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Relationship Determination between Heart Diseases with Endometrial Adenocarcinoma and Ovarian Carcinoma by Evaluating the HPV16 IgG , IgM and p53 in Some Iraqi Female Patients

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Abstract: Heart diseases problems linked to female genitalia associated carcinomas were considered as a major issues and life threat; therefore, it was necessary to determine the relationship between heart diseases problems with HPV16 infection and P53 expression in some groups of female Iraqi patients that suffer from endometrial Adenocarcinoma (EC) and ovarian carcinoma. This study was designed as a two prospective groups. It involved ninety samples of serum were collected from the Medical City of Baghdad from the period of October 2014 to January 2015 as 45 \ 50% of endometrial Adenocarcinoma (EC) patients group and 45 (50%) of ovarian carcinoma patients group then stored in -80°C. All samples were examined for HPV16 IgG, IgM and p53 expression by the enzyme-linked immunosorbent assay (ELISA). Highly significant difference ($p < 0.001$) of Mutant p53 expression has been detected in 32 \ 35.6% out of 41 \ 45.6% patients with heart diseases history in comparing with patients without history of heart diseases. In addition to , highly significant differences ($p < 0.001$) of HPV16 IgM has been detected in 36 patients had previous history of heart problems as 40% out of 41 patients. In the other hand, Endometrial Adenocarcinoma (EC) cases, have been a statistically significant differences with HPV16 IgG in 35 \ 38.9 % and HPV16 IgM in 31 \ 34.4% positive results out of 45 cases ($p < 0.05$) with highly significant differences in the positive cases of 32 \ 35.6% that had been detected with heart diseases problems ($p < 0.001$). Furthermore, the ovarian carcinoma cases have positives HPV16 IgG and HPV16 IgM levels as 24 \ 26.7 % and 18 \ 20% respectively with significant difference regarding to the negative results ($p < 0.05$). An interesting results has been detected recently in this study, as HPV16 infection followed by p53 inactivation may initiate heart diseases in some groups of females Iraqi patients that had been with endometrial Adenocarcinoma or ovarian carcinomas. So that, HPV16 infection plays a major role in the heart diseases progression through it E6 and E7 oncoproteins to the sabotage of the normal p53 and pRb pathways.

Key words: Endometrial Adenocarcinoma (EC), human papillomavirus (HPV) , Protein 53(p53), Triglyceride (TG), CVD.

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Introduction

High risk human papillomavirus (HPV16) is the major causative agent of cervical cancer, also it has been connected with many infections in some others specific sites like: vulva, vagina, and specific epithelium stratified squamous epithelium, heading into epithelial cell proliferation and sometimes malignancy (1). Also some reports have demonstrated the presence of HPV in endometrial adenocarcinoma (2).

Generally, the carcinogenic HPV16 arising carcinoma by it disruption the p53 pathways and the product of the retinoblastoma gene through the action of oncoproteins E6 and E7, subsequently the impaired activity of p53 accelerate the atherosclerotic process as demonstrate in some recent reports (2) (3). Furthermore, there have been scattered studies linking HPV16 with heart diseases, but the role of HPV16 remains controversial, some authors have proposed that HPV16 could play a role in the atherosclerotic plaques progression; however, other studies do not support this hypothesis (3). In addition, the role of HPV16 infection in ovarian cancer is unclear but, many researches reveal that it has the same action as in cervical cancer development with HPV16 infection (4,5).

Materials and Methods

Detection of HPV16 Immunoglobulin's

The ELISA test for both HPV16 IgG and IgM was applied according to manufacturing company leaflet (MyBiosource).The qualitative enzyme immunoassay technique was used. The microtiter plate supplied in this kit has been pre-coated with specific antigens. All specimens were pipetted into the wells with anti-human IgG and anti-human IgM conjugated Horseradish Peroxidase (HRP). If there were any antibodies specific for the antigen present then will bind to the pre-coated antigen in the wells. After that, a washing step to remove any unbound reagents, subsequently, a substrate solution was added to the wells and color develops proportionally to the amount of human HPV16 antibody (IgG) and (IgM) that bounded in the initial step. The color development is terminated and the intensity of the color was measured (6).

