Molecular investigation of Her2 gene in Breast Cancer Women In Babylon City

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KEYWORDS

Breast cancer, Her2 gene, peripheral blood sample, PCR technique.

ABSTRACT

Background: The most prevalent illness in women is breast cancer, "Human epidermal growth factor receptor 2 (HER2)" plays a significant role in the carcinogenesis of breast cancer. (HER2)" overexpression can arise from several causes and is frequently observed in 20–30% of instances of breast cancer.
Aim of the study: The current study was aimed to investigate the correlation between the existence of *HER2* gene expression in the peripheral blood samples with breast cancer females by using the primers of *HER2* gene and PCR technique.
Materials and Methods: In this study, seventy two (72) peripheral Blood samples were taken from the breast cancer ladies from Marjan Hospital's at Oncology Center in Babylon city's who had previously received a confirmatory diagnosis of breast cancer with ten (10) blood samples from clinically healthy women as a control group during the period from June 2022 to January 2023, DNA samples were isolated from the collected blood samples by DNA Extraction kit (FavorPrepTM Blood/Cultured Cells Genomic DNA Extraction Mini Kit), then, conventional PCR technique was performed with specific oligo primers to amplify the *Her2* gene's target sequence in those DNA samples, then, agarose gel electrophoresis was applied for detecting the target sequence in the DNA products with the band size 150bp.
Results: 58 / 72(80.5 %) of the DNA samples were positive and 14/ 72 (19. 5%) were negative for *Her2* gene in the peripheral blood samples of the breast cancer women.
Conclusions: in conclusion the current study's results demonstrated that 58/ 72 (80.5%) of the peripheral blood samples from breast cancer women were positive and the remaining 14/ 72(19.5%) of the peripheral blood samples were negative for *Her-2* gene, "*HER2* gene" can be considered as a useful biomarker and an obvious indicator for breast cancer which could be employed for early and confirmatory diagnosis of breast cancer in women.

1. Introduction

The "tyrosine protein kinase" receptor ERB-B-2 is also referred to as proto-oncogene Neu, CD340 (cluster divides into 340), ERB-B-2 (mice), or ERB-B-2 (human). It might be the human ERBB2 protein-encoding quality, commonly referred to as HER2 (derived from human epidermal growth imaging receptor 2) or HER2/ neu (Janet M. 2015). HER2 protein belong to the human epithelial growth factor receptor (HER/EGFR/ERBB) family. The Upregulation or the over expression for this proto-oncogene has been revealed to act a parting the encouragement and dissemination of multiple forms of advanced breast cancer. With time, this protein was transformed into an essential biomarker and therapy goal for around thirty percent of breast cancer patients (Coussens et al., 1985; Mitri et al., 2012; Debmalya 2017).

HER2 gene entitled due to it is structurally look like to the "human epidermal growth receptor" or HER1gene. The name Neuis given due to it is consequents of a (mouse glioblastoma) cell line, a type of neuroma. ErbB-2 was called due to its likeness to ErbB (avian erythroid B oncogene), an oncogene that was later set up to encrypt for EGFR. Atomic cloning has shown that HER2 gene, Neu and ErbB-2 are encrypted by identical forms. The ERBB2 gene is a well identified proto–oncogene which is found on human chromosome 17's long arm (17q12) (Keshamouni et al ., 2002).There are four (plasma membrane receptor tyrosine kinases) in the E r b B family ring. Among them are e r b B – 2; the others are e r b B – 3 (binding neuregulin; desired area kinase); and e r b B – 4 (epidermal enhancer receptors). All 4 incorporate a professional extracellular ligand region, a trans membrane region, as well as an intracellular region that can bind to a wide variety of signal atoms and display each ligand-based and ligand-independent actions. Except, no ligand for it has been determined that HER2 (Rusnak et al., 2001; Olayioye 2001). HER2 is a well-thought-out common dimerization buddy of the E r b B substitution receptors, and it can hetero dimerise within the least of the three replacement receptors. In

the cytoplasmic area of receptors, dimerization mediates an auto-phosphorylation of tyrosine accumulations and initiates a number of signaling pathways (Roy 2009).

Human epidermal growth factor receptor 2 (HER2) is overexpressed in about 20% of cases of breast cancer (Barnard et al., 2015). Amplification of the HER2 gene results in overexpression of the HER2 protein. The poorest prognosis of all the breast cancer types is related with HER2 gene overexpressing cancer, which frequently exhibits an extremely aggressive character and is linked to metastases to distant organs and lymph nodes (Perou et al ., 2000) , (Slamon et al., 1987) , (Sørlie et al., 2001). Anti-HER2 antibodies, a molecular target-based therapy, may help improve the prognosis of breast cancer with overexpression of the HER2 gene (Lobbezoo et al ., 2013) .

