Study of TP53 Gene-Codon 72 Polymorphism in Epithelial Ovarian Cancer (EOC)

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Abstract

Introduction: The tumor-suppressor gene (TP53) encodes p53 protein, the vital protein in the apoptotic cascade pathway which has been presented to be of crucial potential in the progression of cancers. Genetic variation SNP that were revealed to affect the regulatory biochemical functions of p53 are the exon 4 codon 72, (G)Arg/(C)pro polymorphisms. Aim: the present study aims to analyses genetic variation (SNP) of Tp53 gene, codon 72 and p53 protein level in Epithelial Ovarian Cancer (EOC). Methods: the study achieved on 26 EOC cell line specimens and 26 specimens as control group, using PCR-RFLP genetic analysis method and cell lysate p53 level determined by ELISA technique. Results: the percentage of allele polymorphism come as homologous GG variant 85% and 23% in EOC specimens and control respectively and (CC) (8% and 46%) in EOC case and the control in order. Heterologous pattern CG was (34% and 23%) for experimental and control group respectively.

Conclusion: our study proposes that 72 codon polymorphism (GG) is closely related to EOC progressions.

Keywords *: P53 Gene, p53 PCR-RFLP, Polymorphism, Ovarian cancer*

Introduction

Epithelial ovarian cancer (EOC) is one of the highest harmful inherited malignancy disorders and the fifth source of malignance-associated death of women. The global incidence of this disease has been estimated, about 239,000 case per year with 152,000 deaths record, through the year 2012[1]. The possibility of epithelial ovarian cancer development appropriates with age. Women with over 50 year age are more visible to develop ECO condition, even, other age can affected with this malignant cancer. Substantial attempt aims to improve the prognoses guidelines of EOC and detailed the early staging, along with invasive and chemo-therapeutic administrations, have enhanced the short-term progression of epithelial ovarian carcinoma. Modern records for incidence and death rates caused by cancer, indicate that the EOC related death cases have dropped about 14% percentage. The survival proportion for EOC during the last 5 years has been climbed from (36%) to (44%) of total cases of cancer. Among colorectal, women breast cancer conditions, and men prostate cancers conditions, epithelial ovarian cancer is the only cancer companied with significantly dropping of death curve [2]. Epithelial ovarian cancer subdivided into tow type according on the histological transformations and genetic variations, first type with slow-grading tumors combined with mutate form of PTEN , K-RAS and BRAF cell cycle controlling genes. The second type of EOC characterized by mutated TP53 genes and harmful high-grading clinically [3].

Tumor suppressor protein p53 have dominant role in genomic stability and integrity with a conventional defensive role against malignant transformations [4]. RNA Transcription regulatory protein (P53) contributes in control many cellular activates, including the gene

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expression of several genes included in cell cycle and genomic disturbance. When DNA damage is detected p53 protein blocks cell cycle by suspends cell division at point of transition from G1phse to S phase (G1/S checkpoint) and promoting DNA repairing, if genomic damage verified to be irreversible, p53 triggers programed cell death (apoptosis) [5]. P53 protein consists of four structural domains: N,-terminal transcription activating domain, a domain that binding to specific DNA sequence, a domain of tetramerization and transcription regulatory C-terminal region. [6-7]. functional disturbance of the p53 protein is a distinctive hallmark for different types of malignant tumor conditions [8]. P53 switching The pathways-traffic of several activates that determine the future of the cells dividing under extreme endogenous or exogenous conditions and inactivation of the p53 protein signaling cascade pathway is approved in most human cancers [9]. Several recent Studies have verified that different genetic changes and many abnormal environment aspects can be triggering of cancer development, so TP53 gene polymorphism has been consider a study matter due to it is importance as a key tumor suppressor gene [10]. Genetic alterations in the sequence that encoded for p53 which leads to synthesis of inactive or truncated protein, the abnormal production of proteins that interfere TP53 gene expression although, gene amplification that cause increasing in MDM2 level and other proteins contribute in normal metabolism of p53 in the cell, both of it cause a functional disturbance in p53 function and influence malignant tumors development [11].

There are well established links of possibility of cancer progression, onset of cancers and inclusive surviving of tumors with TP53 gene allelic C/G variant at codon 72 (12). Single nucleotide polymorphism in TP53 gene tightly connected with Epithelial ovarian Cancers (EOCs) case, as 97% of High Grad Serous Ovarian Cancer (HGSOCs) case show an polymorphism in TP35 gene sequence [13].

