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Research Article

HER2 mRNA Expression Profile and Its Correlation with ICC and FISH in FNAC Samples from Breast Cancer Patients

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Abstract

Context: Around 20-30% of breast carcinoma patients have HER2 overexpression, and they usually have a poor prognosis. With the advent of specific molecule targeting therapies, it is very important to determine overexpression or hyperactivity of the target in focus. Considering to these facts, diagnostic accuracy is very important to project the response of tentative candidate to Trastuzumab therapy because it specifically targets HER2. Although, all known tools for diagnostic purposes are more or less satisfactory but none of them have excellent accuracy and some, like tissue biopsy, are very invasive in nature. Fine-needle aspiration (FNA) is a reliable, quicker, and less invasive technique, widely used for diagnosis of invasive breast carcinoma. Immunohistochemistry (IHC), combined with different methods for in situ hybridization, is currently used for routine assessment of HER2 levels in tissue specimens. In the current study, we assessed the use of quantitative real-time reverse

transcription-PCR (qRT-PCR) as a potential method for the accurate relative quantification of the HER2 gene in FNA specimen from breast carcinoma patients and its correlation with both immunocytochemistry (ICC) and Fluorescence in situ hybridization (FISH) performed on cell blocks.

Methods: Total mRNA was isolated by TRIZOL method from FNAC samples from 83 breast cancer patients. The mRNA profiling of HER2 was done by qRT-PCR. Protein profiling on cell blocks, made for analysis of both ICC and FISH from FNAC samples, was done by ICC and amplification of DNA by FISH. Correlation between qRT-PCR with ICC and FISH was performed separately.

Results: The concordance rate between qRT-PCR and ICC results was 83.13% whereas between qRT-PCR and FISH was 91.6%. The diagnostic performance for preoperative diagnosis of HER2 status using RT-PCR on FNAC specimens as compared to standard approach was as follows: sensitivity 91%; specificity 67%; positive predictive value 85%; negative predictive value 78.26%.

Conclusion: The use of qRT-PCR on FNA material may offer a very useful and easy tool to enhance chances of early diagnostic determination of HER2 positive breast cancer patients who are more likely to derive benefit from Trastuzumab therapy.

Introduction

HER2 protein is a member of human epidermal growth receptors, also known as erbB family of receptor tyrosine kinases, which includes the HER-1 (EGFR or c-erbB-1), HER-3 (c-erbB-3) and HER-4 (c-erbB-4). HER2 gene is located on chromosome 17 (17q21) that encodes a 185 kDa transmembrane growth factor receptor with tyrosine kinase activity [1]. HER2 protein participates in carrying out many functions in normal cells which include the regulation of cell growth, differentiation, and survival [2]. On the other hand, HER2 can act as a proto-oncogene [3-5] and its over-expression can play important roles in the development and proliferation of certain types of cancer cells including those leading to breast carcinomas. Comparatively higher level of HER2 protein due to some reasons like gene amplification has often been associated with poor prognoses in breast carcinoma patients as it renders the cancer cells more invasive and thus leading to a worse prognosis translating into a comparatively shorter survival time [6]. Recently, a number of specific drugs targeting this protein have turned out to be an important tool but are effective only in approximately 30% of breast carcinomas patients [7]. Therefore, diagnostic accuracy is important for selecting the tentative patients likely to receive such a drug as a treatment of breast cancer since these are specifically related to HER2 overexpression. Different techniques in current clinical use are less reliable, more invasive and expensive, especially in cases of the developing countries. FNA cytology (FNAC) is a valuable alternative method to obtain specimens for diagnostic purpose and for evaluation of the level of HER2 protein and HER2 gene amplification. FNAC is an admirably simple, modestly invasive, quick, and a cost-effective technique to diagnose and simple as well as metastatic breast carcinoma [8]. Although a variety of cytological preparations including direct smear, cytospin, liquid-based monolayer preparation, and

cell block have been used in previous studies, cell block appears to be the preferential preparation method for assessing the diagnostic factors in the FNAC material [9,10]. Since very little is known about the expression pattern of HER2 at protein or mRNA level or at gene amplification level in samples obtained by FNAC from breast cancer patients, we hypothesised to evaluate it in our clinical set up and asses the relationship between three different techniques; ICC, FISH, and qRT-PCR with a hope to find a combination of techniques which could provide us a better chance for early diagnosis of breast cancer in comparison to present techniques practiced in clinics.