The HER2 gene is one of the main agents involved in breast carcinogenesis, and breast cancer is the most common cancer among women. Many factors lead to the overexpression of Human Epidermal Growth Factor Receptor 2 (HER2), which is a characteristic that typically affects 20 to 30 percent of breast cancer cases. The HER2 gene, also known as HER2/neu, c-ErbB2, and P185 HER2 genes, is found in the human genome at location 17q21. A 1255 amino acids transmembrane glycoprotein weighing 185 kDa are encoded by the HER2 gene (Coussens et al., 1985; King et al., 1985; Sasso et al., 2011).

On the cellular surface, the HER2 product and other HER2, HER1, HER3, and HER4 family members interact to produce active tyrosine kinase receptors (Hung et al., 1986). The structure shared by all members of the HER family is comprised of an intracellular region that has tyrosine kinase activity, an extracellular domain that serves as a ligand binding area, and a lipophilic trans membrane domain. However, because the HER2 and HER3 genes lack active tyrosine kinase and ligand binding domains, respectively, they do not exhibit this pattern. The interaction of Epidermal Growth Factor-Like Growth Factors (EGFs) enhances the activation of these tyrosine kinase domains, even though no specific ligand has been shown to bind to HER2 receptors (Beerli & Hynes 1996). Remarkable ERBB2 overexpression, occurs in (15-30%) of breast cancer cases (Coussens et al., 1985; Barnard et al., 2015). It is intensely linked with reappearance of severe infection and deprived prognosis (Slamon et al., 1987). Overexpression is further diagnosed in the ovary (Sorlie et al., 2001). Stomach, lung adenocarcinoma (King et al., 1985) .and hostile pattern of cancer of uterus, including serous carcinoma of the endometrium (Coussens et al., 1985). In individuals with maxillary gastric cancer (King et al., 1985; Hung et al., 1986) , and HER– 2 is overexpressed in about (30%) of salivary duct carcinomas (Beerli et al., 1996). HER2 gene is co-localized and mainly co-amplified with the remarkable GRB7, to become an oncogene associated with tumors of the breast, testicular germ cells, stomach and esophagus. The HER2 protein is thought to induce cell membrane clumps that may take a part in tumorigenesis (Brandt-Rauf et al., 1990; Yonesaka et al., 2011). The next screen that traps HER2 gene signaling in its ability to fight most cancers focused on the EGFR drug cetuximab. Dimerization is spheroidal during autophosphorylation that accumulates tyrosine in the cytoplasmic region of receptors and begins by developing a combination of signaling pathways (Mazières et al., 2013). In liquid biopsy, “circulating tumor DNA (ctDNA)” is now the most often utilized biomarker. (Keller et al., 2021). Liquid biopsy is a minimally invasive technique for tumor identification that is capable of quantitatively analyzing blood molecular alterations that are intimately linked to the onset and progression of malignancies. The most significant gene marker for breast cancer is” human epidermal growth factor receptor 2 (HER2)” and HER2 amplification is a known risk factor for a poor outcome. (Swain and Associates, 2015; Ma and Associates, 2019). The clinical utility of circulating HER2 levels in comparison to pathological findings which are still Research on these levels has typically focused on the gold standard for cancer diagnosis (Sakai et al., 2018; Mishima et al., 2017).

Nevertheless, a more sensitive technique than biopsy has not yet been developed to identify circulating HER2 amplification. Previous research has demonstrated that the tumor burden 6 has a major impact on ctDNA levels; as a result, variations in tumor size cause ctDNA levels to vary during therapy. It is proposed that ctDNA levels serve as a useful indicator of therapy response in this particular scenario. In solid tumors, radiographic imaging is still the gold standard for assessing therapy effectiveness.

Nonetheless, it is impossible to completely rule out the restrictions posed by variations in imaging technology and the subjective opinions of doctors during imaging. Patients are further discouraged by frequent exposure to radiation from imaging tests. Consequently, to ascertain which individuals would gain the most from a specific treatment, further techniques for the early prediction of treatment response are required. These techniques will assist in assessing if altering the course of therapy to enhance results or starting a maintenance program to reduce toxicity is appropriate. One intriguing biomarker for tracking the effectiveness of a treatment and maybe even forecasting the optimal course of action is the amount of ctDNA. Prior research has shown that the levels of the HER2 extracellular domain in patients with metastatic breast cancer were indicative of their HER2 disease status (Carney et al., 2013; Eppenberger-Castori et al., 2020). This quantitative indicator may aid in the identification of individuals who benefit from a sequential treatment paradigm but do not have visceral illness. (Li and et al., 2020). Additionally, according to (Shoda et al. 2017), Dynamic changes in circulating HER2 have also demonstrated potential predictive utility in determining how well gastric cancer therapies work. Therefore, further research is necessary to determine if dynamically monitoring plasma HER2 is a useful tool for predicting therapeutic response in patients with advanced breast cancer.

