HER2/neu and α-Methylacyl Coemzyme A Racemase Overexpression in Prostatic Cancer as Indicators for Prognosis

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Abstract

prostatic cancer is a malignant neoplasm characterized by elevated level 7-8 folds in both mRNA and protein levels during tumorigenesis of the prostate. Also, HER2/neu expression could be detected in prostatic intraepithelial neoplasia (PIN) ,It affects androgen receptor function and the progression of prostatic cancer and the determination of Association between HER2/neu and α-methylacyl coemzyme A racemase (AMACR) overexpressions in prostatic cancer as prognostic factors. This study included 50 patients aged from 50- ≤ 70 years and 10 healthy men as control group matched with patient group ages. Then, paraffin-embedding procedure was prepared on tissues biopsy and then, hematoxylin and eosin staining (H. and E. staining) procedure was done . Finally, immunohistochemical procedure is done on both control cases and prostate cancer blocks. This immunohistochemical procedure detects her2/neu and AMACR overexpression according to DAKO protocol scoring system. The study showed that there is no significant difference in ages (P >0.05) between prostatic cancer patient and control group (68.46±6.26 years , 62.79±8.68 years) respectively and the prostatic cancer is more prevalence in ages >69 years old which represent 56% of cases.

Also, the overexpression of AMACR represent weak positive (46%), moderate positive (34%), strong (20%) overexpressions. While, the overexpression of HER2/neu gives weak positive (60%), strong positive (40%) and the death occurred to 50% of prostatic cancer patient that have strong positive HER2/neu overexpression. Those whose ages are more than 69 years old who have liability for prostatic cancer . Immunohistochemical overexpressions of α-methylacyl coemzyme A racemase enzyme and HER2/neu have prognostic marker for prostatic cancer .

Key words: prostatic cancer, Human epidermal growth factor receptor2 (HER2/neu), α-methylacyl coemzyme A racemase protein, immunohistochemistry .

الخلاصة

سرطان البروستات هو ورم خبيث الذي يتميز بارتفاع انزيم (AMACR) بمقدار 7−8 ضعف عن طبيعي وايضا ارتفاع مستوى عامل النمو البشراوي (HER2/neu) الذي وجد في التنشؤ داخل الظهارة في بروستات ويؤثر على وظيفية مستقبل اندروجين وتقدم في مرض سرطان البروستات . تحديد علاقة التعابير بين عامل النمو البشراوي (HER2/neu) وانزيم (AMACR) في سرطان البروستات كعوامل لتكهن السرطان. هذه الدراسة شملت 50 مريضا الذين تتراوح اعمارهم بين (50− ≤70) سنة مع 10 اشخاص سليمين من المرض كمجموعة مقارنة منسجمة مع اعمار مجموعة مرضى السرطان. ثم تتم تحضير عملية تغميس البارافين من خزعة النسيج والتي ثم تصبغ بصبغة الايوزين و هيماتوكسيلين (H. and E.) .اخيرا, طريقة الفحص المناعي النسيجي كيميائي تجري على بلوكات البارافين لمرضى سرطان البروستات والاشخاص السليمين . طريقة الفحص المناعي النسيجي كيميائي تعطي نتائج التعابير لكل من عامل النمو البشراوي(HER2/neu) وانزيم (AMACR) وفقا لنظام بروتوكول داكو (DAKO). بينت هذه الدراسة انه لا توجد علاقة بين اعمار الاشخاص المصابين بسرطان البروستات (68.46±6.26 years) و الاشخاص السليمين (62.79±8.68 years) وسرطان البروستات هو الأكثر انتشارا في الاعمار التي هي اكبر من 69 سنة والتي تمثل 56% من الحالات المرضية . وأيضا, تعبير انزيم (AMACR) يمثل ضعيف موجب بنسبة 46% ومتوسط موجب بنسبة 34% و قوي موجب 20% بينما يمثل تعبير عامل النمو البشراوي(HER2/neu) يمثل ضعيف موجب بنسبة 60% و قوي موجب 40% وحالات الوفاة للمرضى يمتد الى 50% من حالات مرضى سرطان البروستات الذين

لديهم تحليل قوي موجب لعامل النمو البشراوي .الاعمار التي هي اكبر من 69 سنة لديهم قابلية الاصابة بسرطان بروستات . تعابير الفحص المناعي النسيجي كيميائي تعطي نتائج التعابير لكل من عامل النمو البشراوي(HER2/neu) وانزيم (AMACR) لديها علاقة تكهن لسرطان البروستات .