In this study, we compared the HER2 expression at all three levels i.e., DNA, mRNA, and protein on FNA samples. We explored the possibility of using qRT-PCR as a potential tool for the accurate relative quantification of the transcripts of HER2 gene on specimens obtained by using FNA. The main aim of the study was to assess the concordance of qRT-PCR based quantification of HER2 overexpression with that determined by both IHC and FISH. Our data suggested that the mRNA level expression of HER2 by qRT-PCR, might be useful early diagnostic tool in case of breast cancer and it also can help in monitoring those patients who do not show positive diagnostic features with present widely used techniques like ICC and FISH.

Materials and Methods

Patients and Samples

The study was approved by the Institutional Ethical Review Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences in Lucknow (SGPGIMS), India. From the pool of patients referred to SGPGIMS between 2014 and 2017 with suspicion of breast carcinoma usually diagnosed by primary clinical manifestations and by mammography, 83 were selected, based on the criteria listed below, for the study and FNAC samples were collected by using 23-gauge needles.

Case Selection

Female patients, irrespective of their age group but with frank clinical symptoms including mammographic and cytological (displaying positive HER2 staining) manifestation of breast carcinoma were included in the study. Patients receiving neoadjuvant or adjuvant chemotherapies were excluded from this study. FNA sample was processed separately for cell block (10% formalin fixation) and for RNA isolation by Trizol method. At the same time, specimen smears were made for the routine diagnosis and for adequacy of the material.

Cell Block Processing

The FNAC samples were kept overnight at 40C with 10% formalin. On the following day, the sediment containing the cell button of the FNAC sample was scooped out on a filter paper and then sample was processed according to the ASCO/CAP guidelines (between 6 and 48 hours) [11].

The paraffin embedded cells button (cell block, CB) sections of 3µm thickness were prepared and stained with the haematoxylin and eosin stain. Immunocytochemistry of HER2 was carried out on all 83 cases while FISH was performed on 24 cases (2+ and 3+ HER2). Level of CEP17 gene expression, also present on chromosome 17, was used as a reference for evaluating HER2 gene amplification. We correlated ICC staining of HER2 protein with FISH findings on cell block sections. ICC results were also compared with IHC on corresponding histological samples.

Immunocytochemistry (ICC) for HER2 Protein

Paraffin sections, on poly-L-lysine coated slides, were kept overnight at 60˚C before proceeding onwards. After deparaffinization and blocking of endogenous peroxidase, HER2 immunostaining was performed using a polymer-based detection system (Envision plus) and rabbit monoclonal antibody (Thermo Fisher Scientific, Fremont, CA, USA) (dilution 1:100) against HER2. After washing, color development was achieved by addition of the diaminobenzidine (DAB) reagent as a chromogen. Known Positive controls of breast carcinomas and negative controls (3% skimmed milk powder instead of primary antibody) were run with every batch of ICC. HER2 results were reported according to the ASCO/CAP guidelines (2013) [11].

Florescence in Situ Hybridization (FISH) for Assessing HER2 Gene Amplification

FISH for HER2 gene was performed on CBs by using the Path Vysion HER2 DNA Probe Kit (Vysis Inc., Downers Grove, IL) according to the manufacturer protocol. In brief, following deparaffinization and rehydration, the CB sections were washed twice with SSC buffer (pH 7.5). After pre-treatment (Sodium Thiocyanate 1M) and digestion of the sections with protease buffer, 10µl of HER2 probe was directly added on the target areas and sealed and then denaturation was carried out in a ThermoBrite system (Abbott Molecular, Santa Clara, CA) at 80ºC for 7 min which was followed by the hybridization step at 37ºC for 12-16 hrs. After hybridization slides were washed in post-hybridization buffer. Then after nuclear counterstaining with 4, 6-diamidino-2-phenylindole (DAPI), slides were examined under the fluorescent microscope. At least 30 HER2 positive invasive tumor nuclei were evaluated at high power and the number of red (HER2gene) to green (CEP 17) signals were expressed as a single ratio.

