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COMBINED EVALUATION OF CYCLIN E AND P^{53} EXPRESSION IN GALLBLADDER CANCER

Alsheyab Fawzi , Morouge Ziada

Jordan University of Science and Technology, Dept. of Biotechnology & Genetic Engineering
E-mail: fawzish@just.edu.jo

Summary. *This study was to investigate the role of Cyclin E and p53 as a useful prognostic indicators in cancerous process of gallbladder carcinomas, and to correlate the expression of these factors with clinicopathological parameters: age, sex, stage, invasion and grade. Thirty two gallbladder carcinomas specimens were surgically resected and were examined immunohistochemically. All patients were diagnosed histopathologically according to the criteria of pathology department. Levels of cyclin E and p53 were found as 34.4% and 65.5% among the patients respectively. Frequencies for p53 expression were high, which suggests that p53 may play an important role in GBC.*

Key words: *Cyclin E, p53, immunohistochemistry, gallbladder cancer*

Introduction

Several risk factors for gallbladder carcinoma have been studied. Some of these risks are attributed to genetic factors (1). These genetic factors had not been widely studied because of the rarity of the tumor and difficulties arise due to late development and location of gallbladder. However, several studies showed deregulation and overexpression of some proteins in GBC such as mamoglobin B, K-ras, Growth factors, p53, MDR1, and cyclin E (2-7).

Cyclin E is 395 amino acid protein derived from a gene on chromosome 10q12. It is one of the most important cell cycle regulators that play an important role in normal cell proliferation and development through promotion of the S phase. Cyclin E deregulation are general events in cancer, irrespective to tumor origin (8).

P53 tumor suppressor gene coded for a 53 KDa phosphoprotein with 393 amino acids long encoded by the 17p13 location. The functional form of p53 created when it binds as a tetramer through a C-terminal domain comprising amino acids residues 325-356 to a p53–binding site (69), and activates the transcription of the adjacent genes (69). The wild type protein is usually unstable and has a short half life. The mutated form is more stable and leads to intracellular accumulation and can be detected by IHC method (www.p53 tumor protein.com, March 19th 2005).

The gallbladder is a small muscular organ that lies in the undersurface of the liver. It stores bile that is used mainly to emulsify fats (9). Gallbladder, histologically, includes lining of simple columnar epithelium, lamina propria that is a thin layer of smooth muscle, serosa of loose fibrous tissue and mucosa (1).

Al-Kayed reported in the cancer incidence registry in Jordan that GBC for the year 2000 constituted 1% of all cancers (10). On the other hand, reported on the epidemiological, clinical, and pathological features of gallbladder cancer (they depended on the histopathological reports and the hospital records for all cases) they found that prevalence was 33/4502 cholecystectomies performed between 1994-2000 (11).

In the Middle East and North Africa only scattered reports are available. Of 7352 cholecystectomies performed, 89 cases of gallbladder carcinoma were found in Libya over a period of 16 years (12).

Gallstones and cholecystitis are the most common disorders of the gallbladder. Gallstones are of two types; cholesterol and billirubin calcium salts, Cholecystitis is inflammation of the gallbladder and it is one of the most indications for abdominal surgery (13).

The objective of our study was to evaluate the expression of p53 and cyclin E in GBC. On the other hand, we aimed to correlate the expression of the previous markers with clinicopathological parameters of the patients.

Material and Methods

Tissue

Paraffin embedded tissues from surgical specimens of 32 GBC patients were retrieved from Pathology Department at Jordan University of Science and Technology. Specimens were collected between 1994-2001 from different hospitals. Patients included Tissues belong to 25 women and 7 men's, ages 45-78 years.

Antibodies

Mouse polyclonal antibodies against all cyclin E (clone m-20) and p53 (clone O-1) were purchased from Sant cruz Biotechnology Incorporation, USA.

Preparation of Sections

Paraffin embedded GBC tissues were cut into 3-µm thick sections

Staining Procedure

Sections were floated on vectabond treated frosted glass slides (Vector Laboratories, USA) and heated in oven at 70°C for 5 mins. Tissue sections were cleared in xylene for 2 mins and rehydrated in a descending series of ethanol (100%-70%). Thereafter, sections were cocked under pressure in the reveal solution (Biocare Medical, (location)) 10x for 2-4 mins in the Decolaking chamber (Biocare Medical, (location)). Sections were left to cool to room temperature then washed and incubated with PBS for 15 mins.

Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 5 min. After that sections were incubated for 1hr. at room temperature. This was followed by incubation with biotinylated secondary antibody for 10-20 min, then immunoperoxid- ase staining was carried out using streptavidin biotin reagent (Biocare Medical LSAB kit) for 10-20 mins at room temperature. However, blocking of endogenous peroxidase activity incubation with secondary antibody and immunoperoxidase staining must be followed by PBS washing.

DAB chromogen was used to develop staining (7 mins incubation) and sections were counterstained with hematoxylin (Gain Land Chemical Company / UK) for 20-40 seconds. Dehydration, clearing, and mounting were carried out then slides examined under light microscope.

Controls

Positive controls for cyclin E and p53 were prepared from breast cancer tissue known to express these markers. Negative controls were performed by incubation of sections with PBS buffer only instead of the primary antibodies.

Assessment of Staining

Expression of investigated markers was evaluated by intensity of staining and incidence of positively stained cells. The intensity was graded as; absent (0), mild (1), moderate (2), and intense (3). The incidence was scored for p53 and cyclin E as; absent (0), less than 10% (1), 10-50% (2), and more than 50% (3). Intensity and incidence of positively stained cells were added to derive a staining score range from 0-6. A score equal to or greater than 3 was considered as overexpression (11).

Statistical Analysis

Frequencies of cyclin E and p53 overexpression were compared with clinical characteristics and pathological parameters. Correlation between markers expression and clinicopathologic factors was determined by using Pearson's chi-square test. Statistical analysis was performed using the statistical software SPSS version 10.0, $p < 0.05$ were considered statistically significant.

Results

During this study two molecular markers (cyclin E and p53) were investigated in 32 cases of GBC using immunohistochemistry.

A. Cyclin E

Table 1. Expression of cyclin E according to clinicopathological characteristics

Clinicopathological characteristics		Cyclin E expression				p
		No. patients	Positive %	No. patients	Negative %	
Age	≤ 64	6	37.5	10	62.5	0.500
	>64	5	31.3	11	68.8	
Gender	Female	7	28	18	72	0.162
	Male	4	57.1	3	42.9	
Grade	1&2	7	35	13	65	0.617
	3&4	4	33.3	8	66.7	
Stage	I, II	3	23.1	10	76.9	0.233
	III, IV	8	42.1	11	57.9	
Invasion	Presence	9	57.1	12	57.1	0.158
	Absence	2	18.2	9	81.8	

Cyclin E expression in association with the age, stages (according to WHO), grading and invasion was studied. Cyclin E expression (Positive staining) was visible in 11 cases out of 32 (34.4%) (Fig. 1). Table 1 summarizes the association between cyclin E overexpression and various clinicopathological variables. 37.5% of patients with age less than 64 years old show cyclin E expression while 31.3% with age more than 64 years old show cyclin E expression. Regard gender 57.1% of male patients have Cyclin E expression while only 28% of female patients show Cyclin E expression. On the other hand it was found that grade (1&2) have 35% Cyclin E expression while grade (3&4) 33.3%. Cyclin E expression in stages III and IV (42.1%) in comparison to stages I and II (23.1%). Also high expression is present in the cases of invasive carcinoma compared with non-invasive cases with no statistical significance.

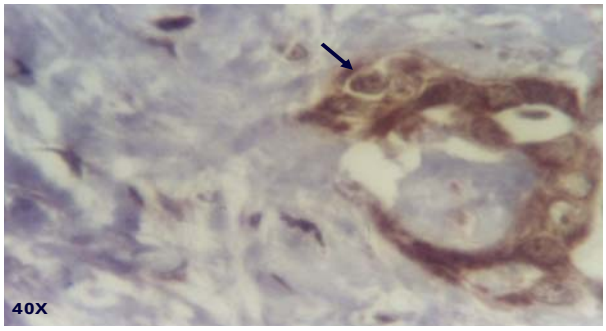

Fig. 1. Strong cyclin E expression in gallbladder cancer

B. p53

Table 2. Expression of p53 according to clinicopathological characteristics

Clinicopathological characteristics		p53				p
		No. patients	Positive %	No. patients	Negative %	
Age	≤ 64	10	62.5	6	37.5	0.110
	>64	11	68.8	5	31.3	
Gender	Female	16	64	9	36	0.423
	Male	5	71.4	2	28.6	
Grade	1&2	14	70	6	30	0.344
	3&4	7	58.3	5	41.7	
Stage	I, II	8	61.5	5	38.5	0.271
	III, IV	13	68.4	6	31.6	
Invasion	Presence	15	71.4	6	28.6	0.425
	Absence	6	54.5	5	45.5	

The p53 expression was significantly correlated with GBC development, 65.5% of the 32 patients show p53 expressed with no significant association with respect to the gender (male: 71.4%; female: 64%), grade (grade 1&2 : 70%, grade 3&4: 58.3%), stage (stage I, II:61.5%, stage III,IV:68.4%) and invasion (presence: 74.4%, absence: 54.5%).

