Biochem. Cell. Arch. Vol. 18, No. 2, pp. 2165-2172, 2018 | www.connectjournals.com/bca | *ISSN 0972-5075*

MOLECULAR HIGHLIGHTING ANALYSIS OF MUTATIONAL P57 GENE IN ASSOCIATION WITH BAMHI Z _EPSTEIN-BARR VIRUS REPLICATION ACTIVATOR INFECTION IN TISSUES FROM IRAQI WOMEN WITH OVARIAN TUMORS

Ruqaya M. J. Awadh[1] and Shakir H. Mohammed Al-Alwany[2]

[1]DNA Research Center, University of Babylon, Iraq.
[2]College of Science, University of Babylon, Iraq.

(Accepted 11 July 2018)

ABSTRACT : Epstein-Barr virus (EBV) has been classified as a group 1 carcinogen associated with a variety of lymphoid and epithelial malignancies by the international agency for research of cancer {IARC}. In last years, EBV has been linked to the development of variety of human malignancies including ovarian tissues that range from benign ovarian tumors to ovarian carcinoma. The mutations in p57 gene have been detected in ovarian cancer cells and are involved in progression steps of ovarian carcinogenesis. To detection the distribution and impact of mutation of p57 and BamHI Z_Epstein-Barr virus replication activator (ZEBRA-EBV) infection on a group of ovarian cancer, borderline ovarian tumor and benign ovarian tumor. This study was designed as retrospective case control study. It involved 150 selected formalin fixed, paraffin embedded ovarian tissue blocks; (45) biopsies from ovarian carcinoma (OC); (45) from benign ovarian tumors (BT); (20) borderline ovarian tumor (BOT) as well as (40) apparently normal ovarian control group. Detection of ZEBRA-EBV and mutation p57were done by ultra-sensitive version of chromogenic *in situ* hybridization technique. Detection of EBV-ZEBRA-CISH reactions in tissues with OC was observed in 29 out of 45 (64.4%), while in the benign ovarian tumors group was 37.8% (17 out of 45 tissues) followed by borderline ovarian tumors & the apparently healthy ovarian control tissues were 30% (6 out of 20 cases) and 7.5% (3 out of 40 cases), respectively. The differences between the percentages of EBV-ZEBRA detection in tissues OC and each of BT & BOT as well as control groups were statistically significant (P value = < 0.0001). Positive mutated P57 –CISH reactions were observed in 17 OC cases (37.8%); 24 BT cases (53.3%) and in BOT was 50% (10 cases). Our results indicate that the EBV-ZEBRA might contribute to the development of subset of ovarian tumors. In addition, the significant percentage of mutated expression of possible p57 gene as well as ZEBRA-EBV in ovarian carcinoma could indicate for an important role of these molecular and viral factors in ovarian carcinogenesis.

Key words : ZEBRA (BamHI Z)-EBV, P57, ovarian tumors, borderline tumor, CISH.

INTRODUCTION

Ovarian tumors comprise a heterogeneous group of tumors arising from different cell types. The surface epithelial ovarian carcinoma (EOC) are regarded the most frequent types represent 90% of all malignant neoplasms. Based on the Ovarian Cancer National Alliance, ovarian cancer occurs in about one out of every 57 women and is on the rise (Carroll Jennifer *et al*, 2001). According to Iraqi cancer registry 2005, ovarian cancers rank the fifth of female cancers. Like other types of cancers, ovarian carcinomas follow a multistep pathway involving activation of certain oncogenes and inactivation of tumor suppressor genes, as well as involvement of other genes and external mutagens (Tachibana *et al*, 2003).

However, researches have focused some factors that may contribute to the development of ovarian tumor, including genetic factors, impaired immune system, viruses, exposure to chemicals and heavy smoking. Therefore, the involvement of *EBV* infection in ovarian tumors has been an interesting issue (Littman *et al*, 2003).