الكلمات االمفتاحية: سرطان البروستات , عامل النمو البشراوي (HER2/neu) , انزيم (AMACR) , طريقة الفحص المناعي النسيجي كيميائي .

Introduction

Prostatic cancer is very common malignancy in the men and represents about one third of all male cancers. So, it is the second cause of cancer death in United State of America (Woods *et al.,* 2010) and causes 27450 deaths in 2015 (American cancer society, 2015) and it represents the seventh most common cancer in Iraqi males and represent 5.06% of total cancers (Ministry of health, 2011). Prostatic cancer is most prevalence malignancy in males in western country but its incidence and mortality rates is lower in Asian countries (Kimura, 2012). The incidence of prostatic cancer in African Americans is about 60% and this incidence is increased in non-Hispanic whites for unclear reasons. The prostatic cancer death rates decreases for all races due to early detection of prostatic cancer by using prostate specific antigen (American cancer society , 2014).

Prostatic cancer is heterogeneous disease with different degrees of aggressiveness, metastasis pattern , and response to therapy, Prostatic cancer is caused by complex etiology that includes exogenous factors(diet, environment etc.) and endogenous factors (hormonal imbalance, family history) . Also, the age is risk factors and it is rare below the age of 40 years but it is commonly after 60-70 years old (60% of cases are found over 65 years) (Woods *et al.,* 2010) . The pathogenesis and progression of prostatic cancer takes years and may be decades to turn from normal epithelial to premalignant to finally become prostatic cancer .

Generally, there are only few signs and symptoms in early stages of prostatic cancer but lower urinary tract symptoms(like urinary frequency and hesitancy, urgency and difficulty in urine out flow) occur in locally advanced cases (Hsiao *et al.,* Walsh *et al.,* 2007). The elevation of prostatic specific antigen with abnormal rectal examination is the most common diagnostic tools to detect prostatic cancer (Robert, 2004). Prostatic specific antigen is most useful marker for prostatic cancer due to its tissue specificity and it can be used for diagnosis , screening, and monitoring patient with prostatic cancer (Ulmert *et al.,* 2012).

AMACR gene is located in chromosome 5q13 and encode a 382 amino acid protein that plays role in bile acid and β oxidation of branched chain fatty acids (Streekumer et al.,2004). The expression of AMACR protein is regarded as new diagnostic marker for prostatic cancer and specially in case of needle biopsies when quantity and quality of tissue are limited (Xu *et al.*,2014) . Zha *et al*., 2003 study has demonstrate a that the increase level of AMACR expression in prostatic cancer leads to proliferation of the cells and this is independent of androgen action.

HER2/neu is a member of the receptor (also called erbB2) and it is transmembrane glycoprotein that contains intrinsic tyrosine kinase activity (Schreoder *et al.,* 2014). HER2 gene is located in chromosome 17q21 and its amplification was discovered in

several tumors . So and expression of HER2/neu was found in prostatic intraepithelial neoplasia , affected androgen receptor (that play important role in growth of prostatic cancer and maintained the libido) , and progression of prostatic cancer (Kankaya *et al.*,2008).

Signoretti *et al.* , 2000 showed 60% of prostatic cancer are associated with HER2 amplification and/or overexpressed , the sensitivity of fluorescent in situ hybridization to HER2 amplification was lower in prostatic cancer (10%-25%) compared with other types of malignancies (Kankaya *et al.*,2008).

Methods

This study was conducted in Al-Hariri general hospital /Baghdad and Al-amel hospital /Baghdad , the period of study extended from Septemer /2012 to May/2014. clinical data of patients include age, sex , and clinical features are taken with paraffin blocks .The practical side of the study was done in Al-Hilla governorate and at Baghdad/ Biotechnology Research Center/ Al-Nahrin University . This study included 50 patient with prostate cancer with 10 control .

The prostate paraffin block tissues were taken through radical removal of prostate or biopsy via ultrasound ultrasound guided needle biopsy.