Detection of p53 Expression

Expression of p53 was measured according to the procedure of manufacturing company leaflet (abcam). Abcam's Human p53 in vitro ELISA is a quantitative kit for measuring the p53 in plasma , serum and cell lysates. Microtiter plate wells pre-coated with monoclonal antibody specific p53. All Samples, including standards of known p53 concentrations, control specimens were pipetted into these wells. Simultaneously all the standards or samples and a biotinylated monoclonal antibody specific for p53 were incubated. Following the washing step, the enzyme Streptavidin-HRP conjugate biotinylated antibody was added, then incubated and washed. A TMB (3,3',5,5'-Tetramethylbenzidine) substrate solution was added which acts on the bound enzyme for color production. The concentration of p53 that present in the specimens reflect the intensity of this colored product. The mutant p53 expression was found in a wide variety of transformed and tumor cells, where its concentration was increased about 5-1000 fold over the concentration in the normal cells. Over all, p53 was also frequently mutated or inactivated in about 60% of cancers (7).

Clinico– Pathological Factors

This include evaluation of carcinomas types , blood pressure measuring , history of heart diseases , history of smoking status , cholesterol, triglyceride levels and age of patients, all of these factors were determined by qualified physicians and organized in the medical

reports of each individual that has been selected in this study.

Results and Discussion

A: In Endometrial Adenocarcinoma (EC) cases 45 \ 50%, there has been a statistically significant differences with HPV16 IgG in 35 (38.9 %) and HPV16 IgM in 31 \ 34.4% detection out of 45 of these cases ($p< 0.05$), with highly significant differences in the positive cases of 32 \ 35.6% that had heart diseases problems ($p< 0.001$). While, there were thirteen cases 14.4% without heart diseases problems, thirty seven cases 41.1% with positives mutated p53 and only eight cases 8.9% with negatives mutated P53 as highly significant ($p< 0.001$) differences in the same factors that has been determined, in addition, twenty five 27.8% and 4 \ 45.6% cases with abnormal levels of Cholesterol ($p<0.05$) and Triglyceride ($p < 0.001$) respectively. Moreover, there were 23 \ 25.6% of cases suffer from continues hypertension ($p< 0.001$), (Table 1).

B: According to the table (1), the ovarian carcinoma cases 45 \ 50%, have HPV16 IgG and HPV16 IgM levels as 24 \ 26.7 % and 18 \ 20 % respectively with significant difference ($p < 0.05$). Furthermore, there were highly significant differences in the positive cases of nine 10% of patients that had heart diseases problems ($p< 0.001$) with the negative cases as 36 patients 40 % without heart diseases problems, thirteen cases 14.4% with positives mutated p53 and thirty two cases 35.6% with negatives mutated P53 as highly significant difference ($p<0.001$), in addition, there were 12 13.3% and 20 \ 22.2% cases with abnormal levels of Cholesterol ($p<0.05$) and Triglyceride respectively ($p< 0.001$). Finally, there were 13 \ 14.4% from the studied ovarian cases noticed with continues hypotension ($p < 0.001$).

Table (1): Comparison between Endometrial Adenocarcinoma and Ovarian Carcinoma according to HPV16 IgG , IgM and p53 of ELISA results and some of clinico- pathological factors

Characteristics		Female associated Cancers				*P - value*
		EC		Ovarian Cancer		
		NO.	%	NO.	%	
p53	Positive	37	41.4%	13	14.4%	0.000 HS
	Negative	8	8.9%	32	35.6%	
HPV 16 IgG	Positive	35	38.9%	24	26.7%	0.01 S
	Negative	10	11.1%	21	23.3%	
HPV16 IgM	Positive	31	34.4%	18	20.0%	0.006 S
	Negative	14	15.6%	27	30.0%	
Heart diseases history	Yes	32	35.6%	9	10.0%	0.000 HS
	No	13	14.4%	36	40.0%	
Cholesterol Level	Normal	20	22.2%	33	36.7%	0.005 S
	Abnormal	25	27.8%	12	13.3%	
TG Level	Normal	4	4.4%	25	27.8%	0.000 HS
	Abnormal	41	45.6%	20	22.2%	
Blood Pressure	Normal	0	0.0%	32	35.6%	0.000 HS
	Hypertension	23	25.6%	0	0.0%	
	Hypotension	22	24.4%	13	14.4%	
	No B.P.	21	23.3%	25	27.8%	

S=Significant difference (P<0.05), HS= Highly Significant difference (p>0.0001), NS=Non Significant difference (P>0.05).

C: Positive HPV16 IgG was detected in 23 patients out of 41 cases with pervious history of heart diseases as 25.6% with no significant differences regarding to the negative cases without pervious history of heart diseases (p > 0.05), while HPV16 IgM was detected in 36 patients that have some of the heart problems as (40%) out of 41 patients with highly significant differences (p < 0.001) (Table 2).