For FISH analyses, a minimum of 20 cells of each sample were selected and HER2 gene amplification was decided according to ASCO/CAP guidelines (2013) [11]. HER2 to CEP17 signal ratio greater than 2.2 considered to be positive for gene amplification (Fig 1.A), while less than 1.8 as negative and signal ratio between 1.8 and 2.2 as equivocal (Fig 2B).

RNA Extraction

For total RNA from FNAC of the breast cancers patients, cells were stored in TRIzolTM (Invitrogen) and lysate was stored at -20˚C till further processing. Samples were thawed and resuspended in 1ml of TRIzol. After complete resuspension, 0.2 ml of chloroform was added, and the solution was vigorously vortexed. Following the centrifugation, (15 min at 12,000 xg at 4˚C), the aqueous layer was transferred into a new tube and mixed with 1 ml isopropanol (SIGMA). Total RNA was collected by centrifugation for 10 min at

12,000 xg at 4˚C. RNA pellets were washed twice with 75% alcohol (SRL, EMSURE®). Air-dried pellets were resuspended in 20µl Elution buffer (QIAGEN). The RNA was quantified by spectrophotometer (NanoDrop-2000, Thermo Scientific, USA).

Quantitative Reverse Transcriptase PCR (qRT-PCR)

One microgram total RNA was reversed transcribed into cDNA using Superscript II reverse transcriptase system (Invitrogen, Carlsbad, CA, USA) according to the manufacturer protocol. Validation of the ICC and FISH results were performed using qRT-PCR with TaqMan Gene Expression Assays (Life Technologies) specific for HER2 (Hs01001580_m1) and GAPDH (Hs00427620_m1). The qRT-PCR reactions (20 µl total) included 5 µl of cDNA template which was diluted with RNase free water (1:5 dilution), 2x TaqMan Universal PCR Master Mix (ABI, Foster City, USA), and 1x FAM labelled gene-specific assay. All qRT-PCR reactions were performed in 96 well plates using the ABI 7500 real time PCR system (Applied Biosystems, Carlsbad, USA), with an initiation step at 95˚C for 10 minutes, followed by 40 cycles at 95˚C for 15 seconds and at 60˚C for 1 minute. The qRT-PCR reactions for each sample were performed in duplicate in independent experiments. Comparisons of gene expressions were calculated after normalizing cycle thresholds against the housekeeping gene GAPDH and presented as the relative fold change by comparative 2-ΔΔCt method [12].

Statistical Analysis

HER2 level determined by ICC were compared with FISH. Statistical analyses, performed by using Statistical Package from the Social Sciences software version 16.0 (SPSS Inc., Chicago) and GraphPad Prism 5, included positive and negative agreements. Discrepancy rates were calculated using the Spearman rank correlation analysis (ρ) and Cohen kappa (κ) test of agreement. Statistical tests like sensitivity, specificity, and Odds ratio were also calculated to ascertain robustness of the findings. The Kruskal–Wallis test (one-way ANOVA) was used to evaluate differences in the mean value of qRT-PCR ratios according to different IHC and FISH categories. A p-value <0.05 was considered statistically significant. One way ANOVA test was used in all comparisons.

Results

Age of all 83 breast cancer patients, whose samples were used in the study, ranged between 30 to 70 years, with an average of 50-53 years with median age of 50 (SD ± 10.36 years). The patients included in the study met the following criteria: (i) had primary breast carcinoma; (ii) had complete set of clinical, histological, and biological data; and; (iii) had no prior radiotherapy or chemotherapy. Level of HER2 staining by ICC on all 83 formalin-fixed paraffin-embedded (FFPE) breast carcinoma CB samples was objectively scored on a scale ranging from 0 to 3+. Slightly more than half of the samples (54.2%; n=45) were 3+, and another almost one fifth (18.1%; n=15) were 2+. About one-seventh (13.3%; n=11) of those scored 1+ and remaining 14.5% (n=12) of the samples with no staining was noticed and therefore they were scored as 0. The same breast cancer samples were evaluated for the level of HER2 gene amplification and mRNA expression in cell blocks by using FISH and ICC, respectively (Fig 1).

