BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Triple Negative Breast Cancer Association With Basal Molecular Subtype In The Middle Of Iraq

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ABSTRACT

The study aim to determine the role of vitamin D and BMI in basal expression of TNBC patient. TNBCs are a diverse set of tumors with one usual clinical character, a markedly aggressive behavior, greater proportions of relapse, lesser overall survival in the disseminating cases matched with further groups of breast malignancy. Cytokeratin 5/6 proteins are important in the classification of tumor cells as basal type. The current study was designed to determine the percentage of basal type by examination of cytokeratiein 5/6 and determining the role of BMI in the basal expression of patients with triple-negative breast cancer. Triple-negative cancer was diagnosed in Al-Sadr Teaching Hospital in AL- Najaf by a pathologist. cytokeratiein 5/6was determined by using the immunohistochemistry method. The WHO classification was determined to measure the body mass index. The number of patients involved was 70 patients. The study founded that the percentage of basal cancer was (77.1%) 54 and found a significant statistical difference in the age of menarche between basal type with other breast cancer subtype and there are no differences of statistical significance in the basal type and other cancer with subtype in tumor site and menopausal state and in BMI, The study concluded that basal subtype was more common than luminal subtype in studied TNBC and basal subtype of TNBC is associated significantly with age of menarche.

Keyword: breast cancer, cytokeratin 5/6, body mass index.

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Received 05 June 2017, Accepted 11 September 2017

Please cite this article as: Alsaabery EA *et al*., Triple Negative Breast Cancer Association With Basal Molecular Subtype In The Middle of Iraq. British Journal of Medical and Health Research 2017.

INTRODUCTION

Triple negative breast cancers (TNBCs) indicate a different subdivision of breast malignances with an immunohistochemical phenotype that is characterized by lacking of progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER-2)[1]. TNBC represents around 15-20% of all cases with breast malignancy and matched with other breast cancer types, is linked with a in elevation in recurrence percentage and short survival duration [2]. Basal subtype consists from cells that actively dividing and relating to proliferation increment and loss of cell cycle checking together with the increased expression of genes of DNA damage response. Increased Ki67 mRNA expression due to the proliferation rate so it is better in responding to antimitotic medications of cell cycle targeting [3]. CK5/6 are intermediate-sized basic keratins". CK5/6 ,In typical tissue are frequently expressed in squamous epithelium of non keratinizing (mucosa) and keratinizing (epidermis) type, in addition to basal myoepithelial cell sheet at breast , salivary glands, and prostate. CK5/6 are similarly founded in malignant and benign growths of squamous mucosa and epidermal cell [4].Near to 80%-90% of TNBCs lie into the group of basal like molecular subtype when correctly experienced for immunohistochemistry of GEP and malignant biomarkers but these expressions are overlap and non-identical [5]. At the DNA level, these tumors show the second highest number of mutations across the genome, mostly are hypomethylated, and 80% and 9% of Basal-like tumors are TP53 and PIK3CA mutated [6].

MATERIALS AND METHOD

Samples used in this study were triple negative breast cancer patient and formalin fixed paraffin embedded (FFPE) tissue .The samples totaled 70 of different invasive stages of breast cancer. All samples were embedded in paraffin from June 2015- January 2016, the samples were obtained from AL-Najaf Governorate Histopathological private laboratories and from AL- Sader Teaching hospital histopatholgical laboratory. All samples were managed by slicing and renumbering of the tissue block for slide preparation. One slide was prepared from each block. Slides were observed through light microscope using40X power lens. Immunohistochemistry was used for detection of cytokeratin 5/6, and mouse anti-human cytokeratin 5/6 clone D5/16B4 product M727 Dako, Denmark was used and the procedure was performed according to Dako Envision Flex system- Manual protocol briefly, after slicing of FFBE into 4-5 micron thickness using microtome, the slides were prepared by de-waxing by using a decreasing concentration of ethanol from (100% to 70%). Body mass index (BMI) was evaluated by this equation:

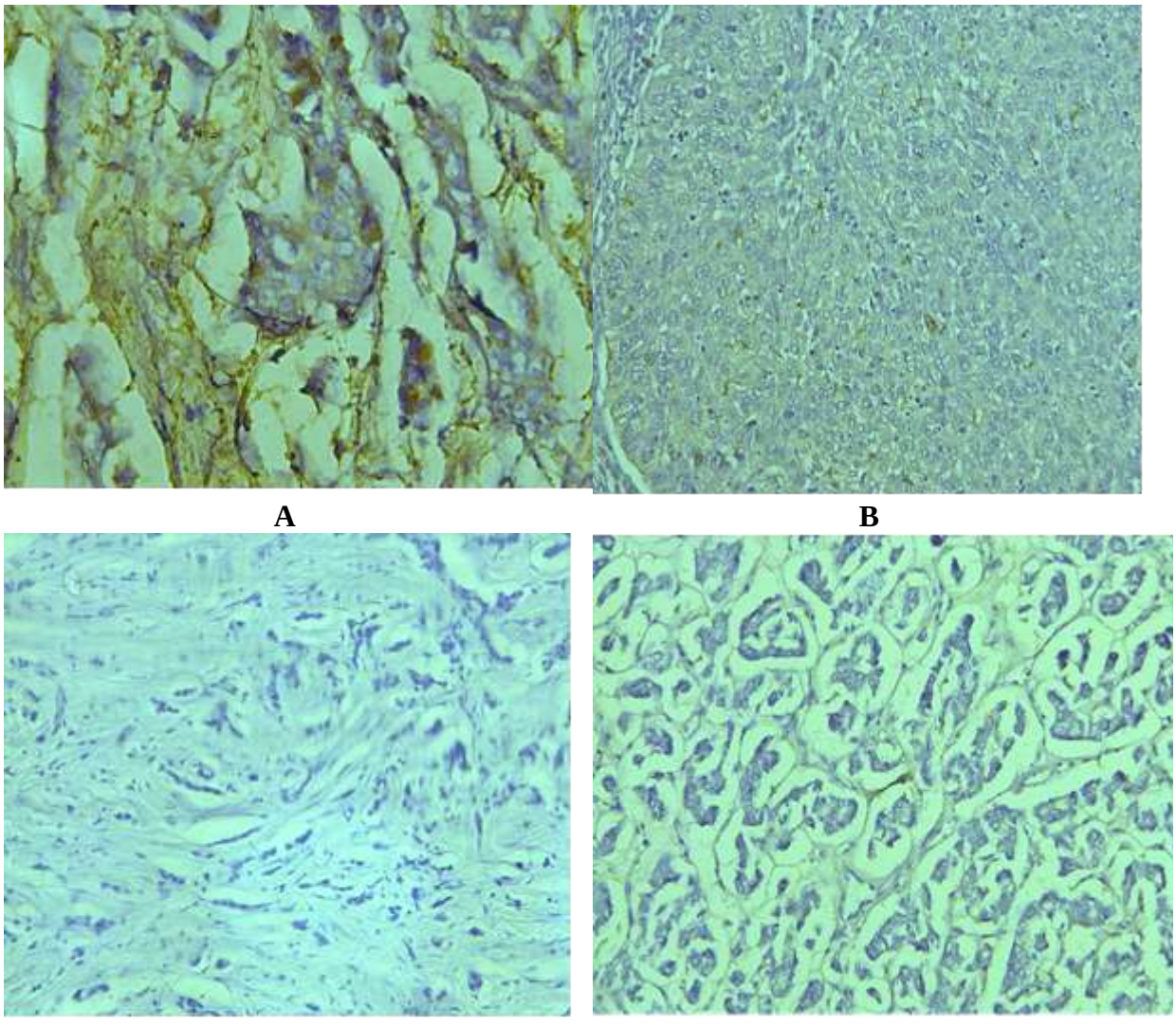
BMI = weight (kg) / height (meters)2 ,weight was categorized according to BMI of WHO classification [7].The study was carried in accordance with medical ethical committee in the college.

Statistical analysis

The chi – square and T test of SPSS version 20 (Chicago, Illinois, USA) was used to determine the p value equal to or below 0.05 level.

