HER2 Amplification in Hydatidiform mole patients

Nawras Najah Mubark, Kareem Hamed Ghali, Mohammed Naji Karim*

University of Wasit , College of Science, Wasit , Iraq

* Unit of clinical Immunology and Immunohistochemistry, Imamein Kadhimein Medical City, Baghdad , Iraq

تضاعف الجين HER2 في مريضات الحمل العنقودي

نورس نجاح مبارك ,كريم حمد غالي ,محمد ناجي كريم*

قسم علوم الحياة, كليه العلوم ,جامعة واسط

*وحدة المناعة السريرية والمناعة النسيجية الكيميائية , مدينة الإمامين الكاظمين (ع) الطبية, بغداد ,العراق

المستخلص

الحمل العنقودي هو حمل غير طبيعي في المشيمة يكون على شكل حويصلات صغيرة تشبه خلية العنب وعادة يمكن رؤيتها بالعين المجردة .

المواد وطريقة العمل: أنجزت هذه الدراسة في مختبرات كلية العلوم \ جامعة واسط بالتعاون مع مستشفيات الزهراء والكرامة التعليميين اعتبارا من شهر تشرين الاول 2014 الى شهر نيسان2015 وتضمنت الدراسة عشرين مريضة مصابة بالحمل العنقودي جمعت عشوائيا وست مريضات غير مصابات بالحمل العنقودي بوصفها عينة سيطرة للمقارنة بين العينتين .استخدمت تقنية التهجين الموضعي الملون في دراسة تضخم الجين HER2 .

النتائج: أظهرت النتائج زيادة معنوية في تضخم الجين *HER2* في مريضات الحمل العنقودي مقارنة بعينة السيطرة ($P\leq0.05$) كما لوحظ نفس الفرق المعنوي عند المقارنة بين مريضات الحمل العنقودي الكلي وعينة السيطرة , وبين مريضات الحمل العنقودي الجزئي وعينة السيطرة. بينما لم يظهر هذا الجين أي فرق معنوي عند المقارنة بين مريضات الحمل العنقودي الكلي والجزئي .($P\geq0.05$)

الاستنتاج :

تضخم الجين HER2 ربما يؤدي دورا مهما في سلسلة من الاشارات التي تسيطر على تنظيم الحمل الطبيعي

الكلمات المفتاحية : جين مستقبل عوامل النمو ,التهجين الموضعي الملون للدنا ,الحمل العنقودي

Abstract

Hydatidiform mole is an abnormal pregnancy in which the placenta contains grape cell like vesicles (small sacs) , that are usually visible with the naked eye .

Patients and methods : This study was carried out in Laboratories of College of Science, Wasit University , AL Zahraa and AL Karma Teaching Hospitals, from October 2014 to April 2015. The study included twenty Hydatidiform mole patients were taken randomly, and six non hydatidiform mole patients as control group. The patients with HM included 10 samples of complete H mole , and 10 samples of partial H mole. Chromogenic In Situ Hybridization (CISH) technique was used to detect the amplification of HER2 gene.

Results: Regarding the HER2 amplification (over-expression of HER2) was significantly more frequent in H mole patients than in control ($p \leq 0.05$), also the same difference was indicated between complete H mole and control group. As well as between partial H mole and control group . While, HER2 amplification did not differ between complete H mole and partial H mole ($p \geq 0.05$) .

Conclusions:

In conclusion, the HER2 gene amplification may play a crucial role in the network of cell-signaling processes controlling normal pregnancy .

Key words : HER2 , Chromogenic in situ hybridization , H mole

Introduction

Hydatidiform mole is an abnormal pregnancy in which the placenta contains grape cell like vesicles (small sacs) that are usually visible with the naked eye. The vesicles arise by distention of the chorionic villi by fluid. When inspected in the microscope, hyperplasia of the trophoblastic tissue is noted(1).HM is characterized by varying degrees of trophoblastic proliferation (cyto-trophoblast ,syncytiotrophoblast and intermediate trophoblast) ,and vesicular swelling of placental villi associated with an absent ,or anabnormal embryo(2).Although ,the majority of HMs spontaneouslyregress after suction evacuation, some may develop gestational trophoblastic neoplasia (GTN) ,and thus require chemotherapy. Metastasesmay develop occasionally (3).The separation of hydatidiform mole into two subtypes ;complete and partial, which represents a significant advance in our under-standing of molar pregnancy. These two forms of hydatidiform mole have different cytogenetic patterns ,that are accompanied by different clinic-pathologic profiles ,and different degrees of risk for the development of GTD. Both forms of mole typically presented in the first trimester (4). Classification of hydatidiform moles as

complete and partial is important not only for clinical management but also for accurate ascertainment of the risk of gestational trophoblastic disease (GTD).HM carries a significant risk for developing persistent GTD , with the higher incidence in patients with CHM (10%-30%) than patients with PHM(0.5%-5%) (5).

