RESEARCH ARTICLE

No Evaluation of Serum P53 Levels in Iraqi Female Breast Cancer Patients

Ferdous A Jabir[1], Wisam H Hoidy[2]*

Abstract

Breast cancer is the most common cancer diagnosed and the second leading cause of cancer death among Iraqi women. The population was exposed to high levels of depleted uranium following the first and second Gulf Wars and this might be a risk factor. Protein 53 (p53) or Tumor protein 53 (Tp53) was originally defined as an oncogenic protein. The aim of the study was to evaluate P-53 serum concentrations in fifty Iraqi female breast cancer patients and twenty five healthy volunteers using the ELISA technique. All these patients attended the Teaching Hospital of AL Diwaniyah during the period between June 2016 to March 2017. The mean values for TP53 concentration in patients with breast cancer and apparently healthy groups were 47+33.5 U/ml and 27. 8+12.7 U/ml, respectively. The results showed no significantly difference , in contrast to most studies conducted elsewhere in the world.

Keywords: P53- breast cancer- Iraqi women- ELISA

Introduction

Breast cancer is the most common cancer diagnosed and the second leading cause of cancer death among United State (US) women (Robert et al., 2007). Also breast carcinoma is the most common malignant tumor in Iraqi women (Iraqi Cancer Board., 2010). Cancer is caused in all or almost all instances by mutation or by some other abnormal activation of cellular genes that control cell growth and cell mitosis. The abnormal genes are called oncogenes. As many as 100 different oncogenes have been discovered (Mehta, 2002). The p53 gene is a tumor suppressor gene and the most frequent site of genetic alterations found in human cancers (Hollstein., 1991). Guardian of the genome","Death star" " Good and bad cop", are just a few of the names that have been attributed to the p53 gene (Dena et al., 2009).

The p53 gene is localized on the short arm of chromosome 17 and encodes a 393-amino acid phosphoprotien, which is present at very low levels in normal cells. This molecule appears to play a major role in the maintenance of genomic integrity (Lane., 1992). There are two types of p53 protein: normal or wild type and mutant type. The wild type is located in the nucleus, and it functions primarily by controlling the transcription of several other genes. It has a short half life of only 20 minutes (Mehta et al., 2002).

p53 is the most commonly mutated gene in human cancers and more than 50% of human cancers contain p53 mutations.(Philomena., 2001). Induction of growth arrest or cell death upon activation of p53 prevents the replication of damaged DNA and the division of genetically altered cells. Therefore, p53 is thought to play an important role in maintaining the integrity of the genome (Lane., 1992). This activity is central to its role as a tumor suppressor and is thought to determine the response of tumor cells to anti-cancer drugs that trigger apoptosis by inducing DNA damage. Indeed, inactivation of p53 due to deletion, mutations or to the interaction with cellular and viral proteins is recognized as the key step in the development of half of all human cancer (Levrero et al., 2000). P53 gene is not reactive in cells where DNA is undamaged, when there is DNA damage, the gene suspends the cell cycle until the damage can be repaired. If there is a mutation in P53, the cell cycle continues unrestrained and reproduces the damaged DNA, leading to uncontrolled cell proliferation and cancer tumors. Cancer results as the cell with damaged DNA divides, the damaged DNA is replicated and each daughter cell's cycle is also unrestrained (Constantinos et al., 2014). All cancer cells contain mutations in combinations of tumor suppressors and oncogenes. The removal of functional P53, from a cell allows for the accumulation of even more DNA damage and the division of cells that contain damaged DNA (Arindam et al., 2016). The mutation of P53 is one of the most frequent genetic changes seen in cancer cells. In addition to mutations that arise during the growth and development of individuals (sporadic mutations), there are forms of cancer associated with the inheritance of a damaged version of P53. In addition, several viruses have evolved ways of inactivating the P53 protein (Noa et al., 2011) Lu.et al, elaborated the DNA damage checkpoint

[1]College of Medicine, [2]Chemistry Department, College of Education, University of Al-Qadisiyah, Al-Qadisiyah, Iraq. *For Correspondence: wisam.hoidy@qu.edu.iq

and P53 signaling pathways in human tumorigenesis (Lu et al., 2008). Lynette et al., (2007) revealed aging associated truncated from of P53 which interacts with wild-type P53 and alt rs p53 stability, localization, and activity. The anti-P53 antibody (P53-Ab) assay is based on the initial results of Crawford et al, who detected P53-Abs in the sera of patients with BC. Because mutation in the P53 gene and the consequent over expression of P53 are associated with tumor tissues, both wild-type and mutant P53 may act as targets of tumor specific humeral and cellular immune responses(Liu et al., 2006)

The aim of this study is instead of was to evaluate P53 levels in patients with malignant breast tumors compare with healthy women.