RESULTS AND DISCUSSION

70 patients of triple negative were included in the study, all cases were females with local and distal metastasize stages and all patients are married and have no history of infertility. Age mean of case was 48.9±10.07, lobular carcinoma was15.% (11), ductal carcinoma was 84.2%(59). 54(77. 1%) triple negative breast cancer cases were positive for cytokeratin 5/6 antibody.



A **B**

C **D**

Figure (1). A. demonstrates the positive staining of cytokeratin antibody 5/6 in triple negative breast cancer in 100X. B. demonstrating the positive staining of cytokeratin antibody 5/6 in triple negative breast cancer in 10X. C. represents the negative control

staining of cytokeratin antibody 5/6 in triple negative breast cancer in 10X. D. Represents negative control of cytokeratin5/6 in triple negative breast cancer in 40 X.

molecular types of TNBC was not significantly associated with both premenopause or post as in

Table 1: The association between molecular TNBC types and menopausal state.

		post Menopause	Pre menopause	P value
CK5/6	positive	29(53.7%)	25(46.3%)	0.484
	negative	7(43.8%)	9(56.2%)	
Total		36(51.4%)	34(48.6%)	

The mean and stander deviation of age of menarche in 70 TNBC patient was 12.1±0.49 . there was significant association between basal and luminal TNBC and age of menarche as in

Table 2: The association between molecular TNBC types menarche age

				Std. Deviation	P value
Menarche	Positive	54	12.20	.49	0.005
	Negative	16	11.81	.40	

The association between the basal and luminal TNBC and tumor origin site (ductal and lobular) revealed non significant association between these two parameters as p value 0.052 as in

Table 3: The association between molecular TNBC types and tumor site

		DCT	LOBULAR	
CK5/6	positive	48(88.9%)	6(11.1%)	0.052
	negative	11(68.8%)	5(31.2%)	
Total		59(84.3%)	11(15.7%)	

There was no significant association between BMI of TNBC patient and cytokeratin 5/6 expression as p value was 0.329 as in Table (4)

Table 4: Represent the relation between cytokeratin 5/6 and BMI

		CK5-6 P(basal)	N(luminal)	Total	P value
BMI	Normal	20(69.0%)	9(31.0%)	29(100%)	0.329
	Overweight	32(82.1%)	7(17.9%)	39(100.0%)	
	obese	2(100%)	0(0%)	2(100%)	
Total		547(7.1%)	16(22.9%)	70	

DISCUSSION

The triple negative phenotype of breast cancer has been reported to have different incidences amongst different ethnic groups. The basal phenotype was used to define as a clinically relevant subtype of breast cancer a tumor that has a triple negative status but which also expresses CK5/6 and other marker which may have a worse outcome than the breast cancers that are negative for all these markers [8].

This study reveals that C5/6 was positive in 54(77.1%) and negative in 23.9% and it is near to Bertucci et al 2014 result which was 75% positive of C5/6 and differs from Gazinska et al 2013 result which was 56% positive for C5/6[8][9].

Current study shows no significant difference in expression of C5/6 in pre menopause and post menopause age group and this is similar to Barakat et al 2015 and Gazinska et al 2013 [8][10] and does not meet with the result of Rao et al 2013 who finds more expression in pre menopause age group with significant difference [11]

This study shows close to be significant association between C5/6 and tumor site and this like the result of Haupt et al 2010 as most basal TNBC are invasive ductal carcinoma as other breast cancer site [12] and does not agree with the result of Sood et al 2014 as find p value more than 0.05 as and Barakat et al 2015 who finds the p value 0.008[13][10].

The thesis resulted that there is no significant association between C5/6 expression and body mass index and is agree with Stead et al 2009 and Eichholzer et al 2013[14][15] and disagree with Millikan et al 2008 who indicates that the women that get obesity later in their life differ from female who initially obese since yang et al 2007 who revealed that increase BMI will increase risk of basal expression [16][17].

Current study shows significant association in C5/6 expression and age of menarche and is similar to Millikan et al 2008 as they show that age of menarche was less than 13 years and significantly differ from luminal TNBC and Anders et al 2009 who resulted that the late menarche age was associated with decrease risk of basal subtype[16][18].

CONCLUSION

The study concluded that basal subtype was more common than luminal subtype in studied TNBC in Iraqi patients and basal subtype of TNBC is associated significantly with early menarche.

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