Epstein-Barr virus (EBV) is an enveloped, ubiquitous gamma herpes virus with a double-stranded DNA genome encoding more than 85 gene. ZEBRA (BamHI Z Epstein-Barr virus replication activator, also known as Zta and BZLF1) is an early lytic protein of EBV encoded by BZLF1(Miller *et al*, 2007). ZEBRA is a homodimer. Each subunit has 245 amino acid residues. It has a basic leucine zipper domain, a characteristic of many transcription

factors (Miller *et al*, 2007; Petosa *et al*, 2006).

Infection by EBV begins with a short replication phase. The virus remains in a latent phase, only entering the lytic phase in response to a cascade of transcriptional signals. These signals are triggered by the ZEBRA protein (BZFL1) along with Rta (BRLF1) two immediate-early transcription factors of the EBV, that activate eachother's promoters for lytic cycle intitiation, as well as their own. The key structural feature of ZEBRA that allows this initial binding to occur is a b-ZIP domain, or basic leucine zipper (Rosenberg *et al*, 2001). Previous study have shown evidence of *Epstein–Barr-virus* in ovarian cancer (Littman *et al*, 2003).

$P57^{kip2}$ (p57) is a maternally expressed imprinted gene regulating growth arrest which belong to the CIP\KIP family of cyclin- and cyclin- dependent kinase complexes, p57 activity has also been linked to differentiation, apoptosis, and senescence. In addition, p57 has recently been shown to be involved in tumorigenesis and cell fate decisions in stem cells (Mademtzoglou *et al*, 2017). CDK inhibitor p57 has been previously reported to be inactivated in a varity of human cancers (Li *et al*, 2002). It was reported that p57 is a direct target of EZH2 and repressed in breast cancer by multiple epigenetic mechanisms (Yang *et al*, 2009).

Lu *et al* (2007), who examined EZH2 gene expression difference in purified endothelial cells from invasive epithelial ovarian cancers and five normal ovaries and found EZH2 was elevated three to 4.3 fold in tumor-associated endothelial cells.

PATIENTS AND METHODS

This study was designed as retrospective case control study. It involved 150 selected formalin fixed, paraffin embedded ovarian tissue blocks (45) biopsies from ovarian carcinoma (OC); (45) from benign ovarian tumors (BT); (20) borderline ovarian tumor (BOT) as well as (40) apparently normal ovarian control group. Detection of ZEBRA-EBV and mutation p57 were done by ultra sensitive version of chromogenic *in situ* hybridization technique.

Proviral Probes (DNA) Probes for integrated DNA of *ZEBRA-EBV Gene & Probes of P57*

We design and request a probes for detection of ZEBRA-EBV Gene and Mutated P57 by CISH complementary to a sequence of ZEBRA-EBV and P57 I & II genes and design a probe for detection of ZEBRA I & II -EBV Gene, P57 I & II by CISH test from Zytovision company, Germany. Each probes of them were labeled with Digoxigeninand applied in a colometric *in-situ* hybridiazation on paraffin embedded tissues.

Primer selection

1. ZEBRA-EBV Gene Probe

-Sequences of First Probe

5'-NCTTCATGAGTCAGTGCT-3'

A	G	C	T
3.3	4.3	4.3	6.3

2. P57 Mutated Gene Probe

-Sequences of mutated p57 Probe

GCGgctccggtcgcggctcCGGTCGCGGT

A	G	C	T
6	7	5	5

RESULTS

I. Some Clinico-pathological Findings

1. Histological types of *Malignant Ovarian Tumors*

Frequency distribution of histological types according to biological behavior was Serous epithelial (71.11%), Mucinous (20%) and Endometrium (8.89 %) (Fig. 1).

There were significant statistical differences ($p<0.05$) between malignant ovarian tumors group according to histological types (Table 1).

2. Histopathlogical Grades of *Malignant Ovarian Tumors*

The results of present study shows that moderately grades ovarian carcinomas (grade II) constituted 35.5%, whereas with poorly differentiated grade ovarian carcinomas (grade III) constituted 33.3% and well differentiated (grade I) 31.2% (14 out of 45 tissues), respectively. The results reveal non-significant differences at ($P>0.05$) between poorly differentiated grade and well differentiated grade, also non-significant difference was noticed between poorly differentiated and moderately differentiated ovarian carcinomas (Table 2).

