://doi.org/10.54112/bcsrj.v2024i1.793]

of hormone therapy. It has a nuclear function and is a steroidogenic hormone receptor (11). On chromosome 1q22 is where the gene code for PR is located. The human PR gene is split into two isoforms, PR-A and PR-B, but the inductive proteins are distinct. PR has two domains, two amino and carboxyl-terminal ends, which are its structural components (12). One domain is a DNA-binding regulatory domain, and the other has an activation function. (AFs). The AF-1 isoform of PRs is located at the N-terminal area and does not require a binding protein for activation. In contrast, both isoforms of PRs retain their unique activation functional domain (12). The C-terminal region is home to AF-2, which binding proteins activate. While PR-A controls transcription by inhibiting the PR-B gene, estrogen receptors (ERs), and other steroid hormone receptors, PR-B isoforms primarily participate in transcription and act on a promoter. PR-B isoforms have an additional activation function domain three at the amino acid terminal. A balance between PR-A and PR-B must exist to execute certain functions and trigger hormonal responses in particular tissues. Both PR isoforms regulate the transcription of breast cancer cells; however, PR-B acts as the primary regulator (13).

HER-2, HER-Etrieu, ERBB2, erbB2, erbB- 2, neuic-erbB-Voncogene neu, neu protein, and neu are some of the various names for this gene. Slamon et al. and other researchers have found a link between a poor prognosis and the expression of this marker on breast cancer cells (14). By nature, the ERBB2IFIEFi2 oncogene located on chromosome 17q21 is the source of the transmembrane tyrosine kinase receptor known as HER-2. This receptor functions as a second messenger; following dimerisation, it promotes intracellular tyrosine kinase activity, which triggers the auto-phosphorylation of cytoplasmic tyrosine residues critical for cell proliferation (15). Because it participates in mitotic phase activity and strong binding even without a ligand, which may potentially result in chemotherapy resistance and be linked to a worse prognosis, it is regarded to be an aggressive marker. This oncogene serves as a marker when applied to abnormal cells, but fortunately, only 20%-30 cases of breast cancer are favourable for it (16).

This nuclear protein, linked to cellular proliferation, was discovered for the first time by Gerdes et al. utilising a mouse monoclonal antibody against a nuclear antigen from an early 1980s Hodgkin's lymphoma cell line. Immunohistochemical examination is the most widely used method for analysing the Ki-67 antigen (17). It was determined that the cell cycle's 5th, Gl, G2, and M stages are when the Ki-67 nuclear antigen is expressed instead of in GO. In samples from normal breast tissue, it was discovered that the expression of Ki-67 was non-significantly higher (N3 9.8 of cells) in ER-negative than ER-positive cells. Using the monoclonal antibody Ki-57, immunostaining can be used to determine the growth fraction (18).

The objective of this study was to establish the relationship between the likelihood of bone metastases in breast cancer and the levels of molecular markers, namely estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER-2), and Ki-67.