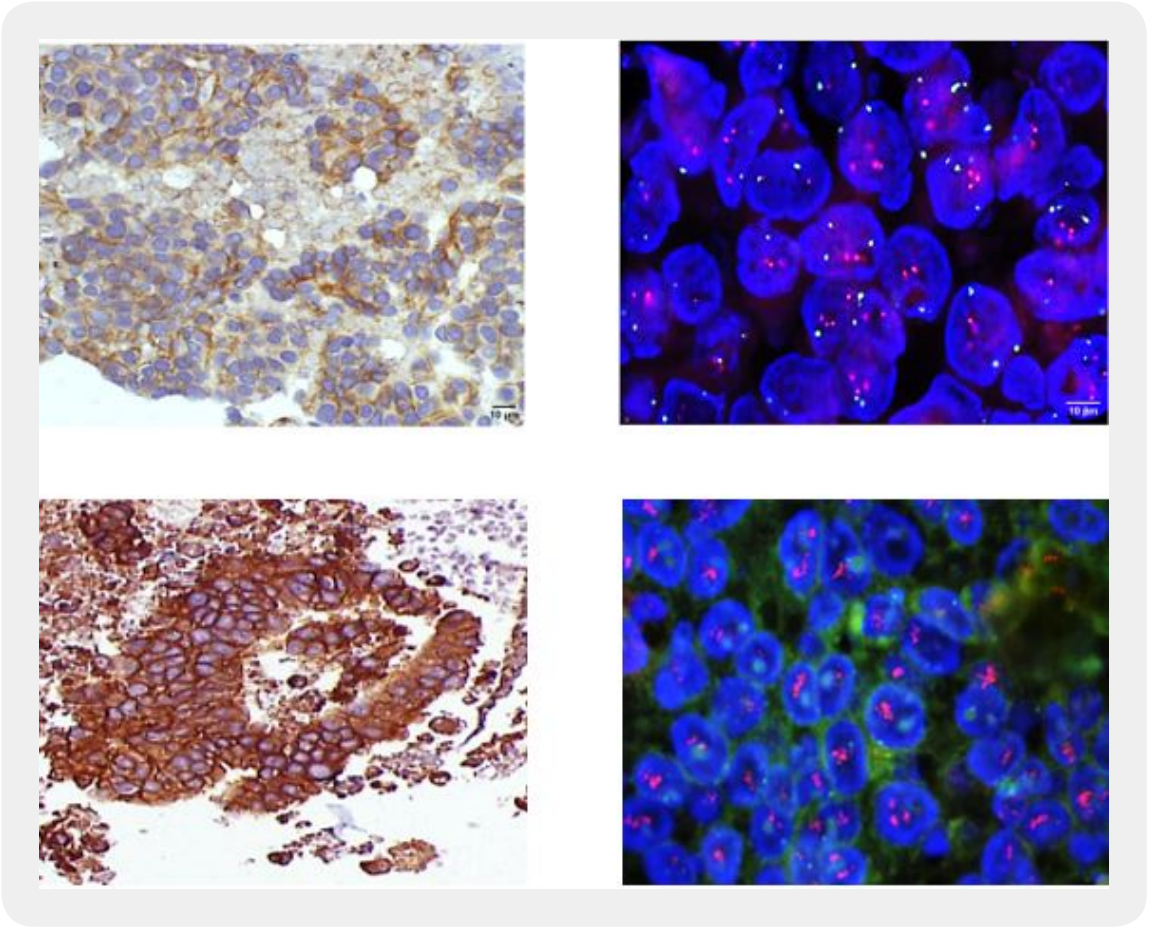

Figure 1: *Representative figures of ICC and FISH. HER2 immunocytochemistry and Fluorescence in Situ Hybridization on Cell blocks of breast cancer Patients. A. HER2, 2+positive for HER2 protein expression by ICC(x40); scale bar 10 µm. B. Equivocal Amplification of HER2 by FISH: HER2/CEP17 ratio < 2.0 (x100); scale bar 10 µm. C. HER2 3+positive for HER2 protein expression by ICC(x40); scale bar 10 µm. D. Positive Amplification of HER2gene by FISH: HER2/CEP17 ratio ≥ 2.0; scale bar 10 µm;(x100).*

FISH Compared to ICC

HER2 gene amplification, if any, was by evaluated FISH in only 24 out of 83 cases because of limited availability of the material. Out of 24, 9 cases were 3+ and 15 cases were 2+ as scored by ICC. All cases 3+ group showed amplification of HER2 gene by FISH [13]. However, in the 2+ group, only 2 cases displayed HER2 gene amplification and the remaining 13 cases did not show any. The overall agreement between ICC and FISH was 91.6%. Sensitivity and specificity were 81.82% and 100% respectively and positive predictor was 100% (Table 1).