P53 gene codon 72 (Arg/Pro) polymorphism, in fourth exon, were inspected in many forms of human cancer, a guanine base replacement with cytosine base in this site results synthesis a contain an arginine amino acid instead of praline with impaired anticancer activity [14-15]. The pointed genetic variations which including exchange of (CGC) by (CCC), affects the tertiary structure assembly of the trans-activating domain of this protein and its critical activity. both allelic variants (Pro72 and Arg72) of the p53 protein [16]. This introduction com to confirm the prospective concern of this substitution on transcriptional level, triggering of apoptosis events and malignant transformation of affected cells [17]. We carried out a cell line based research to verify the frequency of (rs-1042522- G/C) SNP of goal sequence among woman undergoes EOC.

Material and Methods

Study Subject

The present experiments were achieved on EOC and control tissue culture provided by cell culture unit, collage of medicine, university of Babylon as a gift. A total 26 cell lines of EOC and control specimens was included in the study.

Determination of p53 level

Cell lysate was prepared by frozen at (4˚C) and resolving at (45˚C) for two times, cell lysate level of p53 protein was determined by ELISA according to manufacturing procedure of ELISA kit (Elabsit/ China).

TP35 codon 72 polymorphism (SNP) analysis

Genomic DNA from cell line culture for both EOC and control group were extracted using DNA extraction kit manufacturing procedure of (favorgen/korea). The segment of the TP53 gene covering the codon 72 (G/C) polymorphism (199 bp) was amplified by (PCR) using the following primers pairs : Forward primer , 5'-TTG CCG TCC CAA GCA ATG GAT GA-3' and reverse primer , 5'-TCT GGG AAG GGA CAG AAG ATG AC-3'. PCR achieved by using premix (promega/ Spain) separated in finally volume (20 µl). PCR reaction program carried denaturation at (94˚C/30 sec) and annealing at (55˚C/1 min), followed by extension at (72˚C/1 min),the thre steps repeated for35 cycle [18]. PCR products about (199bp) were processed with restriction enzyme (BstUI) concentration 0.1 µl (10 U/µl), RFLP reaction applied at 60˚C for 18 hour, then electrophoresed for analyzing. C Allele is not cleaved by BstUI producing a single band with size about (199bp). The G allele is cleaved by BstUI producing of double restriction fragments of (113 and 86 bp), heterozygous (CG) produce three bands of (199, 113 and 86 bp) [19].

Statistical Analysis

Statistical analysis out carried by SPSS version 23. as a mean ± SD for P53 level. allele frequency tested by Hardy–Weinberg equilibrium.

Results and Discussions

P53 protein level, cell lysate p53 level was determined by ELISA (figure 1), present study show that (38.4%) of EOC and (11.5%) have an detectable level of p53protein, there are non-significant increasing of p53 protein level mean in ECO lysate in contrasting with control (0.216pg/ml and 0.166pg/ml), respectively.