The purpose of this study was to determine whether the HER2 gene is present in peripheral blood samples and whether it is correlated with tumor prognosis in cases of breast cancer. Our ultimate goal was to gather clinical data supporting the viability of HER2 dynamic monitoring as a method for forecasting association with tumor prognosis in breast cancer cases. Analyzing a tumor sample at the time of initial diagnosis is how regular clinical practice determines whether an amplification is present. Personalized cancer therapy has significant challenges since amplifications can be "acquired" and "lost" due to tumor growth and previous treatments (Thompson et al., 2010; Houssami et al., 2011). For example, in around 2-3% of metastatic breast cancers that started as primary malignancies with HER2 not amplified, HER2 amplification is "acquired." Tumor cell DNA may be present in the plasma of cancer patients, providing a potential source for non-invasive tumor DNA analysis (Johnson and Lo 2002). In fact, good concordance with cancer mutational status has been demonstrated by high sensitivity investigations of coding mutations on plasma DNA, also known as cell free DNA or circulating free DNA. (Li et al., 2006; Board et al., 2010). Digital PCR, as opposed to conventional quantitative PCR, may significantly more accurately and precisely determine the amount of nucleic acids in a sample by counting individual DNA molecules (Vogelstein and Kinzler 1999). studies on the potential use of digital PCR to detect minute changes in the copy number of the plasma DNA gene that correspond with amplification unique to cancer, similar to the detection of foetal aneuploidy using maternal plasma DNA analysis (Lo et al., 2007).

Aims of the study:

The purpose of the current study was to use the HER2 gene primer in PCR method to examine the relationship between HER2 gene presences in peripheral blood samples and instances of breast cancer

2. Methodology

Materials

In the present study, seventy two (72) peripheral blood samples were collected from previously diagnosed Breast Cancer women from Oncology Center of Marjan Hospital in Babylon city, with 10 blood samples from clinically healthy women as a control group during the period from June 2022 to January 2023.

Devices

“ (Clever Scientific Thermocycler, Eppendorf Micropipette, Sartorius water Deionizer, Mupid agarose gel electrophoresis system, Atta gel documentation system, GFL water bath, Eppendorf centrifuge, freezer, and Memmert oven)”.

Chemical Materials

Agarose powder of Analytical grade ,Master Mix (Promega) , Ethidium Bromide(Promega),TBE Buffer, Ethanol Isopropanol, "FavorPrepTM Blood/Cultured Cells Genomic DNA Extraction Mini Kit" ,and Deionized Distal Water (pioneer) Oligonucleotide primers (Alpha DNA).

Methods:

Collection of Samples

collection of blood samples was done with 3-5 ml blood with anticoagulant tubes, then, DNA samples were isolated from the collected blood samples

DNA Isolation

DNA Extraction kit "FavorPrepTM Blood/Cultured Cells Genomic DNA Extraction Mini Kit" , Detection the concentration and quality (purity) of the DNA was measured by calculation the absorbance at OD260 nm. with a Nanodrop spectrophotometer (Thermo Scientific, Willington, DE, USA)(Model ND1000). while DNA purity was measured by calculation the ratio of absorbance at OD260 - OD280 nm.

After that, conventional PCR technique was performed for amplifying the target sequence of the Her2 gene in those DNA samples using specific oligo primers. After that, agarose gel electrophoresis was performed to detect the target sequence in DNA products with the band size 150 bp.

Molecular Investigation

(Oligonucleotide primers and PCR Conditions)

"The specific oligo primers were used to amplify the target sequence of the HER-2 gene, Forward primer (5'-CCAGCCCTCTGACGTCCAGCT-3'), and Reverse primer (5'-CGTGTACTTCCGGATCTTCTGCTG-3'), was according to (Bugi and Desriani 2016). As in table 1.

Primer-based DNA amplification was carried out in a 25 µl reaction Eppendorf tube. To achieve the required reaction volume of 25 µl, it was included 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each F and R primer at a concentration of 10 p.mol, 5 µl of DNA template, and 3.5 µl of deionizer water. Using the PCR cycling conditions given in Table 2, the PCR reaction was conducted in a Clever Scientific heat cycler equipment. Later, gel electrophoresis by running the PCR products on a concentration (1.5%) agarose gel and gel documentation system with UV light was used to detect the expected bands, and the results were recorded.

Statistical Analysis

Social science software Version 25 of statistical package was utilized for the comparison of the percentages of different samples of positive Her2 gene , Chi-square tests. Statistical analysis at P values less than, 0.05 were statistical significant in consideration.