II. Results of ZEBRA-EBV in Ovarian tumors

The DNA genome of ZEBRA - EBV was detected in tissue blocks from ovarian tumors patients by using

Table 1 : Distribution of malignant ovarian tumors group according to histological types.

Ovarian cancers		Total (N=45)	%	P-value
Types	Endometrium	4	8.89%	$\div^2$ test P=0.004 Sign. (P>0.05)
	Mucinous	9	20%	
	Serous	32	71.11%	

Table 2 : Grading ofovarian cancers group.

Ovarian cancers		Total (N=45)	%	P-value
Grades	I	14	31.2	$\div^2$ test P = 0.843 Non-sign. (P>0.05)
	II	16	35.5	
	III	15	33.3	

digoxigenin-labeled probes. The signals of CISH were detected as bright blue discoloration with blue stain and counter stained with nuclear red solution in referring to ZEBRA-EBV at the sites of complementary sequences. The positive results of *ZEBRA - EBV*-CISH detection in malignant ovarian tumors, where 64.4% showed positive signals. While, in the benign ovarian tumors group was 37.8%, followed by borderline ovarian tumors & the apparently healthy ovarian control tissues were 30% and 7.5% (Table 3 and Fig. 2).

III. Distributions of Positive *ZEBRA I – EBV*DNA - CISH Signal score and Signal Intensity among study groups

The malignant group showed weak signal intensity were (28.9%), moderate in (26.7%) and strong intensity (8.8%). While, in benign group high percentages of ZEBRA-EBV were found in weak intensity (20%) followed by (17.8%) in moderate signal intensity .Whereas, in borderline group high percentages of ZEBRA -EBV were found in moderate intensity (15%) followed by (10%) and (5%) in low signal intensity and strong signal intensity, respectively. Lastly, control group the positive cases for ZEBRA – EBV were higher in moderate score 5% and weak intensity 5% (Fig. 2).

Significant differences ($p<0.05$) were found on comparing the percentage of ZEBRA–EBV DNA in study groups according to signal score and intensity (Table 4).

IV. Assessment of Mutated P57 – CISH

Regarding malignant ovarian tumors group ,the total percentage of positive mutated P57 –CISH detection was 37.8%. While, in the benign and borderline ovarian tumors groups were 53.3% & 50%, respectively (Table 5).

V. Results of P57 - CISH Signal Scoring

A high percentage 22.2% was involving cases with malignant ovarian tumors that have moderate score (score II). While, in benign ovarian tumors group, 26.7% was found to have low score (score I). Wheras ,the high percentage 30% was involving cases with borderline ovarian tumors that have low score (score I) (Figure 3).Statistically, significant differences ($p <0.05$) were found on comparing the results (according to score) among the study groups (Table 6).

VI. Signal Intensity Results of P57 -CISH Testing

Among fourty-five of malignant ovarian tumors, 37.8% (17 out of 45 cases) showed positive reactions to *P57* - CISH test. Moderate signal intensity was found in (20%), followed by strong signal intensity in (13.3%) and

Table 3 : Distribution of *ZEBRA-EBV*DNA signals with ovarian tumors.

ZEBRA EBV		Studied groups				Pearson Chi-Square (P-value)
		A.H.Control	Benigntumor	Border linetumor	Ovarian cancer	
Positive	N	3	17	6	29	P=0.00 Highly sign. (P<0.01)
	%	7.5%	37.8%	30%	64.4%	
Negative	N	37	28	14	16	
	%	92.5%	62.2%	70%	35.6%	
Total	N	40	43	20	45	
	%	100%	100%	100%	100%	
Z test			P=0.222 (NS)	P=0.344 (NS)	P=0.072 (NS)	

Table 4 : Distributions of Positive *ZEBRA–EBV*DNA-CISH Signal score and Signal Intensity among study groups.