Table 1: *Correlation between ICC and FISH (n=24)*

ICC	FISH		
	Positive	Negative	Total
3+	9	0	9
2+	2	13	15
Total	11	13	24
Kappa=0.830, Spearman correlation=0.842, *P*<0.001			

Assessment of HER2 mRNA Level by RT-PCR

Based on the immunoreactivity score, HER2 mRNA, levels determined by qRT-PCR, was sub-divided into three groups i.e. normal, moderately high, and high expressing group. HER2 mRNA levels in the breast carcinomas scored as 0 (negative) by ICC ranged from 0.03 to 0.79 (mean± SD, 0.34± 0.28) and those samples which scored 1+ with ICC, ranged between 0.78 to 11.55 (mean± SD, 4.07± 3.10) with lower and upper cut-offs at 0.62 and 7.17 respectively. Therefore, we considered values greater than 7.17 (mean± SD) as marker of high HER2 mRNA expression. For the identification of carcinomas with moderate and strong expression from these, we examined the breast carcinomas with 2+ ICC scores. HER2 mRNA values of 2+ carcinomas ranged from 2.2 to 24.76 (mean ± SD, 8.87 ± 7.62); therefore, the upper cut-off level of moderate over-expression was set at 16.49 (mean ± SD), and cases with HER2 levels higher than 16.49 were considered as high HER2 expressing cancers. While, in the 3+ ICC score group, HER2 mRNA values ranged from 2.28 to 120.3 (mean ± SD, 25.29 ± 21.45). The HER2 mRNA levels of the 3+ groups of breast carcinomas were significantly different (P < 0.05; Kruskal-Wallis test) (Fig 2).

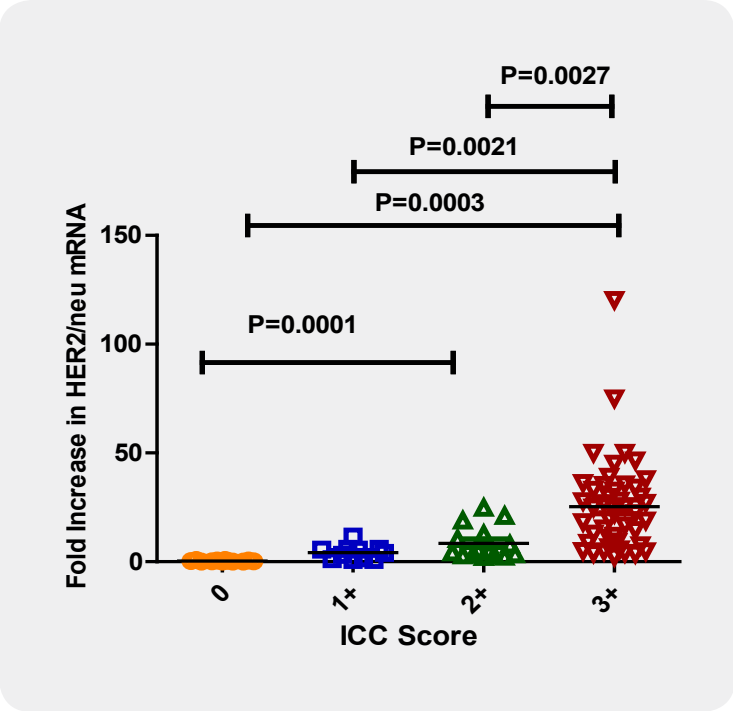

Figure 2: *Chart showing the results of qRT-PCR with ICC score from FNA samples of breast cancer patients.P-values between HER2 IHC negative and positive samples were calculated using one-way ANOVA (P < 0.0001). (0, n=11; 1+, n=12; 2+, n=15; 3+, n=45)*