Then, paraffin-embedding procedure was prepared on tissues biopsy and then, hematoxylin and eosin staining (H. and E. staining) procedure was done and Gleason grading is most commonly dependent scale that used for prostate cancer grading system which based on histological features of the tumor. Finally, immunohistochemistry is done on both control cases and prostate cancer blocks. This immunohistochemistry method detects her2/neu and AMACR expression according to DAKO scoring system.

DAKO scoring system of HER2/neu uses the following categories : 0 is negative result or membrane staining in < 10% of the tumor cells, 1+ is weak and incomplete membrane staining in > 10% of the tumor cells , 2+ is weak to moderate, complete membrane staining in > 10% of the tumor cells , and 3+ is strong complete membrane staining in > 10% of the tumor cells (Lara et al.,2002) .

The negative and 1+ indicate normal HER2/neu expression, 2+ indicate intermediate cases that to be proved by another method (fluorescent in situ hybridization), 3+ shows HER2/neu overexpression.

While, A scale of AMACR are ranging from 0 to 3 was used to grade the expression. 0 indicates no expression , 1(up to 50% of cells with detectable staining) gives weak expression , 2 (50% to 75% of cells with moderate staining) indicate intermediate staining and 3 (more than 75% of cells with intense staining) shows strong expression (Troung et al., 2008).

Results

There is no difference (P >0.05) in the mean of ages between prostatic cancer patient and control group (68.46±6.26 years , 62.79±8.68 years) respectively. The minimum age in patients and control group are 58 years and 50 years respectively while The maximum age in both patients and control group are 80 years as in table (1) .

Table (1): The age of prostatic cancer patients and control group.

Age/years	control group	patients group
Mean±SD	62.79±8.68	68.46±6.26
minimum	50	58
maximum	80	80

The prostatic cancer is more prevalence in age group > 69 years that represents 56% of patients , followed by age groups 60-69 years and 50-59 years which give 38% and 6% respectively as in figure (1). There are no significant differences (P >0.05) between ages in prostatic cancer prevalence .

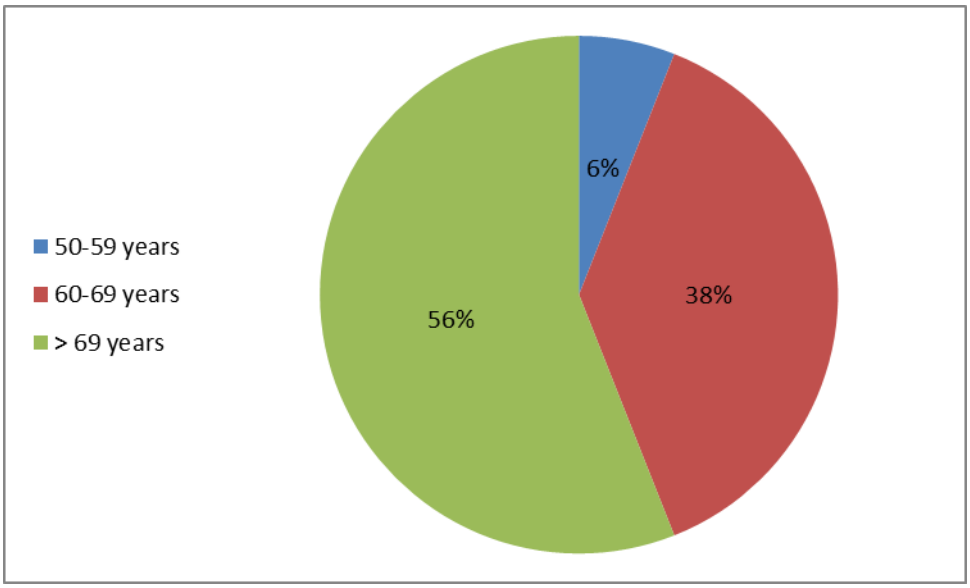

Figure (1): Age prevalence in prostatic cancer patients.

Table (2): Association between Gleason grade levels with histopathological types, HER2/neu, and AMACR overexpressions.