Groups	Negative for ZEBRA 1 EBV	Positive according to score			Positive according to intensity			Total positive ZEBRA 1 EBV	Chi-Square tests
		Low	Moderate	High	Weak	Moderate	Strong		
Malignant	16/45 35.6%	12/29 26.7%	11/29 24.4%	6/29 13.3%	13/29 28.9%	12/29 26.7%	4/29 8.8%	29/45 64.4%	According to score- P value = 0.026 -P value <0.05 -Significant According to intensity -P value=0.042 - P value < 0.05 -Significant
Benign	28/45 62.2%	9/17 20%	5/17 11.1%	3/17 6.7%	9/17 20%	8/17 17.8%	0/17 0.00%	17/45 37.8%	
Borderline	14/20 70%	4/6 20%	2/6 10%	0/6 0.00%	2/6 10%	3/61 15%	1/6 5%	6/20 30%	
Control	37/40 92.5%	1/3 2.5%	2/3 5%	0/30 0%	2/3 5%	1/3 2.5%	0/30 0%	3/40 7.5%	
Chi square		chi-square: 7.38, DF: 2, p-value: 0.025, p<0.05, Significant							

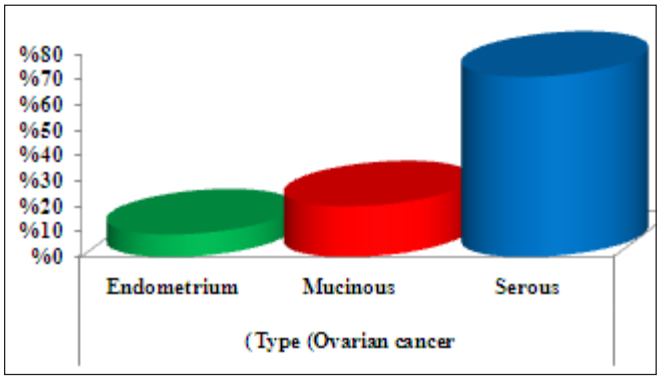


Fig. 1 : Distribution of malignant ovarian tumors group according to histological types.

Table 5 : Distribution of Chromogenic *in situ* hybridization signals for *Mutated P57* DNA among study groups.

P57		Studied groups			Pearson Chi-square (P-value)
		Benign tumor	Border line tumor	Ovarian cancer	
Positive	N	24	10	17	P=0.002 sign. (P>0.05)
	%	53.3%	50%	37.8%	
Negative	N	21	10	28	
	%	46.7%	50%	62.2%	
Total	N	45	20	45	
	%	100%	100%	100%	
Z test		P=0.766 NS	P = 1 NS	P=0.135 NS	