Methodology

After the ethical approval from the Board of Advance Studies, this cross-sectional observational study was conducted at the oncology department of a tertiary care hospital in Karachi from October 2022 to July 2023. Through non-probability consecutive sampling, 280 patients with confirmed breast cancer diagnosis, lying in various stages from I-IV of breast cancer, were included in the present study. Patients suffering from primary Bane diseases, Diabetes, Hepatobiliary disease and previous history of any other type of malignancy were excluded from the study. The consent form was described orally to every patient, and a signature was taken. Trained staff were hired for sample collection. Sterilised materials were used. Collections of tumour tissues (as trust biopsy or mastectomy) for histopathology (10 % neutral buffered Formalin-fixed paraffin-embedded tissue specimens). Tissues were examined for tumour hormone receptors at JPMC and BMSI under a light microscope. Test for hormone receptors using paraffin-embedded tissues treated in 10% neutral buffered formalin and employing the Allred scaring technique by ASCO-CAP guidelines. ENVISION FLEX SYSTEM uses monoclonal antibodies to stain sections for the oestrogen (clone EPI, Dako) and progesterone (clone Pgr636, Dako) receptors. (Dako). Each test slide has the appropriate exterior tissues with a positive and negative component. Internal positive controls, such as ducts and lobules from normal breast tissue, are also used when accessible. On a labelled paraffin block, immuno-histochemical staining was carried out. Extrinsic positive controls that are appropriate are positioned parallel to the patient portion on the same slide. Only after verifying that all extrinsic and intrinsic epithelial components contained in the sample or independently provided from the sample have operated as intended are the results reported. Results were determined using the Allred scoring method. (Dako-) by CAP 2010 and ASCO 2010 guidelines. The HER2 status was assessed using FISH and a DAKO antibody. IHC designated grades 0 and 1 for HERZ as negative results and grade 3 as a positive result. If the IHC score was 23- or higher, HER2 amplification was verified by FISH. An absence of ER, PR, and HER2 expressions was called triple negativity. All referral core biopsies were reviewed by experienced pathologists at the tertiary care hospital in Karachi, including IHC staining at the time of initial referral. Immuno-histo-chemical staining is conducted, and the proportion of the malignant cells staining positive for the nuclear antigen Ki-67 is evaluated quantitatively and visually using light microscopes. The percentage of malignant cells is positively marked by the anti-human Ki-67 monoclonal antibody MIB1, one of the most widely used antibodies and the "gold standard," which calculates Ki-67 values. The percentage of tumour cells positively stained out of all the malignant cells analysed is the Ki-67 percentage score. Independent of the level of colouring, only positivity is of interest. Positive control tissues are finished, ensuring that the staining's quality assurance is guaranteed. According to the current experience of various pathologists and suggestions made at the national and international levels, a Ki-67 cut-off point of 15 9.8 was established. The entire material is examined and is searched

[Citation: Rashid, M.N., Huda, N.U., Riaz, L., Shahid, R.A., Siddiqui, A.M., Butt, F. (2024). Correlation of tumor markers HER2 NEU ER PR and KI 67 in breast cancer patients. *Biol. Clin. Sci. Res. J.*, **2024***: 793.* doi: https://doi.org/10.54112/bcsrj.v2024i1.793]

for any immune-staining tumour cell nuclei. Scoring is conducted considering the whole tumour section and not only limiting to the hot spots of the carcinoma or the most evident positive parts within the invasive segment or the front of necrosis. The data feeding and analysis were done on the computer package SPSS (Statistical Packages of Social Sciences) version 21. Clinical characteristics were summarised in frequencies, means ±SD and percentages for qualitative/quantitative! Variables, tumour markers KI 67, HER 2NUE, ER, PR). Regression correlation analysis was applied to characterise the association and strength of association via Pearson parametric calculation and Spearsman's rho non-parametric calculation among quantitative variables. A value of $P < 0.05$ was considered significant.

Results

There were several significant correlations observed between HER 2 NUE, KI 67, ER, and PR. Specifically, a weak negative correlation was noted between HER 2 NUE and KI 67, while weak positive correlations were observed between HER 2 NUE and both ER and PR. Additionally, a weak positive correlation was found between ER and HER 2 NUE, PR, and KI 67, and a weak positive correlation existed between PR and ER, HER 2 NUE, and KI 67. Finally, KI 67 displayed a weak positive correlation with HER 2 NUE, PR, and ER.

Table 1 shows the descriptive analysis of tumour markers in terms of mean and standard deviation. Figures 1-3 show the frequency of positive patients for the tumour markers HER2/reu, ER, and PR. Table 2 shows the Spearman's rho coefficient of the tumour markers.Table 3 shows the Pearson correlation of the tumour markers.