Variables	number of patients	Gleason grade levels
Gleason grades level according to histopathological types	12 10 16 10 2	10 9 8 7 6
Gleason grades level according to HER2/neu overexpression Strong HER2/neu overexpression	2	10
  Weak HER2/neu overexpression	2 1	7 8
Gleason grades level according to AMACR overexpression Weak AMACR overexpression	10 7 6	8 9 10
Moderate AMACR overexpression	9 4 3 1	6 7 9 10
Strong AMACR overexpression	5 3 1 1	6 7 8 9

The Table (3) shows that there is weak overexpression of α-methylacyl COA racemase in 23 patients that represent 46% of all prostatic cancer patients. Moderate overexpression in AMACR is 17 patients that correspond to 34% of all prostatic cancer patients. Strong overexpression in AMACR is 10 patients that correspond to 20% of all prostatic cancer patients.

Table (3): The number of overexpressed prostatic cancer patients to α-methylacyl COA racemase and HER2/neu .

Gene	overexpression					
	Weak positive		Moderate positive		Strong positive	
	number	%	number	%	number	%
α-methylacyl COA racemase	23	46	17	34	10	20
HER2/neu	3	60	-	-	2	40

In table (3), there is weak positive overexpression of HER2/neu in 3 patients that represent 60% of all prostatic cancer patients. Strong overexpression of HER2/neu is 2 patients that represent 40% prostatic cancer patients. There are 5 prostatic cancer patients diagnosed with HER2/neu that represent 10% from 50 prostatic cancer patients.

Table (4): The percentage of prostatic cancer patients diagnosed with α-methylacyl COA racemase and HER2/neu overexpressions .

Gene	overexpression	
	number	percentage
α-methylacyl COA racemase	50	100%
HER2/neu	5	10%

A number of deceased prostatic cancer patients occurs in one patient from the total five patients of prostatic cancer . The deceased patients have been diagnosed with strong overexpression of HER2/neu that represent 20% of the total patients diagnosed with HER2/neu as in table (5) .

Table (5): The percentage of prostatic cancer deaths in overexpression of α-methylacyl COA racemase and HER2/neu .

Gene	Number of deceased patients	percentage
α-methylacyl COA racemase	0	0%
(HER2/neu)	1	20%