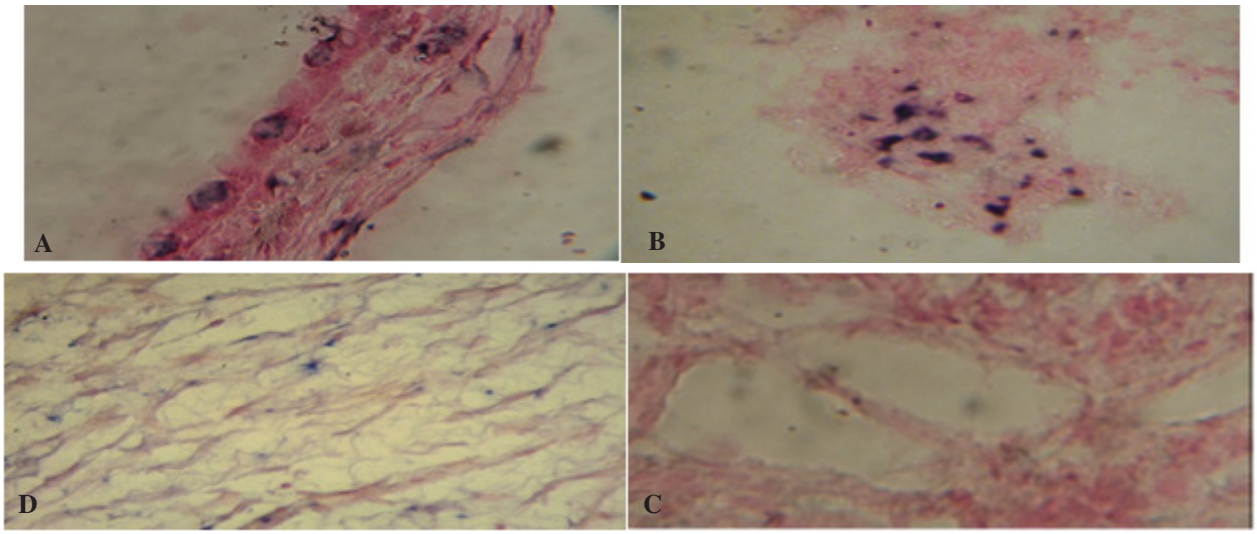


Fig. 2 : Microscopic appearance of *ZEBRA-EBV-CISH* signal in ovarian tumors. Using Digoxigenin-Labeled ZEBRA-EBV Probe; stained with NBT\BCIP (Blue) and Counter stained by Nuclear Red Solution (Red). Blue signal are detected at complementarity sequences sites (arrows).. A) Serous ovarian cancers *ZEBRA -EBV*DNA,low score & moderate signal intensity (X1000).B) Mucinous ovarian cancers *ZEBRA -EBV* DNA, moderate score and strong signal intensity (X1000). C) Benign ovarian tumors *ZEBRA -EBV* DNA, moderate score and moderate signal intensity (X1000). D) Serous ovarian cancers Negative of *ZEBRA-EBV* DNA (X1000).

weak signal intensity in (4.4%). While, the benign ovarian tumor group the moderate signal intensity was found in (31.1) and both weak and strong signal intensity in (6.7%) and (15.6%), respectively. Wheras, in borderline ovarian tumors the highest percentage (20%) of P57-DNA reactions has moderate signal intensity,followed by weak signal intensity in (20%) (Table 7 and Fig. 3).

The statistically analysis shows significant differences at (P<0.05) on comparing the results of these study groups according to their signal intensity.

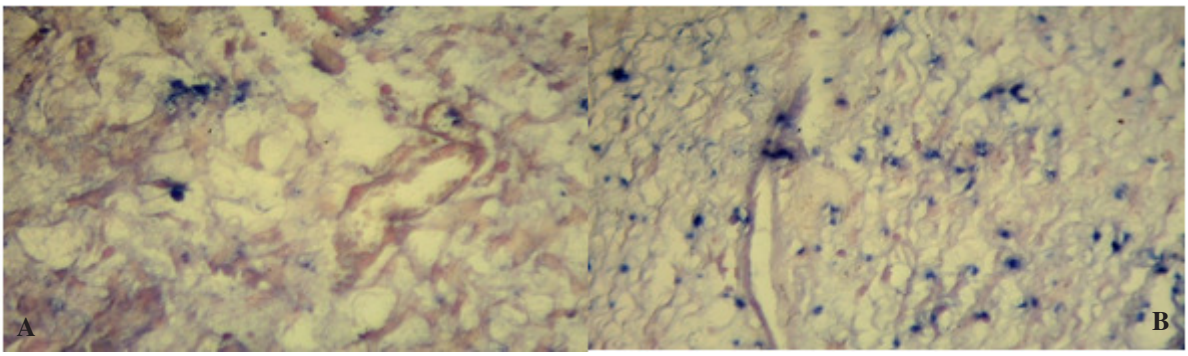
DISCUSSION

In Iraq, ovarian tumors rank the 6th commonest cancer, and it constituted 1.62%, 3.8%, and 4.18% in the years 1976-1978, 1992-1994, and 2001 (Ministry of Health result on Iraqi Cancer Registry1976-1978, 1992-1994 and 2001), while it was 7th commonest cancer and constituted 3.52% according to Iraqi Cancer Board in 2005 (Ministry of Health result on Iraqi Cancer Registry, 2005).