Materials and Methods

A total of 50 women with breast cancer (mean age 47.3 years, ranged between 28-69 years) were included in this study. They were admitted at Al-Diwaniyah teaching hospital, compared with 25 women as control, blood samples were collected from two studies groups. This study was conducted during the period from September 2016 to April 2017. ELISA Technique (Abcam P53 human ELISA kit ab46067) was applied for estimation of P-53 levels as well as apparently healthy.

Statistical analysis

Statistical analysis was performed using SPSS 18, student test (t-test) was used for the quantitative data. The lowest level of significance was when the probability ($p<0.05$).

Results

Initially it was Plot the standard curve on graph paper, with standard concentration on the x-axis and absorbance on the y-axis , as shown in Figure 1 and Table 1.

Then it was calculated the mean absorbance for

Table 1. Standard Dilution Preparation

Standard	Volume to Dilute (μL)	Diluent (μL)	Total Volume (μL)	Starting Conc. (U/mL)	Final Conc. (U/mL)
1	-	-	-	100	100
2	250	250	500	100	50
3	250	250	500	50	25
4	250	250	500	25	12.5
5	250	250	500	12.5	6.25
6	250	250	500	6.25	3.12

Table 2. Mean and Standard Deviation of Serum p53 (U/ml) among the Two Studied Groups

Studied groups	N	Mean	Std. Deviation	Comparison of Significant	
				P-value	Sig
Patients	50	47.0026	33.46758		
Control	25	27.8757	12.68566	0.294	N S
Total	75				

each set of duplicate controls and samples (patients and healthy), and subtract the average zero standard optical density. after that it was drawn the best-fit straight line through the standard points. The concentration values of patients and healthy samples were extracted based on the above diagram.

Level of TP53 among the sera of the studied groups
Level of TP53 has been measured in U/ml using ELISA technique for its estimation among the sera of the studied groups. The result is listed in Table 2.

The patients' age range was 22-84 years (mean 51.29) most of them were in the fourth decade. twenty one patients (42%) were premenopausal and twenty nine (58%) were postmenopausal.

Some of the clinical features and the demographical picture of patients in comparison with controls have been listed in Table 3.

Discussion

Generally, this study showed the demographical features which indicated that the mean of age of the majority of patients were within or above the menopause duration (51.29 ± 12.18 years) with highly significant difference in comparison with control (29.42 ± 10.21 years), this result is comparable to some extent with that of Baghdad previous study (50.9+11.8) (Haider., 2010).

The mean of TP53 of malignant cases was 47 ± 33.5 U/ml in comparison with 27.9 ± 12.7 U/ml for healthy control. By calculating the p value it was found equal 0.294 this value is much higher compared to the p value 0.05,so there is no significant different between malignant breast tumors and healthy , These results differ from those obtained in previous studies (Haider, 2010), The reason for no significant differences may be due to that P53 is tumor suppression gene and it will arrest the cell cycle if it goes wrong. suppression may be due to other genes

Table 3. The Characteristics of Breast Cancer Cases

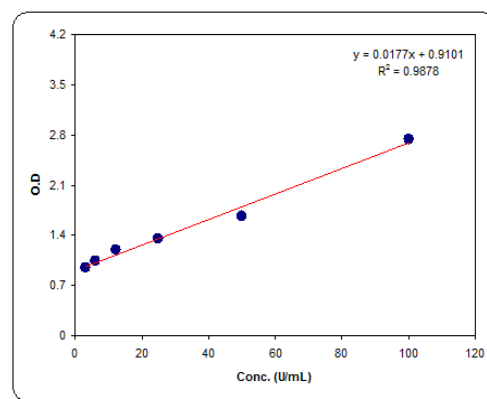| Characteristics	Patients	Control
Age mean (years)	51.29±12.18	29.42±10.21
TP53 (U/ml)	47±33.5	27.9±12.7