Findings by qRT-PCR Stay in Good Agreement with those by ICC:

qRT-PCR for HER2 gene was performed on all samples of the 83 cases used for ICC. Out of 45 cases which had 3+ score by ICC, 43 showed high level of mRNA expression and 2 showed low levels. Out of 15 samples which were scored 2+, 8 were showed high and 7 were showed low mRNA expression. In the group of samples with ICC score of 1+ (n=12), 4 showed high mRNA level while 8 had low. All 11 cases which scored 0 consistently had low mRNA expression (Table 2). These findings demonstrate that conclusions by qRT-PCR were in a good agreement with those of ICC on samples showing either very high or very low HER2 expression but did not agree consistently in samples showing moderately higher HER2 expression (Fig 2).

Table 2: *Correlation between ICC and qRT-PCR (n=83)*

ICC	qRT-PCR		
	High expression	Low expression	Total
3+	43	2	45
2+	8	7	15

1+	4	8	12
0	1	10	11
Total	56	27	83
Kappa=0.600, Spearman correlation=0.604, P<0.001			

qRT-PCR compared to FISH

At present, FISH is the "gold standard" method for evaluation of HER2 gene amplification. Therefore, we wanted to compare the findings by qRT-PCR to those by FISH assay considering findings by ICC. qRT-PCR and FISH investigate HER2 level at two different molecular levels (DNA and RNA). Hence, we compared the findings between the two techniques by calculating agreement measures [14]. Twenty-four breast tumor samples, positive for HER2 protein expression by ICC analyses, were selected for FISH analysis and were compared with RT-PCR. Our results show that out of 24 tumors, 11 (46%) were scored positive with FISH as well as ICC but 2 (8%) negative with FISH but positive with ICC for HER2 gene amplification were scored positive by qRT-PCR whereas, 11(46%) scored negative for both FISH and qRT-PCR but positive for ICC (Fig3). The overall correlation between FISH, and qRT-PCR results was 91.6% (22 of 24 tumors) (Table 3).

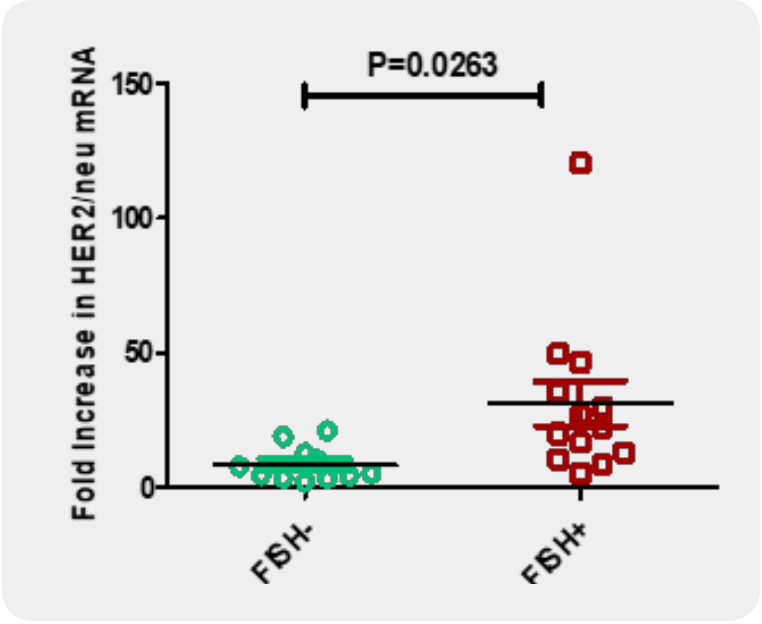

Figure 3: *Scatter chart showing correlation coefficient analysis between the HER2 mRNA qRT-PCR results with FISH results. The correlation coefficient was good as the Spearman rank scored 0.846 with p value <0.001. (FISH-, n=13; FISH+, n=13)*

Table 3: *Correlation between FISH and qRT-PCR (n=24)*

qRT-PCR for HER2 (mRNA)	**FISH**		
	Positive	Negative	Total
High expression	11	2	13
Low expression	0	11	11
Total	11	13	24
Kappa=0.834, Spearman correlation=0.846, P<0.001			

Statistical analysis confirmed the 91.6% concordance between the HER2 level assessed by both methods ($p < 0.001$, two-sided Fisher's test). There was a good correlation coefficient between the two methods used here (Spearman rank 0.846, $p < 0.001$) (Fig 3). The sensitivity and specificity we found in this correlation was 100% and 85% respectively.

