HER2 ILE655VAL AND ALA1170PRO POLYMORPHISMS PATTERNS IN ATIENTS RECEIVING TRASTUZUMAB FOR CARCINOMA BREAST AND HEIR CORRELATION WITH CARDIOTOXICITY- A PILOT STUDY.

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Abstract:

Background: HER2 or is a member of the epidermal growth factor family which if expressed, accounts for extremely aggressive breast cancer. HER2 regulates signaling (Raf/Ras/MAPK and PI3K/AKT) through several pathways associated with cell survival. However, Trastuzumab, an inhibitor of this has shown promising results in patients with HER 2 positive expressions. Though, trastuzumab is well tolerated, some patients report with cardiotoxicity. This may be attributed to mutations or polymorphisms of HER2 gene.

Objectives: The aim of our present study is to find out whether HER2 gene polymorphisms, I655V and A1170P are associated with the drug related cardiotoxicity, to correlate SNPs with the LVEF at 3 month and 6-month interval of time

Methodology: Seventy-two patients with carcinoma breast who were eligible for trastuzumab monotherapy were recruited in the study. Genomic DNA will be performed using phenol-chloroform method with PCR-RFLP method. Assessment of cardiac function will be done on every follow up. Cardiac function primary and secondary outcomes will be recorded.

Conclusion: There is no published data available in the literature regarding the HER2 Ile655Val and HER2 Ala1170Pro SNP among Indian population. The study will help to determine the pattern of HER2 Ile655Val and HER2 Ala1170 Pro SNP, cardiac toxicity of trastuzumab and correlation between them.

Keywords: Trastuzumab, HER2 Ile655Val, HER2 Ala1170Pro, Cardiotoxicity, Carcinoma Breast

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Introduction:

Breast cancer (BC) is second leading cause of cancer deaths worldwide and accounts for 1.7 million new cases diagnosed every year.[1] Breast cancer is highly heterogeneous and ~60–70% of it are estrogen receptor positive which responds to anti-hormone therapy [2] Nearly 20–30% of breast cancers are of Human Epidermal growth factor Receptor2 (HER2) positive and are highly aggressive in nature.

Human epidermal growth factor receptor family members are a group of molecules having tyrosine kinase activity with no natural ligand found till date. HER2 plays major role in the regulation of several pathways such as Raf/Ras/MAPK and PI3K/AKT pathways [3]. Receptor mediated signaling pathways has important role in the regulation of normal cell function, growth and division. Disruption of these pathways might lead to several cancers [4-7]. HER2 positive breast cancers show poor survival rate. However, Trastuzumab an inhibitor of this has shown promising results in patients breast cancer cells expressing the HER 2 positivity [8].

Trastuzumab is a monoclonal antibody that binds specifically to the extracellular domain of the HER2 protein, disrupting the normal regulatory functions of HER2 in cell growth, differentiation, and survival. The addition of adjuvant Trastuzumab to treatment regimens represents a major advance in the treatment of HER2-positive breast cancer. Currently, trastuzumab is indicated in the adjuvant setting for stage I–III breast cancer, metastatic breast cancers, and metastatic gastric and gastro esophageal cancers. Trastuzumab is also being used effectively in neoadjuvant therapy [9]. Trastuzumab is given intravenously, as a monotherapy or in combination with chemotherapy or hormone therapy. The standard duration of adjuvant trastuzumab treatment for breast cancer is for a total of 52 weeks given either weekly or every 3 weeks following an initial loading dose.

Although trastuzumab is well tolerated, the major toxicity is cardiomyopathy, with approximately 10% of patients developing asymptomatic left ventricular ejection fraction (LVEF) decline (defined as >10% decrease in LVEF or decline of EF to or decline of EF to <50%) and 4% developing signs and congestive heart failure following treatment with trastuzumab as a single agent. [10] Studies have suggested that HER2 Ile655Val and HER2 Ala1170Pro polymorphism is associated with cardiac toxicity. [11-12]

Beauclair S et al studied the association of gene polymorphism with tumor response or survival and cardiotoxicity in 61 patients with advanced breast cancer treated with trastuzumab. They concluded that HER2 was an association of the valine polymorphism with cardio toxicity [13].

In the study conducted by ***Chien et al***, Trastuzumab-induced cardiotoxicity ranged from asymptomatic decline in left ventricular ejection fraction (LVEF) to symptomatic heart failure [14]. However according to the study conducted by ***Tripathy et al*** the cardiotoxicity caused by trastuzumab does not appear to be related to HER2 dose or duration. [15]

Ewer et al, studied the cardiotoxicity caused by trastuzumab. They concluded that in the early stages of trastuzumab-induced cardiotoxicity, withdrawal or discontinuation of the drug can reverse the effects with the support of standard cardiac therapy for heart failure. Rechallenge with trastuzumab following discontinuation of trastuzumab and treatment of cardiac symptoms has been reported to be well-tolerated. [16]. As per the study of ***Perez et ql ,*** initiation of β blockers and angiotensin-converting enzyme inhibitors (ACEIs) found to be useful in managing the cardiotoxicity.[17] However the episodes of trastuzumab-induced cardiotoxicity may have long lasting effects on cardiac health, as evidenced by myocyte damage and indicated by cardiac troponin I elevation as per the study of ***Cardinale et al***. [18]

The role of HER2 Ile655Val and HER2 Ala1170 Pro polymorphism in causing cardiotoxicity among trastuzumab receiving breast cancer patients is well documented. However, to best of our knowledge there is limited documented evidence for the association of HER2 Ile655Val and HER2 Ala1170 Pro polymorphism with cardiotoxicity due to trastuzumab among Indian breast cancer patients.