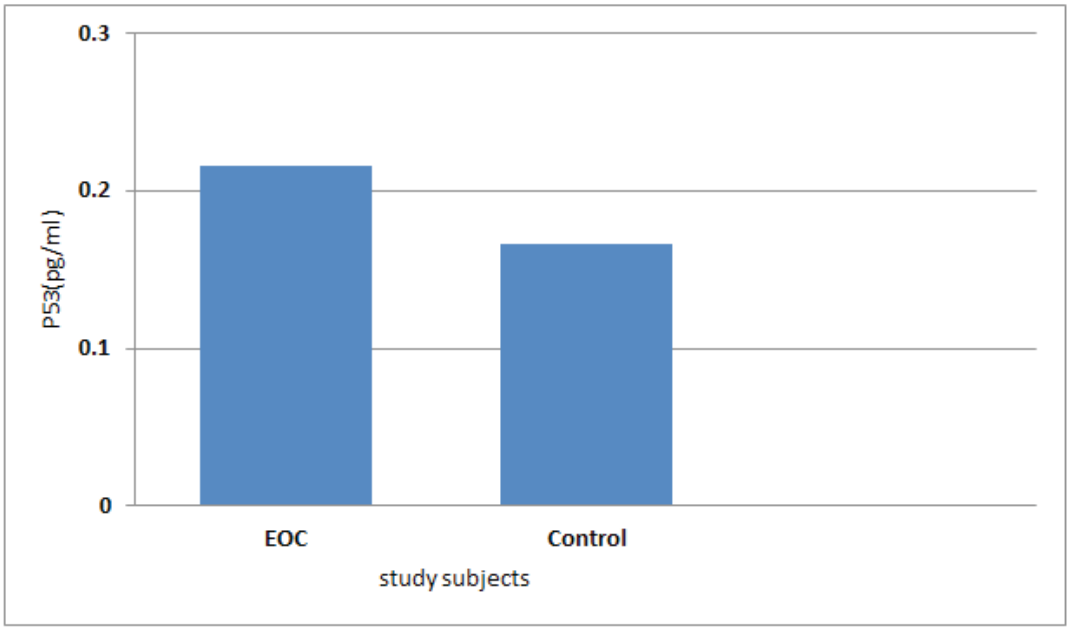

Figure (1) show means of P53 protein concentration of EOC and control specimens

Significant increase of mutant p53, levels in colorectal carcinoma patients compared to the control group [20]. The mean levels of p53 protein of carcinoma of colon were (0.12 ng/ml) [21]. Many experiments show an clear relationships between cytoplasmic p53 concentration and both of triggering of programed cells death and transformation of cancer cells, so this increasing in level considered an powerful clinical of cancers [22]. Extreme rising of cytoplasmic p53 protein ESCC tissue culture about (1.89) fold above that of the normal tissue [23]. Study observations com similar to fondness of Shim et al represented by significantly increasing of p53 in cancer patient specimens [24]. Several excremental analysis based recommendations, suggested that p53 is an important indicator of cancer cells behavior through treatment course [25].

TP53 codon 72 polymorphism, PCR-RFLP genotyping for codon 72 SNP (CG) showed in (figure 2)

The results presented in (table 1), demonstrate an non-significant increasing in Arg alleles patterns (GG) 85% in EOC case in contrast with 23% in control (p-value= 0.09), combined with non-significant decreasing in Pro alleles patterns (CC) in EOC case among the control (8% and 46%) respectively (P-value= 0.069).

Table (1) show genotype patterns of TP35 gene codon 72 polymorphism in EOC and control cases

Genotype	No. (%)		P-value	Phenotype	Mean ± SD of p53 level
	case	control			
GG	15(58%)	6(23%)	0.09	Arg / Arg	0.272 ± 0.072
GC	9(34%)	6(23%)	1	Arg/pro	0.315 ±.096
CC	2(8%)	14(46%)	0.069	Pro/ Pro	0.164 ± 0.042
Total	26	26	------	------	------
LSD value	------	------	------	------	0.899

In aspect of the several researches applied on EOC conditions but there is not recorded instructions about participation of mutated p53 in risk and pathological aspect of EOC[26]. This may be owed to small sizes sampling or mixed population included in study subjects. present study reviles high Arg allele (GG) at TP53 codon 72 in patient of EOC than in control, this result was similar to several studies cured out on TP53 gene codon 72. The percentage of three patterns was homozygote for the Arg allele (33%), homozygote for the Pro allele (17%) and heterozygote Pro/Arg patterns (50%)among women population screened for ovarian cancer [27]. meta-analysis proposes that the TP53 codon 72 CC / CG SNP are not related with an increased risk of breast cancer progression [28]. The percentage of homozygous (Arg=33%), homozygous (Pro=17%), and heterozygous (Arg/Pro=50%) among patients diagnosed for sever ovarian cancer; 62%, 6%, and 32% for health group [29].

These observations administrate that p53 -codon 72 SNPS may attend as peril element for tumors progression and this battle due to distinctiveness of malignant tumor nature, study subjects bulk and ethnic distinction. Our results propose that Arg/Arg pattern is associated with epithelial ovarian cancer progression. The polymorphism in codon 72 of p53 coding sequence is an instance of an risk variation which may have a critical implication in Epithelial ovarian carcinogenesis and stimulus DNA repair in cells and apoptotic cascade events. These outcomes may have a prolific consequence for gene-mediated therapeutic strategy in the treatment of EOC. To certify the link between p53 (codon 72) mutation and EOC, we suggest to achieving an additional cohorts with high volume of international subjects.

