The history about breast cancer biology essay

Science, Biology

ABSTRACT:-

Breast cancer is one of the common diseases diagnosed in females. It causes a large number of deaths worldwide. FRA3B is the common fragile site where Fragile Histidine Triad is located and FRA16D is the common fragile site for the location of WW domain having oxidoreductase. The aim of this study was to observe the loss of common fragile sites with the progression of breast cancer. For that purpose140 samples were taken with informed consent. 55 samples were taken from invasive breast carcinoma, 33 from ductal carcinoma in situ and 28 samples each from both normal breast tissues and atypical ductal hyperplasia. RNA was extracted c DNA was prepared from the samples and then reverse transcriptase polymerase chain reaction was carried out. After this analysis, western blot technique was used to monitor the detectable proteins in all of the samples. Poison regression analysis was done to estimate the rate ratios and 95 % confidence interval and p value <0. 05 was considered as statistically significant. The mRNA and protein expression of WWOX and FHIT changed from 82% to 93% in normal breast tissues and p value was found to be 0. 73 resulting in rejection of null hypothesis. In atypical ductal hyperplasia the mRNA and total protein expression changed from 57% to 75% and p value was 0. 760. Similarly, in ductal carcinoma in situ it changed from 45% to 55% and p value was 0. 879 and in invasive ductal carcinoma the values changed from 27% to 39% with a p value of 0. 595. So, the lost of common fragile site of tumor suppressor gene in breast cancer were observed. If some therapies are invented to stop the deletion of fragile sites then serious situations in breast cancer can be prevented.

KEY WORDS:-

Breast cancer, tumor suppressor gene, common fragile sites.

INTRODUCTION:-

Breast cancer is one of the most common malignancies diagnosed and also a major reason of death among females across the world. 23% of the new cancer cases and 14% of the cancer deaths have been caused with this disease in 2008. Almost 60% of the deaths due to breast cancer was expected to occur in developing countries in 2008 [1]. The risk factors for breast cancer are age, family history, age at first child birth, hormone replacement therapy, smoking, alcohol consumption, age at menarche and menopause, oral contraceptives and radiation exposure [2]. Common fragile sites are unbalanced genomic loci which can split, reorganize and again merge together [3]. In vitro, these fragile sites show amplified rate of gaps and rupture of cells when they are in duplication stress. In vivo, these fragile sites are involved in chromosomal aberration as in cancer [4]. FRA3B and FRA16D are two chromosomal fragile sites where Fragile HIstidine Triad (FHIT) and [4] WW domain containing oxidoreductase (WWOX) [5] tumor suppressor genes are present [4]. Removal of chromosome and loss of heterozygosity in the short arm of chromosome 3p is very common in various forms of epithelial tumors like breast, kidneys and lungs. There are four main regions of chromosomes 3p (3p25, 3p21. 3, 3p14. 2 and 3p12) involved in different disease states. The region 3p14. 2 has a common fragile site FRA3B where FHIT is located [6]. FHIT gene has ten little exons which extend to approximately 2Mb DNA [7]. In tumor cells the homozygous deletion at the region having FHIT produce irregular transcripts which are

lacking in the regions from exon 4 to 9 in comparison with mRNA from normal cells[8]. WWOX gene is located at 16q23. 3-24. 1 and has a common fragile site FRA16D. On genomic study it shows 9 exons which codes mRNA which is 2. 2kb which encodes 46 kDa of WWOX protein having 414 amino acids. Loss of heterozygosity and chromosomal alteration at wwox gene has been observed in prostate, breast, ovarian, hepatocellular and other cancers [9]. This gene has two WW domains at NH2 terminus which play an important role in protein-protein interaction and they are expected to bind ligands and cofactors[10, 11]. The role of WWOX gene was elucidated by Iliopoulos et al. An in vitro study showed an over expression of WWOX exogenous cells causes the inhibition of WWOX deprived cells and cells expressing WWOX has no effect by the over expression. The function of loss of WWOX and its restoration in breast cancer has been studied in vivo [12]. An evident relationship of WWOX and FHIT was observed in a study conducted on breast carcinoma by performing immunohistochemical analysis [13]. The aim of this study to monitor the loss of common fragile sites of tumor suppressor genes (WWOX and FHIT) with the progression of breast cancer from normal breast tissues to invasive ductal carcinoma.