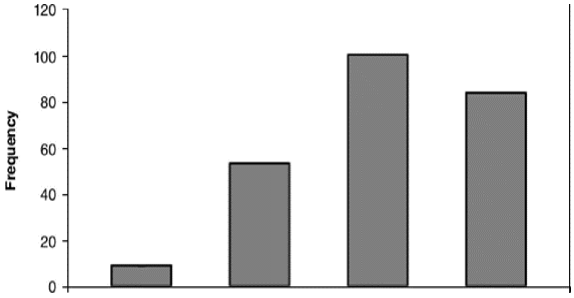

Figure 1: Frequency of HER2/reu

Table 1: Descriptive statistics

Markers	Mean± S. D (n=283)
ER 2 Nue	2.13±0.857
ER	1.50±0.30
PR	1.58±0.45
K1 67 Max	47.47±25.187
Stages	
Stage T	2.80±0.974
Stage N	1.05±0.907
Stage M	1.39±1.73

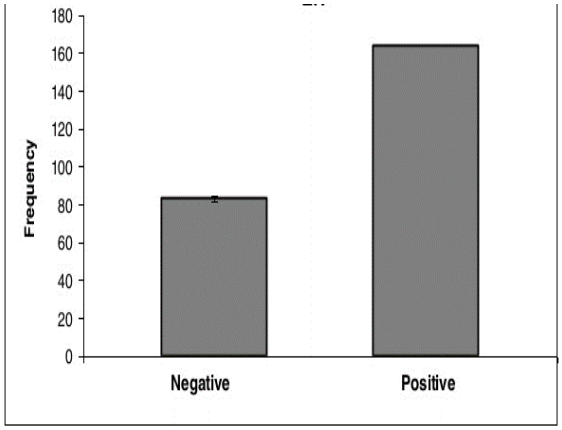

Figure 2: Frequency of ER

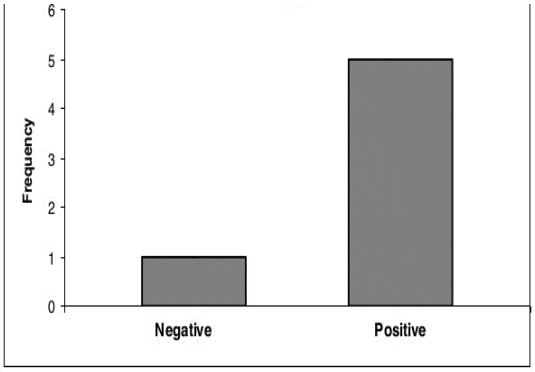

Figure 3: Frequency of PR

Table 2: Spearman's rho of tumor markers

			Her 2 Nue	ER	PR	KI 67 Max	ALP
Spearman's rho	Her 2 Nue	Correlation Coefficient	1.00	.136	.096	-.126	.218
		Sig(2-tailed)	.	.022	.106	.034	.000
		N	283	283	283	283	283
	ER	Correlation Coefficient	.136	1.000	.526''	.266''	.306''
		Sig(2-tailed)	.022	.	.000	.000	.000
		N	283	283	283	283	283
	PR	Correlation Coefficient	.096	.562''	1.000	.225''	.098
		Sig(2-tailed)	.106	.000	.	.000	.098
		N	283	283	283	283	283
	KI 67 Max	Correlation Coefficient	-.126	.266''	.225''	1.000	-.198''
		Sig(2-tailed)	.034	.000	.000	.	.001
		N	283	283	283	283	283

[Citation: Rashid, M.N., Huda, N.U., Riaz, L., Shahid, R.A., Siddiqui, A.M., Butt, F. (2024). Correlation of tumor markers HER2 NEU ER PR and KI 67 in breast cancer patients. *Biol. Clin. Sci. Res. J.*, **2024***: 793.* doi: https://doi.org/10.54112/bcsrj.v2024i1.793]

Table 3: Pearson correlation of the tumor markers

		Her 2 Nue	ER	PR	KI 67 Max
Her 2 Nue	Pearson Correlation	1	.116	.092	-.124’
	Sig. (2-tailed)		.051	.123	.037
	N	283	283	283	283
ER	Pearson Correlation	.116	1	.562’’	.212’’
	Sig. (2-tailed)	.051		.000	.000
	N	283	283	283	283
PR	Pearson Correlation	.092	.526’’	1	.205’’
	Sig. (2-tailed)	.123	.000		.001
	N	283	283	283	283
KL 67 Max	Pearson Correlation	-.124’	.212’’	.205’’	1
	Sig. (2-tailed)	.037	.000	.001	
	N	283	283	283	283