Human epidermal growth factor receptors (HER/erbB) constitute a family of four cell surface receptors involved in transmission of signals controlling normal cell growth and differentiation .(6).The genes for the four members of this family, *HER1,HER2 , HER3 and HER4 .HER1-HER4*, are found on different human chromosomes(7).

HER-2/neu proto-oncogene (human epidermal growth factor receptor 2, also referred to as ErbB-2 or neu) is a member of the type I growth factor receptor gene family , and is located in the long arm of chromosome 17 (17q12) (8). *HER2* is strongly over expressed in many cancerous such as breast, ovarian, stomach and uterine(9).It has been shown in many studies that overexpression of the HER2 protein correlates with amplification of the *HER2* gene (10)

This human gene encodes a protein that has a structure consistent with a growth factor receptor, and has been designated HER2 due to its similarity to the human epidermal growth factor receptor (6).The HER2 receptor has an important role in normal cell growth and differentiation. However, amplification of the HER2 gene leads to over-expression of the receptor, which is linked to the development of many types of human cancers including breast, ovarian and those of

the gastrointestinal tract(11) .The aims of study to determine the *HER2* gene amplification in Iraqi hydatidiform mole patients and investigation the expression of *HER2* in the CHM and PHM .

Materials and methods

Patients and tissue samples

The study involved 26 cases(10 CHM patients ,10 PHM and 6 non HM patients) attended to the AL-Karama Teaching Hospital and AL-Zahraa Teaching Hospital in Wasit Province/ Iraq, during the period between 2010 to April 2015. This study was carried out in Laboratories of the College of Science/ Department of Biology in collaboration with Unit of Histo-pathology in AL-Karama Teaching Hospital in Wasit. Full clinical data were obtained including full medical history and complete clinical examination. The patients with hydatidiform mole included .These cases were collected from laboratory of histopathology in confirmatory histopathological re-evaluation of each obtained paraffin embedded tissue section was done by specialist pathologist. The cases were sectioned (5μm) thickness, from each tissue block.Also,to simplify the statistical analysis, the patients were divided into five age groups and histological types , as CHM and PHM.

ChromogenicIn Situ Hybridization (CISH) technique for estimating the HER2/neu in formalin fixed paraffin embedded sections :

The kit of HER2 gene and chromogenic in situ hybridization staining system are provide by Zytovision

1)Pretreatment (Dewax \ Proteolysis) (Day 1)

Serial tissue sections were cut 5µm thick and were positioned on positive charged slides. The slides were baked in 60˚C oven over night. The tissue sections were deparaffinized; then the slides were dehydrated by graded ethanol concentration (100%, 95%, and 70%) and distal water .

After preparation of tissue sections, slides were incubated for 10 minutes in 3% hydrogen peroxide diluted in PBS, deionized H2O. Each slide was washed2x 1 min in distilled water . Slides were placed in the pre-heated Heat Pretreatment Solution EDTA (PT2) and incubated for 15 minat 75-80°C , then the slides were washed in 2x D.W., for 2 minute. Applied (dropwise) pepsin Solution (ES1) to the tissue section and incubated for 7 min at RT in a humidity chamber, and the slides were washed in 1x D.W., for 2 minute.Dehydration: in 70% , 95% , 100% ethanol, each for 1 min. Air dry sections.

2) Denaturation and Hybridization (Day 1)

Then , added the probe(HER2\neu)on tissue section in slide and place coverslip on slide , as well as using a pipette tip, which has been cut off to increase the size of the opening, when can make the pipetting Process easier.The slides were denatured at 78-80oCfor 5 min, e.g. on a hot plate , and they were transferred to a humidity chamber and hybridize overnight at 37oC (e.g. in a hybridization oven).The slides were denatured at 78-80o C for 5 min, e.g. on a hot plate , and then they were transferred to a humidity chamber and hybridize overnight at 37oC (e.g. in a hybridization oven).