MATERIAL&METHODS:-

SAMPLE COLLECTION:-

Total numbers of the samples were 140. 51 samples were drawn from the woman having ductal invasive carcinoma, 33 samples were taken from ductal carcinoma in situ and 28 samples of precancerous and normal breast tissue paired samples were taken from woman having atypical ductal hyperplasia. Half of the samples from each specimen were stored in freezer

after the surgery and then placed in-80°C. This half of the samples were used for reverse transcription polymerase chain reaction (RT-PCR) and western blotting. The other half of the samples collection was fixed with 10% formalin, paraffin-embedded, hematoxylin and eosin stains for analyzed by a single pathologist. These patients, from whom samples were taken, were admitted in the Department of General Surgery, Örebro University Hospital. Consent was taken from them before sample with drawl.

PCR ANALYSIS:-

By applying the acid guanidinium isothiocyanate-phenol- chloroform extraction method, total RNA was extracted from specimens. 1 µg of total RNA using Moloney murine leukemia virus (MMLV) reverse transcriptase (Invitrogen, USA) with random hexamers cDNA was prepared. A total volume of 20 µl contained 1 µg RNA, 5×buffer 4 µl, 0. 1 mol/L 1, 4-dithiothreitol (DTT) 2 µl, 10 mmol/L deoxy-ribonucleoside triphosphates (dNTPs) 1 µl, 15 nmol/L oligo-dT 1 µl, 10 U/µl MMLV 10 U, and 0. 1% diethyl pyrocarbonate (DEPC) 8 µl. The procedure was conducted at 37°C for 60 minutes, 95°C for 5 minutes for deactivating the MMLV in Biometra PCR equipment (Tgradient 96, Whatman Biometra, Germany). By using primers specific for each target gene and the ß-actin gene in duplex PCRs, PCR was carried out. For an internal control, 22 ß- actin was utilized. The specificity of each primer set was assessed and the primers were used (taken from Wang TT et. al specification) [14] and these primers were:-5'-GATAATCCGACCAAGCCAAC-3' and5'-ACTGCT- TCACTCGCCCTTG-3' (209 bp) for WWOX, 5'-GCCA-ACATCTCATCAAGCCCT-3' and5'-TGGGTCGTCTG- AAACAAATCG-3' (172 bp) for FHIT, 5'-ACTATGTTT- GAGCCTTCAACA-3' and5'-CATCTCTTGCTCGAA-

GTCCA-3' (317 bp) for ß-actin [14]. For PCR amplification, three micro-liter of cDNA was used with the primers which are described above in a volume of 50 µl containing 15 pmol of each primer, 25 mmol/ L of MgCl2, 10 mmol/L of dNTPs mix, 10×PCR buffer, and 1 unit of AmpliTaq Gold (Perkin-Elmer, USA). Biometra PCR equipment was then run according to manufacturers instruction The results were investigated by using a multi-image analyzer (Fluorchem 9900, Germany). The ratio of the intensity of the target gene to the frequency of ß-actin was quantified with the level of gene transcript.

WESTERN BLOTTING:

From frozen tissue total protein extracts were derived. Total cell protein lysates were prepared by using the extracting buffer (50 mmol/L Tris pH 7. 5, 150 mmol/L NaCl, 1 mmol/L ethylene diamine tetraacetic acid, 1 mmol/L Na3VO4, 1% Nonidet P40, 10% Glycerol, and 50 mmol/L NaF; Shanghai Agentia Co, China), containing protease inhibitor (1 mmol/L phenylmethyl sulfonylfluoride) cocktail (Jingmei Biotech Co, China). For the purpose of Western blot 50 µg of total protein was separated by 12. 5% sodium dodecyl sulfate polyacrylamide gel electropheresis (Mini PROTEAN 3 Electrophoresis Cell, Bio-Rad, USA). It was then transformed into the polyvinylidene difluoride (PVDF) membranes (PALL, USA; Mini Trans-Blot Cell, Bio-Rad, USA). Using the reagents and instruments as prescribed by the manufacturer Immunodetection was conducted. WWOX and FHIT proteins were spotted by using affinity-purified anti-WWOX and anti-FHIT rabbit polyclonal primary antibodies developed in GeneTex, USA (1: 500 dilution) and horseradish peroxidase (HRP) conjugated anti-rabbit secondary antibody (Jingmei Biotech Co, 1: 2000 dilution). Then the chemiluminescence autoradiography (ELC,

Jingmei Biotech Co) was done. ß-actin, used as the protein loading control , was sensed by using monoclonal anti-actin antibody (Jingmei Biotech Co, 1: 500 dilution) and HRP conjugated anti-mouse secondary antibody (Jingmei Biotech Co, 1: 2000). Quantitation of X-ray films were conceded out by using a multi-image analyzer (Fluorchem 9900, Germany). When the target gene were developed on the PVDF membrane ß-actin was also developed. Proteins were supposed to be detectable on Western blotting if an appropriately located band was seen

STASTICAL ANALYSIS:-

To calculate point approximation and 95% confidence intervals (CI) of rate ratios, poisson regression was applied by using SPSS version 20. For analyzing the null hypothesis for each histological category, exact two by four contingency table analyses were used . The differences in mRNA and protein expression for WWOX and FHIT would be worthy. For a significant result P <0. 05 was taken into consideration.

RESULTS:-

After RT-PCR, WWOX was detected 93% in normal, 68% in Atypical ductal hyperplasia, 55% in ductal carcinoma in situ and 39% in invasive ductal carcinoma. Whereas, FHIT was detected 86% in normal, 71% in atypical ductal hyperplasia, 45% in ductal carcinoma and 29% in invasive ductal carcinoma as shown in table1 and figure1 below[14].

Table1: Distribution (number and percentage) of samples with detectable and undetectable WWOX and FHIT mRNA by RT-PCR with histological types.

GenesPathological typeDetectable[Number (%)]Undetectable[Number (%)]Total[Number (%)]WWOX

Normal

26(93)

2(7)

28(100)

Atypical ductal hyperplasia

19(68)

9(32)

28(100)

Ductal carcinoma in situ

18(55)

15(45)

33(100)

Invasive ductal carcinoma

20(39)

31(61)

51(100)

FHIT

Normal

24(86)

4(14)

28(100)

Atypical ductal hyperplasia

20(71)

8(29)

28(100)

Ductal carcinoma in situ

15(45)

18(55)

33(100)

Invasive ductal carcinoma

15(29)

36(71)

51(100)

Source: - Wang TT, Frezza EE, Ma R, Hu SY, Liu CZ, Zhang GY, et al. Loss expression of active fragile sites genes associated with the severity of breast epithelial abnormalities. Chin Med J (Engl) 2008 Oct 20; 121(20): 1969-1974.

Rate ratios and 95% CI for mRNA detection were calculated by Poisson regression and the results are shown in figure 2. In comparison of normal and atypical ductal hyperplasia with ductal carcinoma in situ and invasive ductal carcinoma mRNA was greatly expressed in WWOX and FHIT. For