In 2004, in the United States, 50% of all ovarian carcinomas were bilateral. Malignant serous tumors constituted over 40% of invasive epithelial carcinomas. In the present study, was found the most common type the Serous epithelial (71.11%), followed by Mucinous (20%) and Endometrium (8.89 %).

Masafumi *et al* (2018) found that patients, who have invasive serous carcinomas usually acquire more aggressive biological behavior of ovarian carcinoma.

Primary mucinous epithelial ovarian carcinoma (mEOC) is a relatively rare subset of epithelial ovarian cancers. The incidence of mucinous epithelial ovarian cancer is ~12% as exemplified by a recent population



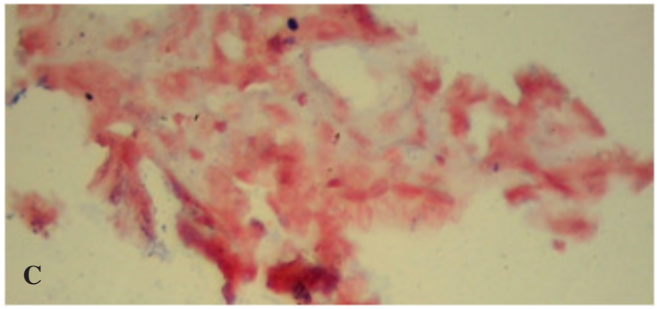


Fig. 3 : Microscopic appearance of mutated p57 - DNA - *CISH* signal in ovarian tumors. Using Digoxigenin-Labeled mutated p57 - DNA Probe; stained with NBT\BCIP (Blue) and Counter stained by Nuclear Red Solution (Red). Blue signal are detected at complementarity sequences sites (arrows). A) Mucinous Ovarian Cancer, mutated p57 - DNA, moderate score & strong intensity(X400). B) Serous Ovarian Cancer, mutated p57 - DNA, high score& strong intensity (X400). C) Benign Ovarian Tumorsmutated p57 - DNAreaction with low score and moderate signal intensity (400X).

Table 6 : Frequency distribution of chromogenic *in situ* hybridization for *Mutated P57* DNA according to signal scoring among study groups.

P57 scoring		Studied groups			Pearson Chi-square (P-value)
		Benign tumor	Border line tumor	Ovarian cancer	
Negative	N	21	10	28	P=0.04 sign. (P>0.05)
	%	46.7%	50%	62.2%	
Low	N	12	6	5	
	%	26.7%	30%	11.1%	
Moderate	N	7	2	10	
	%	15.6%	10%	22.2%	
High	N	5	2	2	
	%	11.1%	10%	4.4%	
Total	N	45	20	45	
	%	100%	100%	100%	
$\div^2$ test		P=0.004 HS	P=0.221 NS	P=0.00 HS	

Table 7 : Frequency distribution of chromogenic *in situ* hybridization for *Mutated P57* DNA according to signal intensity among study groups.

P57 Intensity		Studied groups			Pearson Chi-square (P-value)
		Benign tumor	Border line tumor	Ovarian cancer	
Negative	N	21	10	28	P=0.003 Non sign. (P>0.05)
	%	46.7%	50%	62.2%	
Weak	N	3	4	2	
	%	6.7%	20%	4.4%	
Moderate	N	14	6	9	
	%	31.1%	30%	20%	
Strong	N	7	0	6	
	%	15.6%	0%	13.3%	
Total	N	45	20	45	
	%	100%	100%	100%	
$\div^2$ test		P=0.001 HS	P=0.497 NS	P=0.00 HS	

(Tsz-LunYeung *et al*, 2018).

Endometriosis is a common gynecologic disorder. The estimated frequency among women of reproductive age is 5%–10% and is particularly frequent among women with pelvic pain and infertility. Sampson (1925) was first to describe the malignant transformation of endometriosis to ovarian carcinoma (Michael J*et al* 2013).