3) Post-Hybridization and Detection (Day 2)

Carefully removed the rubber cement or glue, and removed the coverslip by submerging in Wash Buffer SSC (WB1) at RT for 5 min. The slides were washed for 5 min in Wash Buffer SSC (WB1) at 75-80oC then washed 2x 1min in D.W. Immersed slides in 1x Wash Buffer TBS (prepared using WB5) and drain off or blot off the 1x Wash Buffer TBS, for 2min . Put 40µl of Anti-DIG/DNP-Mix(ABI4)to slides and incubated for 15min. at 37°C in a humidity chamber , then washed 3x 1 min in 1x Wash Buffer TBS (prepared using WB5). Put 40µl of HRP/AP-Polymer-Mix(ABI3)to slides and incubated for 15min. at 37°C in a humidity chamber and during the incubation ,AP-Red solution was prepared by added one drop (30 µl) of AP-Red Solution A (SB6a) in a graduated cap (e.g. AP-Red reaction vessel), fill up to 1 ml with AP-Red Solution B (SB6b) and mixed well . then washed 3x 1 min in 1x Wash Buffer TBS (prepared using WB5). Put 100µl of AP-Red Solution to the slides and Incubated for10 min at RT(protect from strong direct light) and during the

incubation, HRP-Green solution was prepared by added two drops (2x 20 µl) of HRP-Green Solution A (SB7a) in a graduated cap (e.g. HRP-Green reaction vessel), fill up to 1 ml with HRP-Green Solution B (SB7b) and mixed well , then washing the slides 2 min in D.W. Put100µl of HRP-Green Solution to the slides and Incubated for10 min at RT (protect from strong direct light) ,then slides washed for 2 min in D.W. Counter stain the tissue samples for 2 min with Nuclear Blue Solution (CS2). Slides were transferred into a staining jar and washed for 2 min in running tap water. Dehydration: 3x 30 s in 100% ethanol (used very pure ethanol), 2x 30 s in xylene (used very pure xylene). Avoided trapped bubbles , covered the samples immediately with a cover slip (22 mm x 22mm; 24 mm x 32 mm) by used Mounting Solution (alcoholic) (MT4) and air dry the slides for approx. 30 min. Evaluated of the sample material was carried out by light microscopy.

Ethical consent

The study was submitted and approved by the Faculty of Science, University of Wasit in collaboration with AL-Karama and AL-Zahraa Teaching Hospitals in Wasit- Iraq.

Statistical analysis

The data were analyzed using the following tests:

- The Statistical Analyses of results were carried out by the help of Minitab using version 16 statistical package .
- Correlation Coefficient test was used to find the correlation between different markerswith other parameters.
- Fisher's Exact test was used to investigate comparison between the expression of each marker in patients group and control group.
- The level of significance was 0.05 (or less) in all statistical testing ,(p value less than 0.05).

Results

Chromogenic In Situ Hybridization (CISH)

HER2 amplification in H mole

The results of HER2 amplification between H mole patients and control group showed that 15(75%) patients from total 20 in this study have positive result for HER2 amplification ,comparing with control,1(16.66%) from total 6 have positive result , with highly significant difference ($p<0.05$) table 1, fig 1

Table (1): *HER2* amplification in H mole patients and control group

Case	Amplification +ve		Non-amplification -ve		Total	
	NO.	%	NO.	%	No.	%
H mole patients	15	(75%)	5	(25%)	20	(100%)
Control	1	(16.7%)	5	(83.3)	6	(100%)
P value ≤0.05						

(highly significant , P= 0.001)

When we compared the HER2 amplification between complete H mole and partial H mole , there was no statistical significance (P value ≥ 0.05) . The data which was obtained from table (1) showed that 7 (70%) out of 10 patientswith complete hydatidiform mole, and 8(80%) out of 10 patients from partial hydatidiform mole have HER2 amplification.

Table (2): *HER*2 amplification in complete and partial H mole patients

Case	Amplification +ve		Non-amplification -ve		Total
	NO.	%	NO.	%	
CHM patients	7	(70%)	3	(30%)	10(100%)
PHM patients	8	(80%)	2	(20%)	10(100%)
					0.05≥P value

(No significant)

In table (3) which was involving HER2amplification in complete H mole patients and control group. The result was detected , that 7(70%) of 10 complete hydatidiform mole patients showed HER2 amplification , compared with 1(16.66) out of 6 of control group. This difference was highly statistical significant (p value ≤ 0.05).