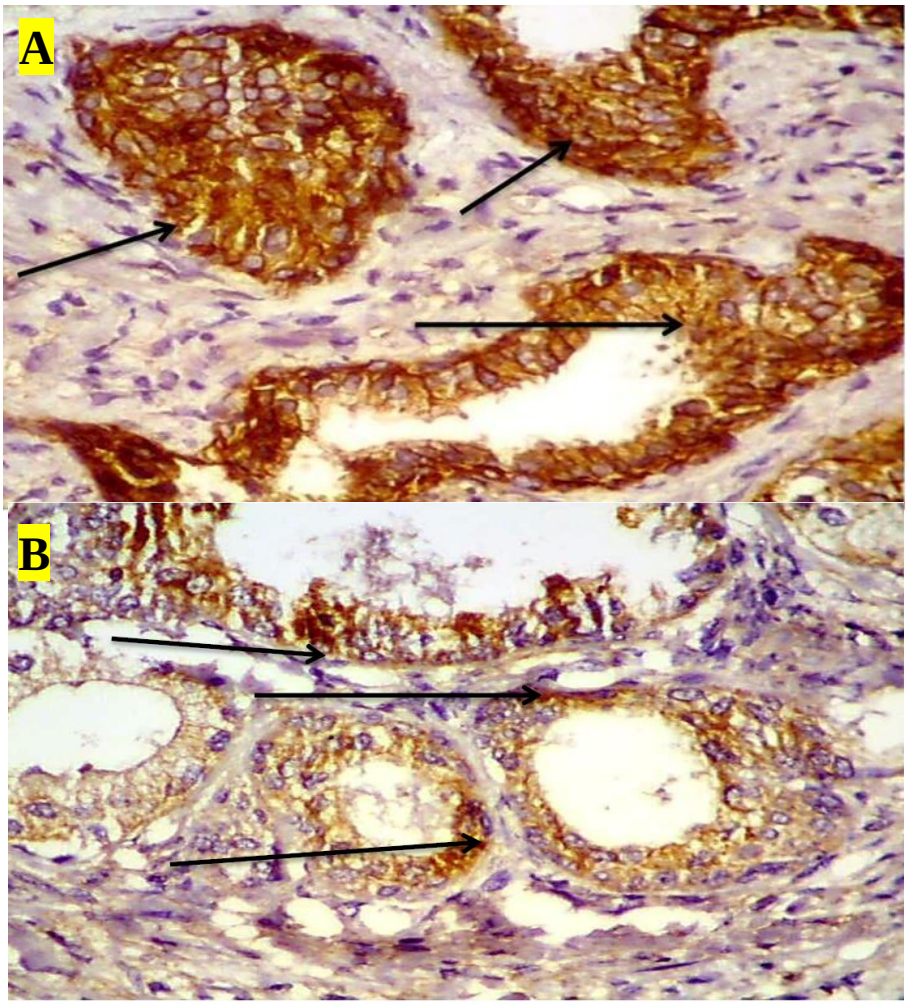

Figure (2): Positive HER2/neu in prostatic cancer (adenocarcinoma) by immunohistochemistry stain (40X) : (A) Intensity 3+, well differentiation Gleason score 4/10, and arrows refer to stained membrane cancer cells, (B) Intensity 2+,moderate differentiation Gleason score 4/10, and arrows refer to stained membrane cancer cells

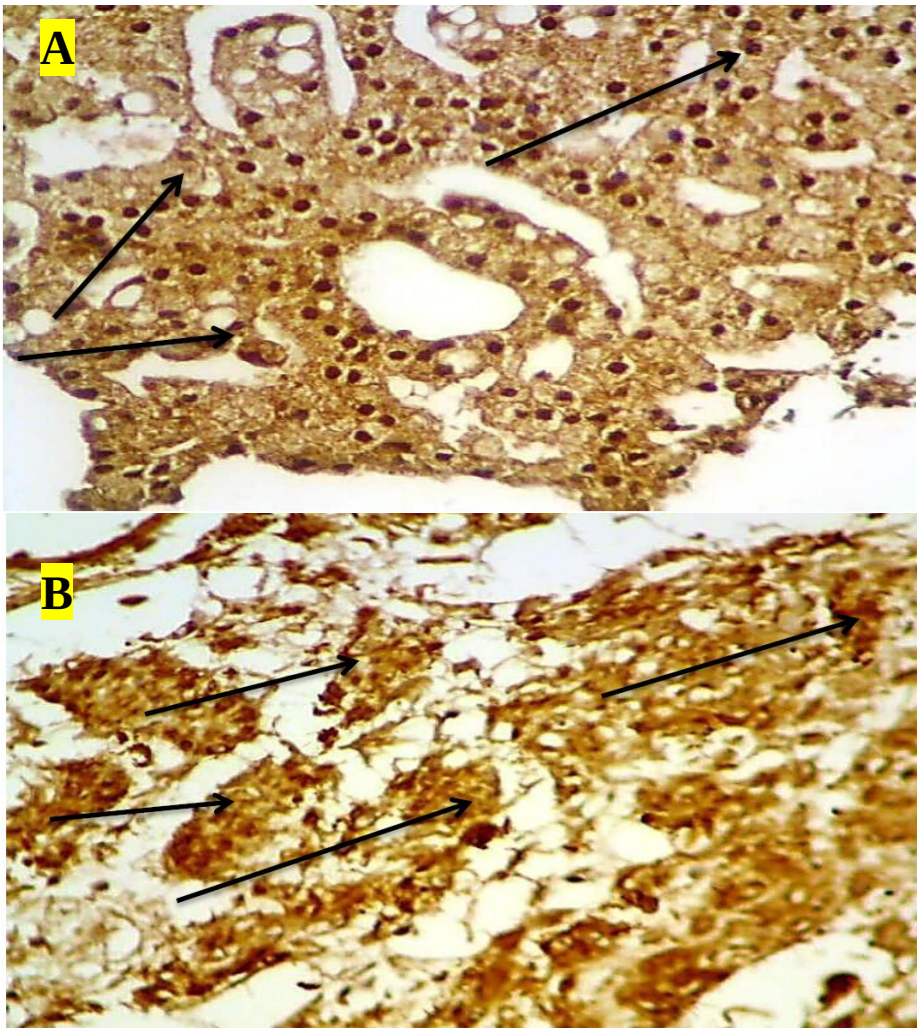

Figure (3): Positive AMACR in prostatic cancer (adenocarcinoma) by immunohistochemistry stain (40X) : (A) Intensity 3+, well differentiation Gleason score 5/10, and arrows refer to stained intracytoplasmic cancer cells, (B) Intensity 3+,moderate differentiation Gleason score 6/10, and arrows refer to stained intracytoplasmic cancer cells.

