WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 12, 125-132. Research Article ISSN 2277–7105

EXPRESSION OF TUMOR SUPPRESSOR GENE P53 CORRELATION WITH GASTRIC ADENOCARCINOMA PATIENTS BY USING IMMUNOHISTOCHEMICAL ASSAY

Noor Al-Huda Ali A. H. Saeed*, Luma Qasim Ali, Areej Abbas Zabbon and Zaid A Saeed

Article Received on
30 April 2018,
Revised on 20 May 2018,
Accepted on 10 June 2018
DOI: 10.20959/wjpr201812-12638

***Corresponding Author**
Noor Al-Huda Ali A. H. Saeed

ABSTRACT

Over expression of **P_{53}**, tumor suppressor gene and alternation may involved in many types of human cancers, one of the most important kind is gastric cancer. In the current study, we shed the light on the relation between **P_{53}** and some aspects of gastric carcinoma patients by using immunohistochemical assay by taking 43 biopsies randomly from patients already have gastrectomy, samples were collected during the period from January 2015 until January 2016, including 26 patients above 50 years old and 17 under that age, male: female ratio 2: 1 with 30 men and 13 women. Histological grade including 21 well differentiated, 13 moderately and 9 poor differentiated types. According to TNM staging, 26 patients out of 43 falling in stages I and II, and the rest 17 patients were falling in stages III and IV. Whereas, the current study involved 27 smoker patients and 16 non-smokers. Here and by using immunohistochemical assay,**P_{53}** were detected in 28 out of 43 patients (65%), in the other hand, the positive results related in highly significant association to each of age, gender, histological grade, stage of the tumor and tobacco smoking of gastric carcinoma patients.

KEYWORD: gastric cancer, adenocarcinoma, p53, tumor suppressor gene, immunohistochemistry.

INTRODUCTION

Worldwide, the second most occurring cancer is gastric carcinoma.[1] It is causes more than 800,000 deaths per year[2], 90% of stomach tumors are malignant, 95% of it were gastric carcinomas[3], and less than 20% of patients with stomach cancer have been 5-years

survival.[4] It is rare before 20 years old[5], recently, the incidence and death of gastric cancer has been a significant decrease in most of developing countries, because of some traditional therapies act an important role for treating many stages and grades for gastric cancer.[6] Smoking, is the most common risk factors that increase incidence of gastric cancer, depends on number of cigarettes as well as the duration of smoking.[7] Variety of abnormalities and molecular events may implicated with gastric cancer, but the most common one is tumor suppressor gene **P_{53}**, were identified with several kinds of cancer, also, it is mutated about 68% in gastric cancer.[8] Any detect or mutation of **P_{53}** suppressor gene, make a damage in replication of DNA and induce malignant transformation and growing[9], these mutations in tumor suppressor gene **P_{53}** may lead to inactivation of biological properties of **P_{53}** protein[10], it is thought that any alteration of this gene can play a major role in multistep carcinogenesis.[11] The mutation was located on chromosome 17p 13, so that detected in primary gastric cancer of human.[12, 13] Inactivity of tumor suppressor **P_{53}** during developing of tumor is a multistep process of its genetic abnormalities accumulation.[11] **P_{53}** mutation can be loss allele or vice versa[14] by both ways the function of **P_{53}** is partially inactivation while the only one allele of **P_{53}** gene is affective, in activation of both allele of**P_{53}**, gene always occurs by mutation in one allele and lose of other one, finally results of complete loss of function.[15]

MATERIALS AND METHODS

Forty-three biopsies of Iraqi gastric cancer patients were taken randomly from Baghdad medical city, whom already have been surgical operation of gastrectomy between the period of time from January 2015 to January 2016, all of them have not received any anti-cancerous therapies before surgery, the gender ratio of male: female was 2 : 1, 30 men and 13 women, age ranged 30-70 years, with mean age 59 years, the biopsies including 21 well differentiated, 13 moderately and 9 poorly differentiated types, 26 of them were falling in stages I and II. And the other 17 specimens were falling in stages III and IV of tumor.

Tumor suppressor gene **P_{53}** was determined on these biopsies by using immunohistochemical method which was recommended in leaflet with kit as the detection system used for **P_{53}** protein universal Dako cytomation labeled streptavidin Biotin 2 system, horseradish peroxidase (LSAB-2 system HRP), detection system ready to use code no. ko673 (CA. USA), ready to use N-series primary antibody monoclonal mouse anti human**P_{53}** protein clone:

DO7. code N 1581. Dako-Denmark. Immunohistochemical procedure based on cutting sections of paraffin embedded blocks into 4 µm thickness, then deparaffinized and dehydrated all of these samples and dewaxing by immersed on xylene, (100, 90, 70%) ethanol, D.W, and put it on endogenous peroxidase block for 25 min, adding $\mathbf{P_{53}}$ primary antibody in each section for 90 min, washing by PBS, adding secondary antibody and incubating for 1 hr., put it on humid chambers, adding streptavidin 30 min, then counterstained with hematoxylin, and finally, dehydrating by immersing on serial 70, 90, 100% ethanol, xylene.