Before taking up wet lab-based approaches, if disease-associated SNPs can be identified from neutral SNPs, it would be of great use. In silico analysis are useful when the disease association could not be established by subsequent independent studies [19,20]. Hence, independent evidence of functionality of SNPs obtained by using prediction tools could also serve as additional resources to discriminate true associations from false positive. Hence In silico analysis was carried out using SIFT, Polyphen 2 and I- mutant 3 bioinformatics tools so as to analyse the the effects of mutation on the function of the protein as shown in Table 1,

Table 1: SIFT , PolyPhen 2 and I-mutant 3.0 Analysis of two selected SNPs of HER2

Coordinates	Codons	Substitution	dbSNP ID	SNP Type	Prediction	SIFT Score	Poly Phen score	sensitivity	specificity	Prediction	SVM2 Prediction effect	DDG Value prediction
17, 37879588,1, A/G,T	ATC -gTC	I655V	novel	Nonsynonymous	TOLERATED	0.95	0.406	0.89	0.90	BENIGN	-1.01 Kcal/mol	Decrease
17, 37884223,1, G/A,C	GCT - cCT	A1170P	novel	Nonsynonymous	TOLERATED	0.43	0.953	0.79	0.95	POSSIBLY DAMAGING	-0.14 Kcal/mol	Decrease

In missense mutations, two SNPs were selected, I655V and A1170P. Both SNPs were found to be tolerant with a SIFT score of 0.95 & 0.43 respectively. These SNPs were analyzed by the PolyPhen tool with a score of 0.406 and 0.953, first being benign and the second being possibly damaging. I mutant 3.0 was used to predict the effects of single point protein mutation on the the protein stability. DDG values of binary classification showed values of <0 implying a decreased stability of the protein HER2 as a result of the gene polymorphism. Hence it is justifiable to find its association with the toxicity effect of the drug and therapeutic outcome.

Objectives:

1. To determine the HER2 Ile655Val and HER2 Ala1170 Pro SNP in carcinoma of breast patients receiving Trastuzumab therapy.
2. To monitor the LVEF at 3 months and 6-month interval of trastuzumab therapy.
3. To correlate the HER2 Ile655Val and HER2 Ala1170Pro with the LVEF at 3 month and 6-month interval of trastuzumab therapy.

Methodology:

Study Site: Medical Oncology Department, Justice KS Hegde Charitable Hospital, Deralakatte, Mangalore, Central Research Laboratory, KSHEMA. Study design: Prospective Cohort study

Inclusion Criteria:

1. Diagnosed patients of carcinoma breast eligible to receive trastuzumab monotherapy
i.e., Node positive/ node negative HRneg / HR + - grade 2-3/ grade 1 with tumor size > 2cm/ < 2cm in patient less than 35 years who are willing to participate in the study.
2. Patients with normal basal cardiac function confirmed by Echocardiogram

Exclusion Criteria:

1. Patients with symptomatic CCF, reduced LVEF at baseline, Renal failure, recently received anthracycline anticancer drugs for carcinoma of breast
2. Patients with known history of anaphylaxis, angioedema, clinically diagnosed interstitial pneumonitis, ARDS

Sample Size Calculation: All eligible patients fulfilling the criteria will be enrolled. Considering an average of 3 patients per month, we are expected to recruit 72 patients in 3 years' time.

Sample collection: 3ml of whole blood sample will be collected with aseptic precaution on the day of enrollment which is stored at -80º C for genetic polymorphism analysis.

Gene polymorphism: Genomic DNA from whole blood was isolated by Phenol-chloroform extraction and ethanol precipitation method. The HER2 Ile655Val and HER2 Ala1170 Pro SNP will be analyzed by the Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP) method.

Amplification was performed in MJ-Mini Thermal cycler (Bio-Rad, Tokyo, Japan). PCR was conducted with an initial denaturation enzyme activation step at 95°C for 5 minutes, amplification step for 35 cycles at 95°C for 30 seconds, an annealing temperature for 30 seconds, treatment at 72°C for 30 seconds and a final extension step at 72°C for 5 minutes. PCR product was digested with suitable restriction enzyme. Amplified product of DNA samples and restriction fragments was separated on a 2% agarose gel with ethidium bromide. The genotype was assigned based on the results of the analysis of the digestion patterns.

Assessment of cardiac function: Clinical assessment of cardio-vascular system will be done at each follow-up (every three week). The echocardiography will be done at 12 weeks and 24 weeks to find the left ventricular ejection fraction.

Cardiac function Primary Outcome measures: (requiring stoppage of Herceptin for more than 4 weeks due to one of the below mentioned

1. Percentage of patients who are having above LLN and developing ≥16% absolute decrease from baseline

2. Percentage of patients who are having LLN and ≥10% absolute decrease from baseline

Cardiac function Secondary Outcome measures:

1. Patients presenting with Congestive heart failure
2. Clinically significant asymptomatic decrease in Left ventricular function
3. Persistent (> 8 weeks) LVEF decline
4. Trastuzumab dosing held on more than 3 occasions.

Statistical Analysis: Quantitative data such as LVEF will be expressed as Mean± SD and qualitative parameters such as age, staging of cancer, histological type of cancer, SNP types will be expressed in frequency or percentages. Correlation between the SNP types and cardiac functions will be assessed by Spearman's correlation. Chi-square test for the association of gene polymorphism and cardiac markers will be used.

Conclusion: There is no published data available in the literature regarding the HER2 Ile655Val and HER2 Ala1170Pro SNP among Indian population. The study will help to determine the pattern of HER2 Ile655Val and HER2 Ala1170 Pro SNP, cardiac toxicity of trastuzumab and correlation between them.

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