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Measurement of Breast Cancer by PCR method

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Short Commentary

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ABSTRACT

The aim of this study was optimization of Real Time PCR condition as breast cancer is one of the most common malignancies among women in various countries. HER2 overexpression occurs in 20-30% of breast cancers. HER2 gene encodes 185kDa transmembrane glycoprotein with 1255 amino acids. This active product triggers downstream intracellular signaling pathways inducing cell proliferation and cell survival. These activities can be done in an uncontrolled manner in the cases which HER2 expression undergoes up-regulation. In this study, total RNA was extracted from fresh tissue samples, first strand of total cDNA was synthesized and in the following steps, Real Time PCR was performed to be optimized.

INTRODUCTION

Breast cancer is the most prevalent cancer among women and HER2 is one the most prominent agents involved in breast tumorigenesis. Human Epidermal growth factor Receptor 2 (HER2) is overexpressed because of miscellaneous reasons and this phenomenon generally occurs in 20 to 30 percent of breast malignancies. HER2 gene, which is called by some other names including HER2/neu, c-ErbB2 and P185HER2. HER2 product, along with other members of HER -HER1, HER3 and HER4- family, form active tyrosine kinase receptors on the surface of cells [1]. All members of HER family have similar structure, an extracellular domain which is ligand binding region, a lipophilic transmembrane domain and an intracellular domain which have tyrosine kinase activity. As exceptions, HER3 and HER2 lack an active tyrosine kinase and ligand binding domains respectively. Some intracellular signal transduction pathways are triggered by HER-2 activation, resulting in cell proliferation and cell survival [2-6]. HER2 amplification occurs in 20 to 25 percent of breast cancers and causes poor prognosis [7]. In principle, HER2 amplification changes normal epithelial tissue into an invasive carcinoma. Estrogen Receptor (ER) and HER2 signaling pathway are the major reasons of proliferation and immortality in 85 percent of breast cancers; therefore, studies on HER2 and ER targeting has developed therapeutic approaches in HER2 and ER positive patients[8-12]. It then causes transphosphorylation of intracellular domains with tyrosine kinase activity. These phosphorylated receptors are becoming anchors for other proteins which play role as secondary messengers in signal transduction pathways. Real Time PCR is one the suitable methods used for assessment of HER2 status in breast tumor cells. Thus, optimization of this technique can improve preciseness of HER2 evaluation for distinguishing HER2 positive tumors [12-16].

MATERIALS AND METHODS

Twenty to thirty milligram fresh frozen specimen was collected from each patient for RNA isolation. Mortar and pestle with liquid nitrogen were recruited for disruption of samples. It is followed by homogenization using a syringe and needle. The procedure of isolation was performed according to the manufacturer's instruction. RNA was then stored in RNase-free water at -70°C [17-20]. The quantity and quality of purified RNAs were measured by

spectrophotometer and electrophoresis in a 2% agarose gel with ethidium bromide staining respectively. Total RNA was then transcribed into total cDNA using RevertAid™First Strand cDNA Synthesis Kit (Fermentas). The manufacturer's instruction of this kit was used for cDNA synthesis. Synthesized cDNA was stored at -70°C [21-24].

PDH was selected as the housekeeping gene. Primers were designed by AlleleID version 7.7 and Oligo version 7 software.

Spectrophotometer, used in order to check extracted RNAs, showed 200 to 10000 nano gram amount of RNA per milliliter [25-30]. Agarose gel and ethidium bromide staining were used for determining the integrity of purified RNAs. These bands confirmed the integrity of extracted RNAs and dearth of excessive and irrelevant substances in final volume of extraction [31-35].

In general, the housekeeping gene (reference gene) and the main gene (HER2) should be amplified simultaneously at the same temperature. Temperature gradient between 54˚C to 58˚C (54˚C, 56˚C and 58˚C) was used for selection the best annealing temperature and 3, 58˚C was the best temperature for both HER2 and GAPDH cDNAs [36-40].

As primer dimer and other excessive bands may cause false positive results, it is necessary to obviate them in Real Time PCR method. Therefore; the concentration of specific primers was reduced in order to eliminate primer dimer bands [41-43]. Thus, a gradient of different concentrations for specific primers (0.16, 0.24 and 0.32 pm/µl) in 58˚C were used to determine the best condition. 4, 0.32 pm/µl was the best concentration of HER2 and GAPDH primers for performance of Real Time PCR using SYBR Green dye [44-48].

DISCUSSION

Optimization of Real Time PCR method for assessment of HER-2 expression in breast cancer specimens is chosen because of two main reasons; first, it is one of the most dependable methods for quantitative measurement of mRNA and second, quantifying of HER2 expression has been used to determine prognosis in breast cancer [49]. Except for HER2 gene amplification which accounts for 92–95% of HER2 overexpression cases, other reasons such as chromosome 17 polysomy and mutations in the HER2 gene or upstream regulator genes [50]. Therefore, it seems logical to assess transcripts of HER2 gene to encompass the whole various reasons that cause HER2 overexpression.

Real time PCR can be a sensitive, credible and cost effective method provided that conditions of its reaction are optimized perfectly. Under these circumstances, this technique can be applied as a dependable and powerful method in clinical laboratories.

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