Discussion

The accuracy of diagnostic assays for HER2 protein in breast cancer is extremely important as HER2 level is not only a diagnostic marker but can also be a predictive parameter for the response of patients to the specific molecule targeting chemotherapies such as trastuzumab [14]. Increased HER2 level and activity is usually caused by amplification of the HER2 gene [15]. This increase in the number of HER2 gene copies could be associated with overexpression of HER2 mRNA leading to higher protein levels. Several studies have reported an almost complete concordance between HER2 amplification detected by FISH and protein overexpression determined by IHC [16-18]. However, in other studies, there a comparatively poorer correlation between IHC and FISH in the IHC2+ category has been reported [19,20]. We, also, did not record a great correlation in findings between ICC and FISH in samples having 2+ or lower on ICC signal scale. As we know that an increase in the protein level of a molecule can take place because of amplification at gene level or by stabilization of the protein itself or transcripts encoding it. On the other hand, amplification of a gene can occur without any manifestation at the protein level. In this case also, an increased level of HER2 protein can be exhibited even in the absence of HER2 gene amplification [21], suggesting that other mechanisms which induced HER2 protein level, and its activation could be playing an active role.

Both, IHC and FISH analyses are microscope-based techniques commonly used in diagnostic laboratories for ascertaining the types of breast carcinomas. IHC is subjected to different variations which could be contributed by types of antibodies and fixatives used and, also because of personal subjectivity at assessment level. Therefore, while IHC staining is easy to perform and relatively inexpensive method for detecting HER2 level, it exhibits a wide range of inter and intra-laboratory variations in its sensitivity and specificity, and thus the results obtained by IHC can be difficult to reproduce in case a patient is evaluated by some other pathology laboratory. Even in the same laboratory, IHC findings are likely to be ambiguous when the scores range from 1.8 to 2.2 [22]. Several studies have suggested that IHC can be used as the first line of screening method for determining HER2 level, only to be verified by FISH which can be considered

as the second line method that to be applied to all weakly positive samples having an IHC score around 2+ [23]. Nevertheless, when combined both FISH and ICC could be time-consuming, expensive, and cumbersome for regular screening of multiple clinical samples in a very time-sensitive circumstances. Moreover, these methods are difficult to standardize across laboratories [24,25]. Molecular tests for determination of HER2 level at DNA or RNA levels have been widely studied for their potential advantages over FISH [26]. PCR-based methods may potentially improve the simplicity and accuracy of HER2 level assessment and may have several advantages over current methods as they are quantitative in nature, do not require extensive training for their interpretation, are not subject to inter-observer variability, and can be standardized, automated and can be performed on small samples [27,28]. Since mRNA integrity could be compromised by several factors, including tissue fixation and processing and storage time [29], the assessment of mRNA encoding HER2 status by qRT-PCR can be challenging using FFPE materials [30]. FNAC has garnered more and more use in the diagnosis of breast carcinomas because it is an excellently simple, modestly invasive, quick, and a cost-effective procedure to carry on [8]. The tissue samples obtained by this method are often quantitatively enough to be used for diagnostic purposes as well as for a multitude of ancillary tests including prognostic and predictive biomarkers. FNAC is a valuable alternative method to obtain specimens for diagnosis and evaluation of the level of HER2 protein and HER2 gene amplification (DNA and mRNA). Testing for HER2 protein level by ICC has been developed and optimized for use on FFPE tissue obtained by FNA. There are very few reported studies which have results validated on cytological specimens. Here we studied HER2 expression on cell blocks by ICC, the amplification of HER2 gene with FISH on cell block and mRNA expression of HER2 on FNA sample.