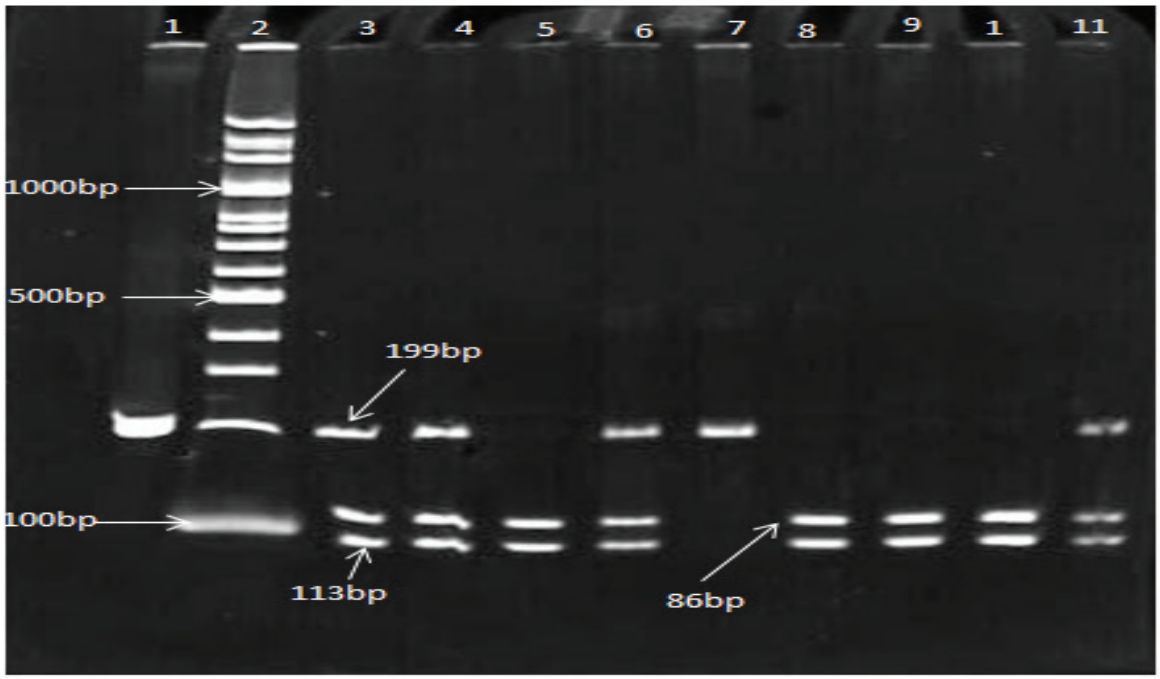

Figure (2) shows gel electrophoresis band pattern of p53 gene allels vesiolized on polyacrylamid gel under UV transumilation, Lin1 undigested product, Lin2 100bp marker, lin(3,4,6,11) CG, lin(5,8.9,10) GG, lin (7) CC pattern.

Conclusions

Epithelial Ovarian Cancer (EOC) is a serious disease that causes mortality in Iraq and worldwide. p53 protein is encoded by the tumor-suppressor gene (TP53) and plays a vital role in progression of EOC. The current study aims to investigate the genetic variation (SNP) of Tp53 gene, codon 72 and p53 protein level in Epithelial Ovarian Cancer (EOC). The obtained results show that the percentage of the homologous allele polymorphism GG variant is 85% and 23% in EOC specimens and control respectively, while (CC) variant gave 8% and 46% in EOC case and the control in order. On the other hand, heterologous pattern CG was 34% and 23% for EOC specimens and control groups, respectively. The findings of this study suggest that the polymorphism in 72 codons (GG) is directly related to EOC progression.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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References

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
2. Goff B. Measuring ovarian cancer care: why are we still failing? Gynecol Oncol 2015;136:1-2.
3. Shih IeM, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol 2004;164:1511-8.
4. 1. Vousden KH, Lane DP. P53 in health and disease. Nat Rev Mol Cell Biol. 2007;8:275–83.
5. Brooks CL, Gu W. New insights into p53 activation. Cell Res 2010;20:614-21.
6. Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. Nat Rev Cancer 2009;9:701-13.
7. Zhou X, Liao JM, Liao WJ, et al. Scission of the p53- MDM2 Loop by Ribosomal Proteins. Genes Cancer 2012;3:298-310.
8. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408:307–10.
9. Siegfried, Nathan A., et al. RNA motif discovery by SHAPE and mutational profiling (SHAPE-

MaP). Nature methods, 2014, 11.9: 959-965.