Discussion

Ki-67 is a nuclear protein that is not associated with histones. It is found in the nucleus and is present during the cell cycle's G1, S, G2, and M stages but not during the G0 phase. Ki-67 is a substantial nuclear protein with a molecular weight 395kDa (19). It is believed to play a role in various cellular operations, including regulating the cell cycle, digesting ribosomal RNA, organising DNA, and maybe having a structural function within the nucleus (20). Ki-67 is a marker that indicates the level of cell proliferation and is linked to the invasiveness and recurrence of tumours. Multiple studies have examined Ki-67 as a prognostic marker for breast. Previous research has shown that the expression of Ki-67 is linked to the low differentiation of tumours and the presence of big tumour sizes in breast cancer (21). This study demonstrated a negative link between the expression of hormone receptors (ER and PR) and Ki-67, while a positive correlation was observed between Ki-67 and HER-2 expression.

Several studies have demonstrated a negative association between the expression of Ki-67 and ER expression (22, 23). To assess the relationship between the expression of Ki-67 and certain clinicopathological characteristics, an analysis was conducted on 356 BC samples, looking back at past data. The study revealed a correlation between Ki-67 expression and tumour size, grade, and lymph node metastases. However, there was an inverse correlation between Ki-67 expression and HR expression, which aligns with the findings of this study (24).

Conclusion

Her two nurses, ER, ALP, KI 67, and breastfeeding are significantly and directly related. PR is not significantly related to other mentioned tumour markers.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department concerned. (NO. IRBEC/21-555-DOW/KHR)

Consent for publication

Approved

Funding

Not applicable

Conflict of interest

The authors declared the absence of a conflict of interest.

Author Contribution

MUHAMMAD NOMAN RASHID (Assistant Professor)
Study Design, Review of Literature.
Conception of Study, Development of Research Methodology Design, Study Design, manuscript Review, and final approval of manuscript.
NASR UL HUDA (Assistant Professor)
Coordination of collaborative efforts.
Conception of Study, Final approval of manuscript.
LUBNA RIAZ (Assistant Professor)
Manuscript revisions, critical input.
Coordination of collaborative efforts.
RIAZ AHMED SHAHID (Assistant Professor)
Data acquisition and analysis.
Manuscript drafting.
ASHHAD MAZHAR SIDDIQUI (Assistant Professor)
Data entry and data analysis, as well as drafting the article.
Data acquisition and analysis.
FAREEHA BUTT (Assistant Professor)
Coordination of collaborative efforts.

References

1. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. The British journal of radiology. 2022;95(1130):20211033.
2. Britt KL, Cuzick J, Phillips K-A. Key steps for effective breast cancer prevention. Nature Reviews Cancer. 2020;20(8):417-36.
3. Milosevic M, Jankovic D, Milenkovic A, Stojanov D. Early breast cancer diagnosis and detection. Technology and Health Care. 2018;26(4):729-59.
4. Pirsaheb M, Mohammadi S, Salimi A. Current advances of carbon dots based biosensors for tumour marker detection, cancer cells analysis and bioimaging. TrAC Trends in Analytical Chemistry. 2019;115:83-99.

[Citation: Rashid, M.N., Huda, N.U., Riaz, L., Shahid, R.A., Siddiqui, A.M., Butt, F. (2024). Correlation of tumor markers HER2 NEU ER PR and KI 67 in breast cancer patients. *Biol. Clin. Sci. Res. J.*, **2024***: 793.* doi: https://doi.org/10.54112/bcsrj.v2024i1.793]