Table (2) : Association among Heart diseases history with HPV16 \ IgM and HPV16 \ IgG in Endometrial Adenocarcinoma and Ovarian Carcinoma Patients

Heart Diseases History	HPV16 \ IgM		Total	HPV16 \ IgG		Total
	Positive	Negative		Positive	Negative	
No	13 14.4%	36 40.0%	49 54.4%	36 40.0%	13 14.4%	49 54.4%
Yes	36 40.0%	5 5.6%	41 45.6%	23 25.6%	18 20.0%	41 45.6%
Total	49 54.4%	41 45.6%	90 100.0%	59 65.6%	31 34.4%	90 100.0%
P - value	0.000 < 0.001 HS			0.084 > 0.05 NS		

S=Significant difference (P<0.05), HS= Highly Significant difference (p>0.0001), NS=Non Significant difference (P>0.05).

D: Mutant p53 expression was detected in 32 \ 35.6% of patients with history of heart diseases out of 41\45.6% of patients with highly significant difference (p <0.001) regarding to the patients without history of heart diseases (Figure1). In the other hand, highly significant difference of the mutant p53 expression was detected in 41 \ 45.6%) of patients with abnormal TG level out of 61\67.8% (Figure2).Lastly, high concentration of mutant p53 was detected in 23 \ 25.6% patients with abnormal level of cholesterol out of 37\41.1% of patients with significant difference according to the normal cholesterol level (p <0.05) (Figure 3).

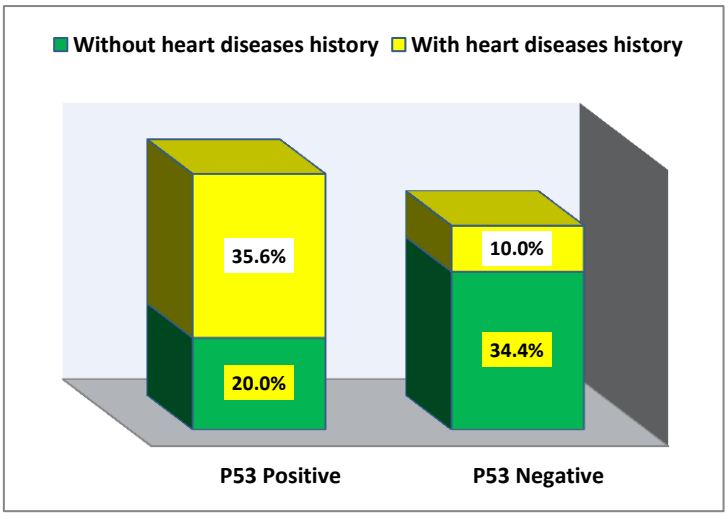

Figure (1): The Relationship between heart diseases history and mutant p53 expression (p < 0.001)

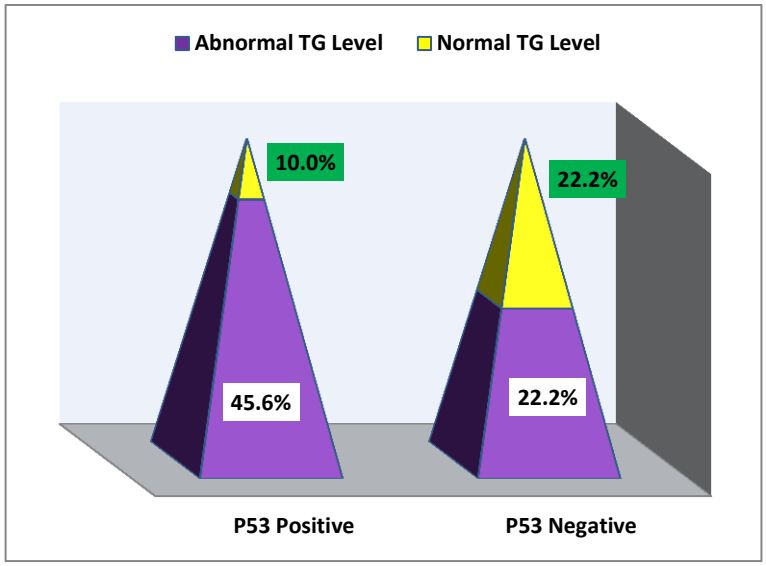

Figure (2): The Relationship between TG Levels and mutant p53 expression ($p < 0.001$)