Discussion

The results of this study demonstrate that the prostatic cancer is more common in ages >69 years and there was no significant difference ($P > 0.05$) in the prostatic cancer incidence in the ages between the subjects in the control group and these in the experimental group. The data of our trail showed that the ages > 69 years represent 56% of all prostatic cancer patients .

Ages 60-69 years represent 38% of all prostatic cancer patients and then the age 50-59 years that represent 6% and this ratio constitutes the lowest percentage in the prostatic cancer patients in this study.

All researchers like Gronberg (Gronberg,2003) , Leitzmann and Rohrmann (Leitzmann and Rohrmann, 2012), and Romero *et al* (Romero *et al.*,2012 considering

age as non-modifiable risk factors for prostatic cancer agree that age is the important risk factors for prostatic cancer . The current trail coincide that age has many features to get the priority in prostatic cancer proposed that the hydroxyl radical assist changes in the structure that resemble the cancer-like phenotype (Malins *et al.*,2003).

There is ratio of mutagenic 8-hydroxyl to non-mutagenic purine lesion in prostate deoxyribonucleic acid (DNA) that increased 3-folds in old men than it is the case with the young men in addition to an increase in base oxidation .

The increase in DNA base lesion concentrate in various tissues , reduced enzyme repair deoxyribonucleic acid nuclease activity, all the finding with age related changes has important role in prostatic development (De Pinho,2000; Brosh *et al.*,2001; Osterod *et al.*,2001) . We supposed that older men have greater chance to get prostatic cancer because they have more exposure to exogenous effects like (diets, smokes, chemicals) and endogenous effects like (free radical, hormonal imbalance) that leads to more lesion for DNA and its repair system.

The diagnosis of prostatic cancer is based on combination of architectural, cytological and additional features (Kumaresan *et al.*,2010). The diagnosis can be difficult due to the presence of either a small focus of cancer or due to the many benign mimickers of malignancy like , adenosis atrophy , partial atrophy , basal cell hyperplasia . In this study, we collected many suspected tissue microarrays with prostatic cancer from the laboratories of Al-Hariri general hospital according to pathologist reports, but all these tissue microarrays give positive result for prostatic cancer when it stained with AMACR by immunohistochemistry (IHC) . Our judgement for that result, is that the AMACR (IHC) is more sensitive than hematoxylin and eosin (H and E stain) in prostatic cancer diagnosis . We cannot find tissue mimicked to prostatic cancer to compare H and E stain with AMACR stain to evaluate accuracy in detection of prostatic cancer. This study showed the deceased percentage is 0% in strong positive overexpression of AMACR .

The most important explanation to this result of the AMACR overexpression is the decreased in hormone-refractory metastatic tissue sample compared to localized prostatic cancer (Kuefer,2002), so strong positive overexpression of AMACR has no relationship to death.

Expression of HER2/neu was shown in the cells membrane and detected by immunohistochemistry technique depending on the scoring system used for the HER2/neu . Alteration in HER2/neu overexpression as tumor progresses from localized to metastatic cancer and from androgen dependence to androgen independence . When hormone-refractory occusr , treatment is limited , the prognosis is poor, and most patients will die within 9-12 month (Carles et al., 2004). The result of our study showed that one men with prostatic cancer deceased as a result of HER2/neu overexpression and might have hormone-refractory. Data showed positive overexpression for HER2/neu was found in 20% of prostatic cancer patients while 80% cases were not expressed or were negative for HER2/neu depending upon cutoff value . Death occurred in one patient who has diagnosed with HER2/neu overexpression by immunohistochemistry and this finding is supported by another researches that the prostatic cancer patients HER2 gene is bad prognosis (Carles *et al.*, 2004).

Conclusion

HER2/neu overexpressions is associated with high Gleason score .HER2/neu overexpressions are regarded as bad prognostic factor. α-methylacyl coemzyme A racemase (AMACR) overexpressions is associated with low Gleason score . α-methylacyl coemzyme A racemase (AMACR) overexpressions are regarded good prognostic factor.

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