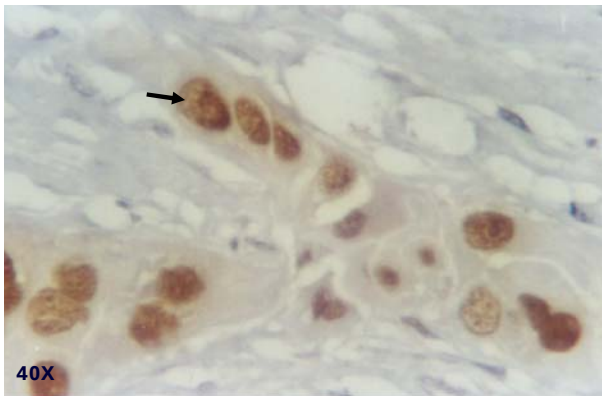

Fig. 2. Nuclear staining for p53 at the tip of the black arrow

Discussion

Cyclin E is amplified and overexpressed in many types of human neoplasms, including cancers of urinary bladder, colon, esophagus, ovary, breast, and stomach have been reported (14,15,16). It has been also reported that cyclin E amplification and overexpression can be used as a prognostic marker for several human tumors (17).

Most of our patients with GBC were females. The relatively high risk of GBC for women may be due to elevated sex hormones (estrogen and progesterone) that may alter the lithiogenicity of the bile and increases cholesterol uptake, thus increasing the risk at early age at first pregnancy, multiple pregnancies and prolonged fertility (18).

Expression of cyclin E in the current study coincides with finding obtained by Jin (9) but it was higher than the results reported by Scharm (16) which may be due to the use of polyclonal anti cyclin E antibody clone (m – 20) in our study and this detected the two isoforms of cyclin E, whereas the other two research groups used monoclonal anti cyclin E antibody (clone 13A3) that detected only one isoform.

Cyclin E overexpression was reported to be higher in invasive gastric cancers, aggressive histological subtypes, advanced stages, and patient who survived less (19). However, our results did not show significant association between cyclin E expression and any of these clinincopathological parameters which might be due to the limited number of cases of our study group.

Combined overexpression of cyclin E and p53 reported rapidly increase the aggressiveness of the disease, and is also associated with poor prognosis (5), however our results did not show similar findings.

Overexpression of p53 in GBC is suggested to be significantly associated with stage, grade, and invasion (20); our results did not confirm such findings, probably because of the small number of the study group.

Conclusions

Combined overexpression of cyclin E and p53 was not associated with poor prognosis. p53 was widely expressed in GBCs positive while cyclin E may play a role in GBC.

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KOMBINOVANA EVALUACIJA CIKLINA E I P^{53} KOD KANCERA ŽUČNE KESE

Alsheyab Fawzi , Morouge Ziada

Jordanski Univerzitet za nauku i tehnologiju, Departman za biotehnologiju i genetski inženjering
E-mail: fawzish@just.edu.jo

Kratak sadržaj: *Ova studija je proučavala utical ciklina E i p53 kao korisnih prognostičkih indikatora kod kancera žučne kese i ekspresiju ova dva faktora u korelaciji sa kliničkopatološkim parametrima: starost, pol, stanje, invazija i stepen. Trideset dva uzorka karcinoma žučne kese su ispitivana imunohitohemijski posle resekcije. Svi pacijenti su histopatološki dijagnostifikovani prema kriterijumu patološkog odeljenja. Nivoi ciklina E i p53 su nađeni u 34,4% i 65,5% pacijenata. Učestalost ekspresije p53 je visoka, što sugeriše da p53 može da ima važnu ulogu u karcinomu žučne kese.*

Ključne reči: *ciklin E, p53, imunohistohemija, kancer žučne kese*