We evaluated accuracy and sensitivity of the qRT-PCR method and com¬pared with ICC/FISH for HER2 detection. We expected that qRT-PCR method on FNA could be standardized to help reduce variations between labo¬ratories for quantification of HER2 expression in clinical samples as qRT-PCR is a robust assay for assessing the level of target gene mRNA expression. qRT-PCR is also recommend¬ed by the Clinical and Laboratory Standards Institute (CLSI) guidelines, and it is highly sensi¬tive and specific because primers and probes are target gene sequence-specific [31]. We found a significant concordance in findings about HER2 level between ICC and RT-PCR which was 83.13%, with a good positive agreement of 85%, and negative agreement of 78.3%. In particular, the concordance was limited in samples with weak positive (score 1+) or strong positive expression (score 3+). Cases with score 0 by ICC were low in mRNA expression also, whereas the group with Score 2+ to 3+ had comparable high mRNA levels except in some cases with 1+ score by ICC which showed high mRNA expression. This finding suggests that further studies could define a clinically relevant cut-off for HER2 overexpression based on qRT-PCR. It also can help in early diagnosis and thus can prompt a clinician to keep the suspected patients under constant supervision as they had higher level of mRNA expression of HER2 as they could be positive in due course. Also, concordance between genomic HER2 amplification and HER2 mRNA expression needs to be clarified by further studies. The correlation of qRT-PCR results was calculated with FISH in 24 cases. The concordance between three procedures i.e., ICC, FISH and qRT-PCR was 83.3% while 4 cases were with discrepancies. Two cases showed high amplification of HER2 mRNA by qRT-PCR while they were negative by FISH. On the other hand, 4 cases of 2+ by ICC showed high HER2 mRNA expression. We found 16.5% and 8.3% discordance when we compared qRT-PCR results with ICC and FISH respectively. A 91.6% concordance

was found between the results of qRT-PCR and FISH. Here we found a good correlation between ICC and FISH which is in a good agreement with our earlier study where we compared the results obtained by ICC and FISH on cell blocks from the FNA of breast cancer patients [32].

A few studies have been also compared qRT-PCR based gene expression assessment with IHC/FISH. A good agreement was found by two groups between FISH or IHC gene expression analysis by qRT-PCR, although those studies are on a small number of patients [33]. Several other studies also found a significant correlation from 80-95% when they compared the results of real-time PCR with FISH [34-37]. Nistor *et al*., [30] conducted a similar study on border line IHC +2 category and compared FISH data with real-time PCR and reported 92% concordance. In general, the results of these studies suggest that the rate of over all concordance between qRT-PCR and IHC was 72.7-93% [38-40]. In our study, the concordance was 81.93% which is in a good agreement of the reported data.

Barberis *et al*., determined that qRT-PCR was a fast, sensitive, reliable, and cost-effective technique to estimate HER2 status in frozen and FFPE breast cancer samples, which could be applied to regular clinical practices [31]. Tvrdík *et al*., [41] showed that FISH, IHC, and qPCR are highly comparable to detect HER2 amplification and over-expression. Also, other studies showed high agreement between FISH and real-time PCR [42-44]. Luoh *et al*.; have shown that qRT-PCR can perform better than FISH in predicting HER2 overexpression. They observed that human breast carcinomas, which were positive for HER2 gene amplification with FISH technique, did not perform HER2 protein synthesis; therefore, it was important to use alternative methods such as qRT-PCR to identify the overexpression of HER2 [45].

Conclusion

Our study demonstrates that the cell block prepared from FNA is a simple, cost-effective, rapid, and minimally invasive procedure for early as well as advance diagnostic tests for breast carcinoma patients especially and has potential to be used for diagnosis of other carcinoma patients. The minimally invasive nature of the FNAC preparation may make it a useful tool which may help in monitoring progress of the recovery of the patients and in a better guessing about their prognoses. The use of qRT-PCR for assessing the expression level of HER2 gene on FNA materials may represent a very useful and easy tool to facilitate early identification and/or confirmation of HER2 positive breast cancer candidates who, probably, can originate a great benefit from molecule-specific therapies like Trastuzumab.

Conflict of Interest

There is no conflict of interest.

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Ethical Statements

The study was approved by the Institutional Ethical Review Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences in Lucknow (SGPGIMS), India

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