Table(3) :*HER2* amplification in complete H mole patients and control group

Case	*Her2* amplification				Total
	+ ve		-ve		
	NO.	%	NO.	%	
CHM patients	7	(70%)	3	(30%)	10 (100%)
Control	1	(16.66%)	5	(83.34%)	6 (100%)
					0.05 < P value

(highly significant, P= 0.001)

Also , the same results demonstrated that HER2 amplification was increased in partial H mole patients comparing with control group with highly statistical significant(p value > 0.05) table 4 . HER2 positive wasdetected in 8 (80%) of 10 partial hydatidiform mole patients compared with 1(16.66) out of 6 of control group.

Table (4):*HER2* amplification in partial H mole patients and control group

Case	*Her2* amplification				Total
	+ ve		-ve		
	NO.	%	NO.	%	
PHM patients	8	(80%)	2	(20%)	10 (100%)
Control	1	(16.7%)	5	(83.3%)	6 (100%)
					0.05 < P value

(highly significant, P= 0.001)

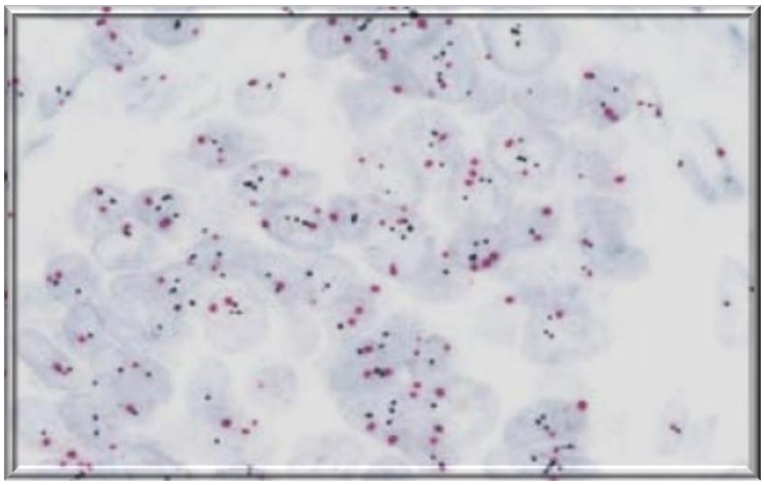

Figure 1: Positive *HER2* amplification in hydatidiform mole tissues (CISH staining, Magnification: 40x10)

Discussion

We postulated that HER2 amplification could be used in the evaluation of hydatidiform mole. These results recorded high percentage from HER2 amplification in patients of HM , an overall success rate of 75% (15/20). while in control group, the HER2 amplification was low. LeGallo, R.D., et al ,(12) , they mentioned that HER2 amplified were available for 20 of the 23 HMs , 3 cases yielded no signal of the 23 cases. HER2 amplification play significant roles in signaltransformation, cell proliferation, development, differentiation,

migration and tumor formation . Our finding indicate a discrepancy to tumerigensis . The amplification of HER2 was few in control group ,

because the expressed of HER2 is low levels in normal tissue ,but there was a high amplification for HER2 in both complete and partial hydatidiform mole. Therefore, did not show any significant differences between the types of HM . In similar study carried out by LeGallo, RD; et al.(12), for HER2 gene where found that in CHM ,HER2 data were positive for 10 of the 13 ; only 3 cases yielded no signal of the 13 cases. while in PHM , HER2 was successfully performed on all 10 cases . Furthermore ,over expression of HER2 is associated with aggressive tumor growth and metastatic activity (6).

We mentioned that there are high for *HER2* amplification in CHM patients and decline in control group. We can explain why amplification of HER2 few in control group , because innormal cells, few HER2 molecules exist at the cell surface. So few heterodimers are formed and growth signals are relatively weak and controllable. When HER2 is over-expressed multiple HER2 heterodimers are formed and cell signaling is stronger, resulting in enhanced responsiveness to growth factors and malignant growth (6)

.In amplification for HER2 gene, differences statistically highly significant were appeared between PHM patients and control group. The finding obtained from tables 3 and 4 refers to hydatidiform mole patients have a tendency to carcinogenesis and need to more follow-up . In conclusion, the HER2 gene amplification may play a crucial role in the network of cell-signaling processes controlling normal pregnancy.

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