Statistical Analysis

SAS statistical analysis system[16] is using to effect different factors of study parameters. Chi square test at the comparation between percentage of this study.

RESULTS

Forty-three specimens of gastric cancer were studied immunohistochemically by using $\mathbf{P_{53}}$ protein monoclonal antibody. Our studied patients mean age 59 years, ranged between (30-70) years, 26 patients aged over 50 years, and the other 17 patients were falling under 50 years, male: female ratio was 2 : 1, with 30 men and 13 women, and the smokers: non-smokers ratio was also 2 : 1, with 27 smokers and 16 non-smokers patients. The histological grade involved 21 well differentiated, 13 moderately differentiated, and 9 poorly differentiated tumors. The dominant specimens (26 out of 43) were falling in the stages I and II, and 17 patients were falling on stages III and IV. As shown in figure (1),$\mathbf{P_{53}}$ tumor suppressor gene expression were immunohistochemical detected on 28 out 43(65.1%) gastric carcinoma patients, according to their general aspects, and as shown in figure (2), 21(70%) out of 26 male were positive relation with $\mathbf{P_{53}}$, whereas, 7(53.8%) women out of 13 were positivity, on the other hand and as shown in figure (3), 16(61.5%) out of 28 were aging above 50 years, and 12(70%) out of 17 under 50 years were showing positive $\mathbf{P_{53}}$ expression. Figure (4) shows that 18(69.2%) patients in stages I and II, were positive $\mathbf{P_{53}}$, whereas, the rest 10(58.8%) patients with stages III and IV have positive results. As we can see in figure (5), 11(52.3%) well differentiated samples, 9(69.2%) moderate and 8 (88.8%)out of 28 were poor differentiated types have $\mathbf{P_{53}}$ positive expression,in figure (6)17 (62.9%) tobacco smoking and 11(68.7%) non-smoking patients have been shown positive $\mathbf{P_{53}}$ relation. As shown in table (1) and regarding to all aspect above, age, gender, histological grade, stage of tumor and smoking habit, there were highly significant association with age at ($p = 0.0271$),

and stage (p = 0.041) at (p ≤ 0.05), while there were highly significant association with gender (p = 0.0128), grade (p = 0.048), and smoking (p = 0.0133) respectively at (p ≤ 0.01).

Table (1): Distribution of Iraqi patients with gastric cancer according to their age, gender, tumor stage, histological grade and tobacco smoking correlation with P_{53} (IHC).

Factor		P_{53} (IHC) +ve = 28 (65%)	P_{53} (IHC) -ve = 15 (34.8%)	P vale x^2 test
Age	≤50	16 (57%)	10 (66.6%)	P = 0.0271
	>50	12 (42.8%)	5 (33.3%)	x^2 = 4.724*
Gender	Male	21 (75%)	9 (60%)	P = 0.0128
	Female	7 (25%)	6 (40%)	x^2 = 9.844**
Stage	I, II	18 (64%)	8 (53.3%)	P = 0.041
	III, IV	10 (35.7%)	7 (46.6%)	x^2 = 4.268*
Grade	Well	11 (39.2%)	10 (66.6%)	P = 0.0048
	Moderate	9 (32.1%)	4 (26.6%)	x^2 = 11.509**
	Poor	8 (28.5%)	1 (6.6%)	
Smokers		17 (60.7%)	10 (66.6%)	P = 0.0133
Non-smokers		11 (39.2%)	5 (33.3%)	x^2 = 9.261**

*P≤ **0.05** ; **P≤ **0.01.**

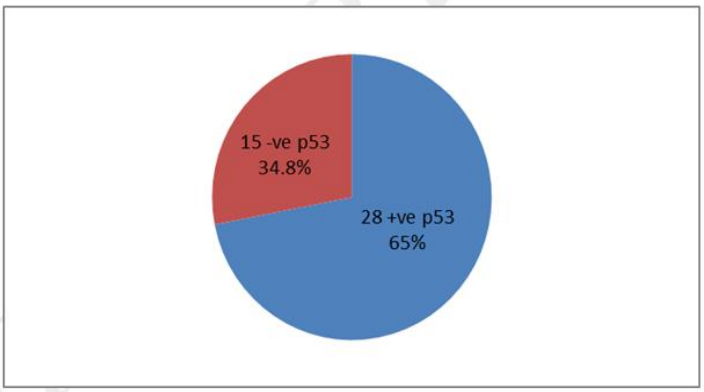

Figure 1: Distribution of gastric cancer patients in relation with p53.