WWOX the mRNA expression rate ratio was 2. 95 and 95% CI was 1. 24-7. 08 and for FHIT the mRNA expression rate ratio was 4. 58 and 95% CI was 1. 82-11. 81[14]. When normal and atypical ductal hyperplasia was compared WWOX rate ratios and 95%CI were 1. 37and 0. 76-2. 51respectively and FHIT rate ratios and 95% CI were 1. 60 and 0. 66 -2. 19 respectively. On comparison of ductal carcinoma in situ and invasive ductal carcinoma WWOX rate ratio and 95%CI were 1. 7 and 0. 73-2. 63 and FHIT rate ratio were 1. 55 and 95% CI was 0. 75-3. 19. So, no statistically, significant values were obtained[14]. Figure1:- Illustration of WWOX and FHIT genes mRNA by performing RT-PCR amplification by using RNA from tissues of normal breast tissue, Atypical ductal hyperplasia, Ductal carcinoma in situ, Invasive ductal carcinoma A: Illustration of β-actin mRNA expression in two cases (case 1, 2) respectively from normal breast tissue, Atypical ductal hyperplasia , Ductal carcinoma in situ, Invasive ductal carcinoma B: WWOX mRNA expression in two cases (case 1, 2) from groups mentioned in A. C: FHIT mRNA expression in two cases (case 1, 2) from groups listed in A. Source: - Wang TT, Frezza EE, Ma R, Hu SY, Liu CZ, Zhang GY, et al. Loss expression of active fragile sites genes associated with the severity of breast epithelial abnormalities. Chin Med J (Engl) 2008 Oct 20; 121(20): 1969-1974. Figure2:- Rate ratios and 95% confidence interval for WWOX and FHIT mRNA by using poisson regression. Source: - Wang TT, Frezza EE, Ma R, Hu SY, Liu CZ, Zhang GY, et al. Loss expression of active fragile sites genes associated with the severity of breast epithelial abnormalities. Chin Med J (Engl) 2008 Oct 20; 121(20): 1969-1974. On western blot analysis, WWOX protein was 86% detected in normal breast tissues, 64% in atypical ductal hyperplasia, 45% in ductal

carcinoma in situ and 29% in invasive breast carcinoma, while FHIT protein in normal breast tissue was 82%, 57% in atypical ductal hyperplasia, 45% ductal carcinoma in situ and 27% in invasive breast carcinoma as shown in figure 3 and Table 2[14].

Table2:- Distribution (number and percentage) of samples with detectable and undetectable WWOX and FHIT proteins by western blots with histological types.

GenesPathological typeDetectable[Number (%)]Undetectable[Number (%)]Total[Number (%)]WWOX

Normal

24(86)

4(14)

28(100)

Atypical ductal hyperplasia

18(64)

10(36)

28(100)

Ductal carcinoma in situ

15(45)

18(55)

33(100)

Invasive ductal carcinoma

15(29)

36(71)

51(100)

FHIT

Normal

23(82)

5(18)

28(100)

Atypical ductal hyperplasia

16(57)

12(43)

28(100)

Ductal carcinoma in situ

15(45)

18(55)

33(100)

Invasive ductal carcinoma

14(27)

37(73)

51(100)

Source: - Wang TT, Frezza EE, Ma R, Hu SY, Liu CZ, Zhang GY, et al. Loss expression of active fragile sites genes associated with the severity of breast epithelial abnormalities. Chin Med J (Engl) 2008 Oct 20; 121(20): 1969-1974.

Figure3:- Illustration of WWOX and FHIT gene protein by performing western blot by using anti-WWOX, anti-FHIT or anti- Actin antibody by taking the protein samples from tissues of normal breast tissues, atypical ductal hyperplasia, ductal carcinoma in situ and invasive ductal carcinoma. A:

Illustration of β-actin protein expression in two cases (case 1, 2) respectively from normal breast tissue, Atypical ductal hyperplasia , Ductal carcinoma in situ, Invasive ductal carcinoma B: WWOX protein expression in two cases (case 1, 2) from groups which are mentioned in A. C: FHIT protein expression in two cases (case 1, 2) from groups which are mentioned A. Source: - Wang TT, Frezza EE, Ma R, Hu SY, Liu CZ, Zhang GY, et al. Loss expression of active fragile sites genes associated with the severity of breast epithelial abnormalities. Chin Med J (Engl) 2008 Oct 20; 121(20): 1969-1974. Rate ratios and 95% CI for protein detection were calculated by Poisson regression and the results are shown in figure 4. In comparison of normal and atypical ductal hyperplasia with ductal carcinoma in situ and invasive ductal carcinoma protein was greatly expressed in WWOX and FHIT. For WWOX the protein expression rate ratio was 4. 12 and 95% CI was 01. 63- 10. 73 and for FHIT the protein expression rate ratio was 3. 76 and 95% CI was 1. 44-10. 06[14]. When normal and atypical ductal hyperplasia was compared WWOX rate ratios and 95%CI were 1. 33and 0. 73-2. 49respectively and FHIT rate ratios and 95% CI were 1. 44 and 0. 76 -2. 77 respectively. On comparison of ductal carcinoma in situ and invasive ductal carcinoma WWOX rate ratio and 95%CI were 1. 55 and 0. 75-3. 19 and FHIT rate ratio were 1. 66 and 95% CI was 0. 80-3. 14. So, no statistically, significant values were obtained[14]. Figure4:- Rate ratios and 95% confidence interval for WWOX and FHIT proteins by using poisson regression. Source: - Wang TT, Frezza EE, Ma R, Hu SY, Liu CZ, Zhang GY, et al. Loss expression of active fragile sites genes associated with the severity of breast epithelial abnormalities. Chin Med J (Engl) 2008 Oct 20; 121(20): 1969-1974. In normal breast tissue, the mRNA

and protein expression of WWOX and FHIT changed from 82% to 93%. After an exact contingency analysis p value was found to be 0. 73. so null hypothesis was rejected. In atypical ductal hyperplasia it changed from 57% to 75% and p value was 0. 760. In ductal carcinoma in situ it changed from 45% to 55% and p value was 0. 879 and invasive ductal carcinoma it changed from 27% to 39% with a p value of 0. 595[14].

DISCUSSION:-

Breast cancer is one of common cause of death in females. It is believed that the common fragile sites of tumor suppressor genes are lost in the progression of cancer from normal breast tissues to invasive ductal carcinoma. Various studies on different cancers involving these two tumor suppressor genes are in progress. A study was conducted on FHIT and WWOX expression in mucoepidermoid and adenoid cystic salivary gland carcinoma. Loss of expression of these genes also showed that these tumor suppressor genes are lost in cancerous state [15]. Similarly, a loss of FHIT and WWOX expression was studied in immunohistochemical analysis of pre-invasive and invasive cervical cancer [16]. Tissue microarrays for the expression of FHIT and WWOX, estrogen and progesterone receptors were studied and the reduced expression of WWOX and FHIT was found to be associated with pathology of the basal like phenotype of breast cancer [17]. Also the genetic changes due to loss of WWOX and FHIT was studied using PCR and direct sequencing in breast carcinoma[18]. In this study, common fragile sites of WWOX and FHIT are studied with the progression of breast cancer. 140 samples were taken, 51 specimens were taken from invasive ductal carcinoma, 33 from ductal carcinoma in situ and 28 each from normal

and atypical ductal hyperplasia. It was observed that mRNA and protein expression of these tumor suppressor genes were changed from p value 0. 73 in normal breast tissues to p value 0. 595 in invasive ductal carcinoma. Rate ratios for mRNA and protein expression for WWOX were 2. 95 and 4. 12 respectively, when normal and atypical ductal hyperplasia was compared with ductal carcinoma in situ and invasive ductal carcinoma. Similarly, the ratio ratios for mRNA and total protein expression for the same comparison for FHIT are 4. 58 and 3. 76 respectively. So, the loss of expression of WWOX and FHIT in invasive carcinoma suggests that if some medications stop the WWOX and FHIT deletion then the more complex stages of breast cancer can be prevented. Different techniques are used nowadays for cloning of WWOX gene in order to cause apoptosis and prevent the cancer cell growth in ovarian tissues [19]. Still some other techniques must be adopted for the confirmation of WWOX and FHIT deletion like microarrays in precancerous and cancerous tissues. Also, therapies must be developed to inhibit the serious cancerous stages if they are discovered in early stage.