5. Hong R, Sun H, Li D, Yang W, Fan K, Liu C, et al. A review of biosensors for detecting tumor markers in breast cancer. Life. 2022;12(3):342.
6. Mutebi M, Unger-Saldaña K, Ginsburg O. Breast cancer: Burden, epidemiology and priority interventions. Noncommunicable Diseases. 2023:91-7.
7. Duffy M, Harbeck N, Nap M, Molina R, Nicolini A, Senkus E, et al. Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM). European journal of cancer. 2017;75:284-98.
8. Begam AJ, Jubie S, Nanjan M. Estrogen receptor agonists/antagonists in breast cancer therapy: A critical review. Bioorganic chemistry. 2017;71:257-74.
9. Martinez-Perez C, Turnbull AK, Dixon JM. The evolving role of receptors as predictive biomarkers for metastatic breast cancer. Expert Review of Anticancer Therapy. 2019;19(2):121-38.
10. Reinert T, Cascelli F, Resende CAAd, Goncalves AC, Godo VSP. Clinical implication of low estrogen receptor (ER-low) expression in breast cancer. Frontiers in Endocrinology. 2022;13:1015388.
11. Trabert B, Sherman ME, Kannan N, Stanczyk FZ. Progesterone and breast cancer. Endocrine reviews. 2020;41(2):320-44.
12. Honma N, Matsuda Y, Mikami T. Carcinogenesis of triple-negative breast cancer and sex steroid hormones. Cancers. 2021;13(11):2588.
13. Woo ARE, Sze SK, Chung HH, Lin VC. Delineation of critical amino acids in activation function 1 of progesterone receptor for recruitment of transcription coregulators. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms. 2019;1862(4):522-33.
14. Fasching PA, Gass P, Häberle L, Volz B, Hein A, Hack CC, et al. Prognostic effect of Ki-67 in common clinical subgroups of patients with HER2-negative, hormone receptor-positive early breast cancer. Breast cancer research and treatment. 2019;175:617-25.
15. Finn RS, Liu Y, Zhu Z, Martin M, Rugo HS, Diéras V, et al. Biomarker analyses of response to cyclin-dependent kinase 4/6 inhibition and endocrine therapy in women with treatment-naïve metastatic breast cancer. Clinical Cancer Research. 2020;26(1):110-21.
16. De Angelis C, Fu X, Cataldo ML, Nardone A, Pereira R, Veeraraghavan J, et al. Activation of the IFN signaling pathway is associated with resistance to CDK4/6 inhibitors and immune checkpoint activation in ER-positive breast cancer. Clinical Cancer Research. 2021;27(17):4870-82.
17. Dodson A. Measuring Ki-67 in Breast Cancer: Past, Present, and Future. Artificial Intelligence Applications in Human Pathology2022. p. 71-98.
18. Bienz M, Ramdani S, Knecht H. Molecular pathogenesis of Hodgkin lymphoma: past, present, future. International Journal of Molecular Sciences. 2020;21(18):6623.
19. Sun X, Kaufman PD. Ki-67: more than a proliferation marker. Chromosoma. 2018;127:175-86.
20. Menon SS, Guruvayoorappan C, Sakthivel KM, Rasmi RR. Ki-67 protein as a tumour proliferation marker. Clinica chimica acta. 2019;491:39-45.
21. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer biology & medicine. 2016;13(4):496.
22. Alco G, Bozdogan A, Selamoglu D, Pilancı KN, Tuzlalı S, Ordu C, et al. Clinical and histopathological factors associated with Ki-67 expression in breast cancer patients. Oncology letters. 2015;9(3):1046-54.
23. Kilickap S, Kaya Y, Yücel B, Tuncer E, AKGÜL BABACAN N, Elagoz S. Higher Ki67 expression is associates with unfavorable prognostic factors and shorter survival in breast cancer. Asian Pacific Journal of Cancer Prevention. 2014;15(3).
24. Al Nemer A. The performance of Ki-67 labeling index in different specimen categories of invasive ductal carcinoma of the breast using 2 scoring methods. Applied Immunohistochemistry & Molecular Morphology. 2017;25(2):86-90.

[Citation: Rashid, M.N., Huda, N.U., Riaz, L., Shahid, R.A., Siddiqui, A.M., Butt, F. (2024). Correlation of tumor markers HER2 NEU ER PR and KI 67 in breast cancer patients. *Biol. Clin. Sci. Res. J.*, **2024***: 793.* doi: https://doi.org/10.54112/bcsrj.v2024i1.793]