10. Hossein Pour Feizi MA: RavanbakhshGavgani R, Pourahmad R, Pouladi N, Azarfam P and Montazeri V: Association of p53 Arg/Pro polymorphism at codon 72 with risk of breast cancer in east azerbaijani women. J Babol Univ Med Sci 14: 31-38, 2012.
11. . Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature. 1991; 351: 453–6.
12. Tommiska J, Eerola H, Heinonen M, Salonen L, Kaare M, Tallila J, Ristimaki A, von Smitten K, Aittomaki K, Heikkila P, Blomqvist C, Nevanlinna H. Breast cancer patients with p53 Pro72 homozygous genotype have a poorer survival. Clin Cancer Res. 2005; 11: 5098–103.
13. ZHANG, Yu, et al. TP53 mutations in epithelial ovarian cancer. Translational cancer research, 2016, 5.6: 650.
14. Lin HY, Huang CH, Wu WJ, Chang LC and Lung FW: TP53 codon 72 gene polymorphism paradox in associated with various carcinoma incidences, invasiveness and chemotherapy responses. Int J Biomed Sci 2008,4: 248-254.
15. Kalemi TG, Lambropoulos AF, Gueorguiev M, Chrisafi S, Papazisis KT and Kotsis A: The association of p53 mutations and p53 codon 72, Her 2 codon 655 and MTHFR C677T polymorphisms with breast cancer in Northern Greece. Cancer Lett 2005, 222: 57-65.
16. Buchman VL, Chumakov PM, Ninkina NN, Samarina OP, Georgiev GP. A variation in the structure of the protein-coding region of the human p53 gene. 1988, 70: 245-252.
17. Thomas M, Kalita A, Labrecque S, Pim D, Banks L, et al. wo polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol. 1999, 19: 1092-1100.
18. Matei MC, Negură L, Liliac L, Negură A and Azoicăi D: Validation of PCR-RFLP techniques for the evaluation of codon 72 of p53 and CYP1A1 gene's polymorphisms in relation with ovarian cancer in a Romanian population. Rom J Morphol Embryol 53: 47-54, 2012
19. HEDAYATIZADEH-OMRAN, Akbar, et al. Association of P53 gene polymorphism with gastric cancer in Northern Iran as a high-risk region. Biomedical reports, 2018, 8.5: 433-438.
20. ABDEL-AZIZ, Mohamed M., et al. Mutant p53 protein in the serum of patients with colorectal cancer: Correlation with the level of carcinoembryonic antigen and serum epidermal growth factor receptor. Cancer Detection and Prevention, 2009, 32.4: 329-335.
21. LUO, Jiin-Chyuan, et al. Levels of p53 antigen in the plasma of patients with adenomas and carcinomas of the colon. Cancer letters, 1995, 91.2: 235-240.
22. HUANG, Kate, et al. Elevated p53 expression levels correlate with tumor progression and poor prognosis in patients exhibiting esophageal squamous cell carcinoma. Oncology letters, 2014, 8.4: 1441-1446.
23. CHAVA, S., et al. Immunohistochemical evaluation of p53, FHIT, and IGF2 gene expression in esophageal cancer. Diseases of the Esophagus, 2012, 25.1: 81-87.
24. Shim K-S, Kim K-H, Park P-W, Lee S-Y, Choi J-H, Han W-S, et al. Increased serum levels of mutant p53 proteins in patients with colorectal cancer. J Korean Med Sci 1998;13:44–8.
25. Broll R, Duchrow M, Oevermann E, Wellm C, Schwandner O, Schimmelpenning H, et al. p53 autoantibodies in sera of patients with a colorectal cancer and their association to p53 protein concentration and p53 immunohistochemistry in tumor tissue. Int J Colorectal Dis 2001;16(1):22–7.
26. Huang SP, Wu WJ, Chang WS, et al. p53 Codon 72 and p21 codon 31 polymorphisms in prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13:2217–24.
27. DHOLARIYA, S., et al. TP53 gene polymorphism in epithelial ovarian carcinoma patients from North Indian population and its Pro/Pro variant is potentially contributing to cancer susceptibility. J Genet Syndr Gene Ther, 2013, 4.5: 1-6.
28. HOU, Jing, et al. p53 codon 72 polymorphism and breast cancer risk: A meta-analysis. Experimental and therapeutic medicine, 2013, 5.5: 1397-1402.
29. DHOLARIYA, S., et al. TP53 gene polymorphism in epithelial ovarian carcinoma patients from North Indian population and its Pro/Pro variant is potentially contributing to cancer susceptibility. J Genet Syndr Gene Ther, 2013, 4.5: 1-6.