The results of present study show that poorly differentiated constituted 35.5% followed by moderately grades 33.3% and lastly, 31.2% for well differentiated .These results gave us an indication the old age women may be more susceptible to get malignancy for several reasons.

Several factors related to these finding, the cell mediated immunity play an important role in the defenses against the cancer. These results are compatible with Stanly report in 2005, who shows the importance of cellular immune responses in the resolution of viruses infection, it is not surprising that deficiencies in cell-mediated immunity increase the likelihood of disease expression (persistence or progression) in groups such as older women (waning immunity), transplant recipients,

patients with HIV and those receiving immune suppressive drugs (Hutt-Fletcher, 2001).

Studies on the prognostic implications of age and ovarian cancer are inconclusive. Chan and his colleague when reported that, the distribution of tumor grade differed between young and old women. They found that in younger women with mean age 40±5.7 years, well, moderately and poorly differentiated carcinoma constituted 11%, 35% and 54% respectively, compared to 4%, 11% and 85% in older patients with a mean age of 61±8.7 (Chan *et al*, 2005). While (Masafumi *et al*, 2018) who found high-grade clinically aggressive neoplasms that are usually diagnosed at an advanced stage. These results consistent with study by Jaffar and Al-Alwany (2018).

During primary infection, EBV initially undergoes a brief replication in the epithelial cells EBV infects human B lymphocytes and epithelial cells via different entry mechanisms. In contrast, the mechanism by which EBV infects human epithelial cells remains unclear (Hisashi *et al*, 2012). ZEBRA plays an indispensable role in driving the lytic cycle of EBV (Ayman El-Guindy *et al*, 2010). EBV-encoded BZLF-1 protein (ZEBRA) downregulates NF-κB and promotes viral lytic growth and host cell apoptosis (Edel Kavanagh, 2011).

Significantly high percentage of ZEBRA detection in ovarian cancer group (64.4%) was observed on comparison to border line, benign ovarian tumors and apparently ovarian control groups. This finding reflects a possible role of the EBV-infection in the carcinogenesis of ovarian malignant tumors group, majority of these patients' tumors are EBV-positive. ZEBRA protein expressed in human T lymphocytes could alter T-cell proliferation and apoptosis during EBV infection (Edel Kavanagh, 2011). Therefore, results of tumor show in this study increased in malignant associated with EBV. These results are consistent to those reported by Lu *et al* (2007), who elevated IgG titers to viral capsid antigen of *EBV*, a marker of a relatively severe (and conceivably, later) initial EBV infection had a 5.3-fold (95% CI 1.5-18.4) increased risk of ovarian cancer.

EBV transformation was successful in all 5 cases (95% confidence interval, 46.3% to 100%). After cryopreservation of EBV-transformed B-cell lines and subsequent thawing, they observed that all cell lines were viable and proliferative (Tachibana *et al*, 2003).

Furthermore, ZEBRA I differ in sequence from ZEBRA II therefor result differ about malignant tumor , it is well documented that the sequences of type I and II genes differ in multiple genes. These sequence variations may directly contribute to subtle differences in the biology of the virus. Indeed have provided strong evidence that the two viral types differ in their ability to spontaneously enter into the lytic cycleMalignant number 52 case give only 27 ZEBRA (Masafumi *et al*, 2018).

Masafumi *et al* (2018), who isolate genomic EBV1&2 DNA(ZEBRA) obtained from all tumor and non-tumor samples was directly used as a template in polymerase chain reactions (PCRs). PCR was used to amplify the fragment from nucleotides –221 to +12 (with respect to the transcription start site) of the BZLF1 promoterIt is well documented that the sequences of type A and B EBVs differ in multiple genes.