3. Result and Discussion

In the current study, concentration of extracted DNA were 217- 320 µg/ml, for Blood samples, as well as, the proportion for OD260/OD280 were at ranges of (1.8 and 2.2) when measured using the Spectrophotometer. Moreover, amplification of specific fragments of Her2 gene with specific primers utilizing conventional PCR and DNA sizing method with agarose gel electrophoresis was displayed the amplicon of DNA products with the length of (150 bp.) for (Her2 gene) from peripheral blood sample of breast cancer cases of women as exhibited in the figures (no.1, 2)

The current study demonstrated that 58/72 (80.5%) of the peripheral blood samples from breast cancer women were positive and the remaining 14/72 (19.5%) of the peripheral blood samples were negative for Her-2 gene, as demonstrated in (table 1 and figure 1) with the characteristic bands of 150 bp. in figure 2, and all of the healthy control group blood samples were negative for Her-2 gene. As in table 3

Discussion:

The results of the current study revealed the presence of Her-2 gene in the peripheral blood samples of breast cancer women as demonstrated in figure 2, and its association with cases of confirmed diagnosis breast cancer which could be employed as an obvious indicator for early detection of breast cancer in women, this was similar with previous studies like (Mates et al., 2015; Bugi Ratno and Desriani 2016; Ryuichi et al ., 2016; Yanlin et al., 2018).

The method has the potential activity to distinguish the tumor cells exhibiting high HER-2 gene expression from those with low expression, as well as differentiate tumor blood from normal blood. Noteworthy dissimilarities were observed in peripheral blood samples obtained from HER-2 positive patients and healthy controls displaying positive HER2 gene expression (Yanlin et al., 2018). It is a predictive biomarker for individuals undergoing neo-adjuvant treatment is circulating HER2. The HER2 gene status of breast cancer tumors varies as they grow, making them extremely diverse malignant tumors (Zhao et al., 2015).

On the other hand, other predictive biomarker for breast cancer are the liquid biopsies that have the capability to detect the presence of HER2 gene, which is released from tumor tissues into the bloodstream, even in patients with multiple tumor sites. Additionally, liquid biopsies enable the identification of cancer-associated genes in the blood, offering comprehensive genetic overview of both primary and metastatic tumors (Wang et al., 2015; De Mattos and Arruda, 2017). As a result, the data from a liquid biopsy could provide valuable additional information to that from a histology. Moreover, liquid biopsies have the ability to dynamically track alterations in breast cancer patients' HER 2 levels following targeted therapy or chemotherapy, offering clinical decision-makers prognostic data (Crowley et al., 2013).

In patients who arrive having original tumor tissue that is HER 2 positive, following anti-HER 2 therapy, Peripheral blood levels of Herceptin (HER 2) may range from positive to negative, and these changes influence whether or not anti-HER 2 therapy should be continued (Raitoharju et al., 2014) .

As it is known, cancer is one of the worst diseases in the world and is spreading more often. Breast cancer is the leading cancer in women, accounting for 23% of cases and 14% of deaths among all cancers. Recent advancements in breast cancer treatment emphasize the significance of adaptable biomarkers for diagnosis, prognosis, and monitoring of anticancer drug therapy, particularly as the HER2 gene mutation is linked to breast cancer development (Ryuichi et al ., 2016).

4. Conclusion and future scope

The current study concluded that the “HER2 gene” is a useful biomarker, and 58/72 (80.5%) of the peripheral blood samples from breast cancer women were positive and the remaining 14/72 (19.5%) of the peripheral blood samples were negative for Her-2 gene, the association of cases that confirmed diagnosis breast cancer which could be employed as an obvious indicator for early detection of breast cancer in women.

Recommendations

Plasma DNA assays are non-invasive, repeatable numerous times during the course of the disease, and have the capacity to evaluate the whole variety of mutations present. Since DNA is often present in plasma at low concentrations and tumor cell-derived DNA may make up just a tiny portion of the total plasma DNA, the remaining DNA may be produced from somatic cells, analysis of plasma DNA necessitates the use of an assay with high sensitivity, as well as, we recommended an increasing the numbers and types of specimens for breast cancer cases and assessment of effective more gens that participate in breast cancers for the sake of prediction, progression, treatment, and direction of such dangerous cases in women population.

Declarations

We attest that this work is unique, unpublished, and not presently being considered for publication anywhere else. The "University of Kerbala" representative of my institute is completely aware of this contribution, and all of the writers concur. This work does not require ethical approval. There are no disclosed conflicts of interest.

Acknowledgments

The technician staff and faculty members of the University of Kerbala - College of Veterinary Medicine-Biology Laboratory

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