Figure 1. The Relationship between Concentration of Standard and Absorption

such as PTEN, P16, P63, P73 and others. Therefore, the suppression can be due to other genes, so the concentration of P53 is not increased. According to IHC research of p53 in human prostatic carcinoma, this protein is highly elevated in cancerous prostatic glands, but not in other surrounding-healthy tissue. Because we did not evaluate the mutational status of the p53, we are not sure of whether this p53 is elevated because it is not functional and thus feed back loop for expression is over stimulated, or it is functional and overexpressed due to its normal function say to kill cancerous cells in apoptosis.

According to this study, it was clear that the TP53 concentration no increased with disease duration which may be due to no increase the accumulation of TP53 due to no increase its half-life when mutation of TP53 gene occur, but there is no significant difference between duration periods which may belong to the small sample size of each period. These results differ from the results of most studies conducted elsewhere in the world (Haider, 2010; Ahmed et al., 2010; Ahmed et al., 2011).

In conclusion, this study showed non statically significant difference (p=0.294) between status of P53 concentration of patients with breast cancer compared with healthy women. the observation that this result is different from the results of many studies inside and outside Iraq noted that there are significant differences (P<0.05) between patients and healthy, where the concentration of p53 was high in patients compared to the concentration of p53 in the healthy but in this study found that concentrations of patients and healthy were found to be close.

References

Ahmed AA, Mosadak I, Batool H, et al (2011). Detection of serum anti-p53 antibodies in breast cancer patients. *Al- Mustansiriya J Sci*, **22**, 7-12.

Ahmed A, Nidhal AM, Batool H (2010). Serum level of p53 antibody and tissue expression of p53 in breast cancer. *IRAQI J Sci*, **51**, 214- 19.

Arindam D, Abhishikta GR, Abhijit C (2016). P53ness in human cancers: An overview. *AJCS*, **2**, 15-24.

Constantinos G, Howard B (2014). DNA damage response genesand the development of cancer metastasis. *Radiat Res*, **181**,111–30.

Dena A, Bedoor A (2009). P53 expression in colonic carcinoma –immunohistochemical study. *Ann Coll Med*, **35**, 111-16.

Haider AH (2010). Level of total tumor protein 53 in the sera of Iraqi breast cancer patients. *IRAQI J Sci*, **51**, 410-14.

Hollstein M, Sidransky D, Vogelstein CC (1991). p53 mutations in human cancers. *Science*, **253**, 49-53.

Iraqi cancer board (2010). Results of Iraqi cancer registry 2004-2005. Ministry of health. Baghdad-Iraq

Lane DP (1992). P53, guardian of the genome. *Nature*, **358**, 15-26.

Levrero M, De Laurenzi V, Costanzo1 A, et al (2000). The p53/p63/p73 family of transcription factors: overlapping and distinct functions. *J Cell Sci*, **113**, 1661-70.

Liu XF, Zhang H, Zhu SG, et al (2006). Correlation of p53 gene mutation and expression of p53 protein in cholangiocarcinoma. *World J Gastrol*, **12**, 4706-9.

Lu X, Nguyen T, Zhang X, et al (2008). The Wip1 phosphatase and Mdm2: Cracking the "Wip" on p53 stability. *Cell Cycle*, **7**, 164-168.

Lynette M, Xiongbin L, Nader G, et al (2007). Donehower. aging-associated truncated form of p53 interacts with wild-type p53 and alters p53 stability, localization, and activity. *Mech Ageing Dev*, **128**, 11-2.

Mehta KU, Goldfarb MA, Zinterhofer LJ (2002) Absent p53 protein in colorectal tumors reflects poor survival. *J Appl Res*, **2**, 1-15.

Noa R, Ran B, Moshe O, et al (2011). Varda R. mutations in the p53 tumor suppressor gene important milestones at the various steps of tumorigenesis. *Genes Cancer*, **2**, 466-72.

Philomena G (2011). P53 how crucial is its role in cancer. *Int J Curr Pharm Res*, **3**, 19-25.

Robert A, Vilma C, Harmon J (2007). Cancer screening in the United States: Review of current guidelines, practices and prospects. *CA Cancer J Clin*, **57**, 90-104.