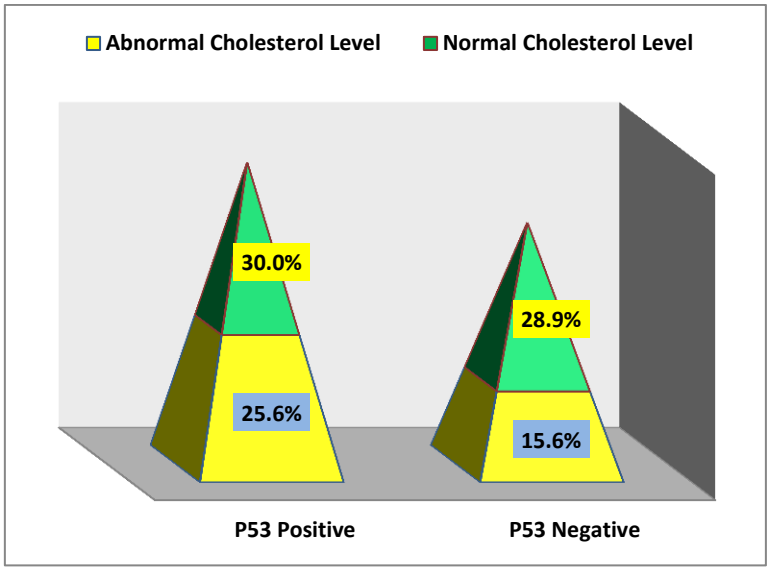

Figure (3): Relationship between Cholesterol Levels and mutant p53 expression ($p > 0.05$)

Statistics

Statistical analyses of research data was performed using SPSS ver. 18.0. The possible interaction of HPV16 infection with heart diseases progression in Iraqi female patients that suffer from endometrial Adenocarcinoma and ovarian carcinoma were analyzed using the Chi-square test.

To the best of our knowledge, there is no previous Iraqi study regarding to HPVs infection linking to cardiovascular disease (CVD). This is the first report to investigate the association of HPVs infection and CVD progression. It has been detected that HPV infection is likely associated with heart diseases among all the Iraqi women that included in this study of age periods belong to (25-69) years, this findings are provocative in this study, but need further validation from larger studies of both men and women. In addition, as Human papillomavirus (HPV16) infection is responsible for the most of cervical cancers there is also some studies refer to whether the presence of HPV16 in endometrial tissues helps to develop the endometrial neoplasm's with a little information regarding to the viral presence in endometrial carcinoma (1) (2) (3). According to some researchers, the mechanisms of raising a woman's risk of heart diseases with genital carcinomas result from the HPV16 infection that might interfere with some cellular genes action in people who do not have such common risk factors as, diabetes , smoking or obesity (1). Other said their work didn't establish that HPV infection caused heart diseases, but instead gave us a scope for more investigation. The researchers set up a studying about the potential role of HPV in the progressing of heart diseases because of some proteins present in high – risk HPVs infection effects on an important tumor-suppressing gene called p53 (3), which also plays a role in blocking of the arteries, called atherosclerosis. Furthermore, patients with impaired p53 gene action or inactivated are more prone to reclogging of their arteries after all the blockages have been surgically removed (3). The current study agree with Fujise and Kuo, 2011 hypothesis, also it bring out several opinions and gives a number of hypothesis for future researches to evaluate most of the causative agents for endometrial or ovarian neoplasm's. HPV16 appears to have roles in the progression of CVD among women. The Evaluating and monitoring the HPV infection may be important for identifying and targeting women at risk for subsequent CVD who's may require more attention to avoid the progressing of cardiovascular events. finally, more studies for the interaction between HPV oncoproteins and p53/pRb has been mostly confined to HPV types 16 and 18 (8) (9), are warranted for better understanding of the molecular action from HPV infection to atherosclerosis pathway, also they made a prospective study of testing whether HPV immunization preventing the cardiovascular outcomes in women. The products of two tumor suppressor genes, p53 and retinoblastoma protein (pRb) might interfere directly with the possible mechanism of the association of HPV infection and heart diseases progression (8)(9)(10). HPV oncoproteins interact with and inactivate both p53 and pRb. Retinoblastoma gene, the first tumor suppressor gene identified molecularly, also plays a pivotal role in regulating cell proliferation then might accelerate the atherosclerotic process (11) (12). However, the role of HPV16 infection with heart diseases progress is unsupported yet, but the current study it found that statistically significant correlation between endometrial and ovarian carcinomas with heart diseases initiation ($P>0.001$). In addition to, highly significant correlation between mutant p53 expression in endometrial and ovarian carcinomas with history of heart diseases in some of females Iraqi patients was found ($P> 0.001$). The final results that

showed the presence of HPV16 in endometrial and ovarian carcinomas patients with heart diseases, suggested that HPV16 infection might play a role in atherosclerotic development.

Recommendation

The Current study recommended a future cautions to explore the reality of endometrial Adenocarcinoma and ovarian carcinoma relationship with heart diseases progression in details. Furthermore, to investigate the mechanisms of other abnormal gene expression like; tumor suppressor genes or other cellular proteins in heart tissue plaques, which must be studied with a large Iraqi sample size.

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