ZEBRA plays a fundamental role in disrupting latency and initiating the EBV lytic cascade. Transcriptional activation of the ZEBRA-encoding BZLF1 gene is the primary underlying mechanism by which activators of lytic virus replication. ZEBRA shares homology with the DNA-binding domain of the cellular transcription factor (Hisashi *et al*, 2012). Because the lytic transactivator protein BZLF1 is necessary and sufficient to induce the lytic cycle differences in this protein could help modulate the responsiveness to autoreactivation signals or to other inducers of the lytic cycle (Masafumi *et al*, 2018).

The reason for EBV to exert its oncogenic influences in a particular patient is unknown but is probably associated with co-factors. Again, it is possible that HPV exerts its oncogenic influences in concert with co-factors including a possible collaboration with EBV (Pateras *et al*, 2012).

The fact that we found viral DNA in healthy ovarian specimens could support, to a certain extent, the hypothesis that the virus might play a role in the etiology of ovarian cancer in only a subpopulation of patients. It is logical, on the other hand, to believe that the presence of EBV alone is not sufficient to implement the full carcinogenesis process and that further changes would accumulate over time in a stepwise manner to cause the disease and in turn suggesting a need for further large cohort studies to explore the role of each contributing factors.

Also, the differences of current results, might be related to the geographic variation, the sensitivity of the probe used for CISH, or differences between the subjects studied, yet a deûnitive reason is not apparent.

Abnormal $p57^{kip2}$ function includes its participation in cancer initiation and progression. Since $p57^{kip2}$ was discovered as a CDKI, research revolved around describing its ability to inhibit proliferation hence rendering it a tumor suppressor gene status. $p57^{kip2}$ has also been shown to influence cell differentiation thus

positively inhibiting cancer progression and preventing their maintenance in an undifferentiated state (Adriana *et al*, 2011).

Evading cell death is a significant ability of cancer cells. Conflicting reports have been put forth relating to the apoptotic role of $p57^{kip2}$. Both pro and anti-apoptotic functions have been described depending on cellular context, regulatory tumor cell pathways and tumor microenvironment. However, this evidence is from mouse models with structural differences compared to humans (Besson *et al*, 2008).

In the current research, the rate of detection of positive results of mutated P57 I & II DNA-CISH reactions in the group of malignant ovarian tumors was 37.8% and 24.4%, respectively. While in the benign ovarian tumors was 53.3% of mutated P57 I and 41.9%for mutated p57 II. Lastly, the positive results of mutated P57 I & II DNA-CISH reactions in the group of borderline ovarian tumors was 50% and 40%, respectively. These results are consistent to those reported by Sui *et al* (2002), who identified $p57^{kip2}$ expression in 40.4% of ovarian carcinoma. While, 63.6% in benign ovarian tumors, followed by 52.2% in borderline ovarian tumors. These findings suggest that decreased $p57^{kip2}$ expression may play a pivotal role in the progression of ovarian tumors and provide an important prognostic implication for epithelial ovarian carcinomas.

Up to our best knowledge, this study is the first in Iraq that investigate the mutated p57 I & II in a group of Iraq patients with malignant & benign ovarian lesions. In addition, and on reviewing the available scientific research works in this entity, we found an extreme shortage of the articles in this respect.

However, the present results are in disagreement with a work done by Rosenberg *et al* (2001) was found 65% of ovarian carcinoma in their study have evidence of p57 infection where their expression was based on immunohistochemistry.

Epigenetic control is important in $p57^{kip2}$ regulation frequently via promoter methylation of CpG islands. Transcriptional and translational down-regulation of $p57^{kip2}$ has been demonstrated by Pateras *et al* (2009) in various cancers with decreased expression associated with aggressiveness. Histone modifications of $p57^{kip2}$ repression helps participate in tumorigenesis in methylation dependent and in an independent manner. Several studies have shown that $p57^{kip2}$ gene reactivation after demethylating agent treatment in many cancers demonstrates the epigenetic mechanism of downregulation (Coley *et al*, 2012).

Another causesp57^{KIP2} is generally not mutated in cancer, but its expression is downregulated through epigenetic changes such as DNA methylation and repressive histone marks at the promoter (Edel Kavanagh, 2011).

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