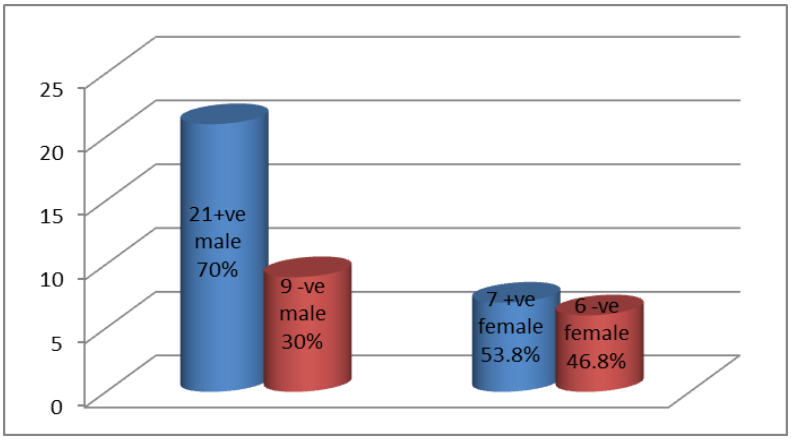

Figure 2: Distribution of p53 according to the gender of gastric patients.

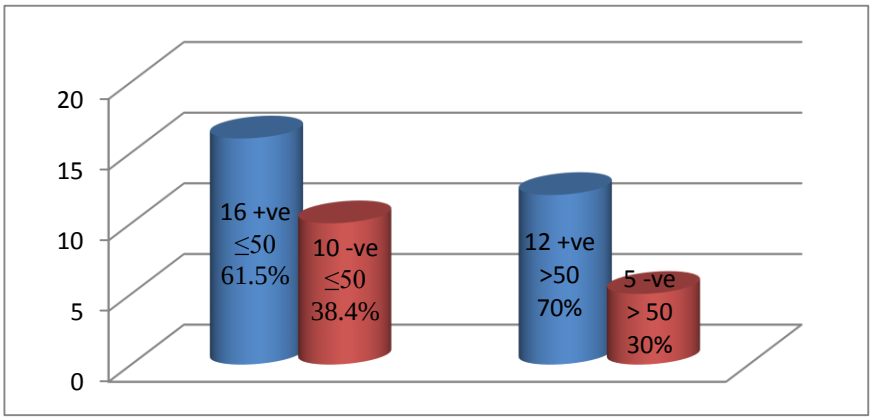

Figure 3: Distribution of p53 according to the age of gastric patients cancer.

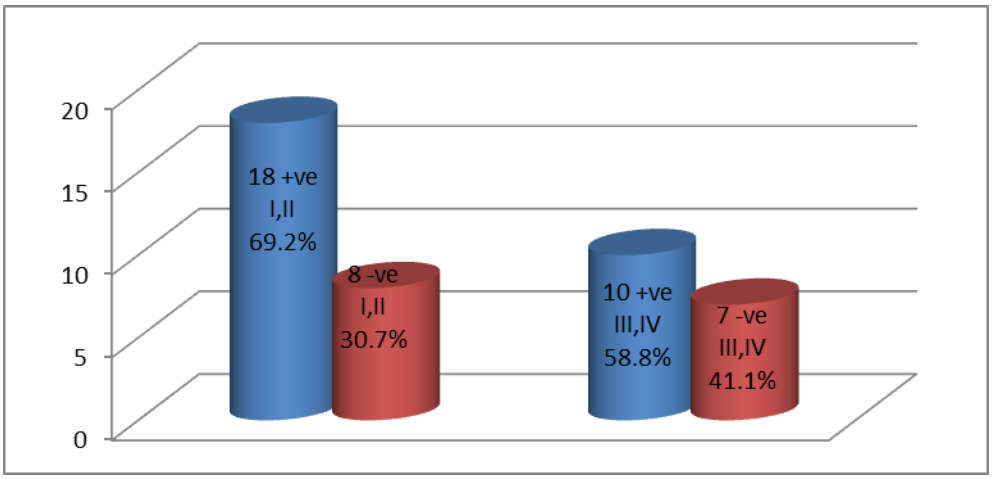

Figure 4: Distribution of p53 according to the tumor stage of gastric patients.

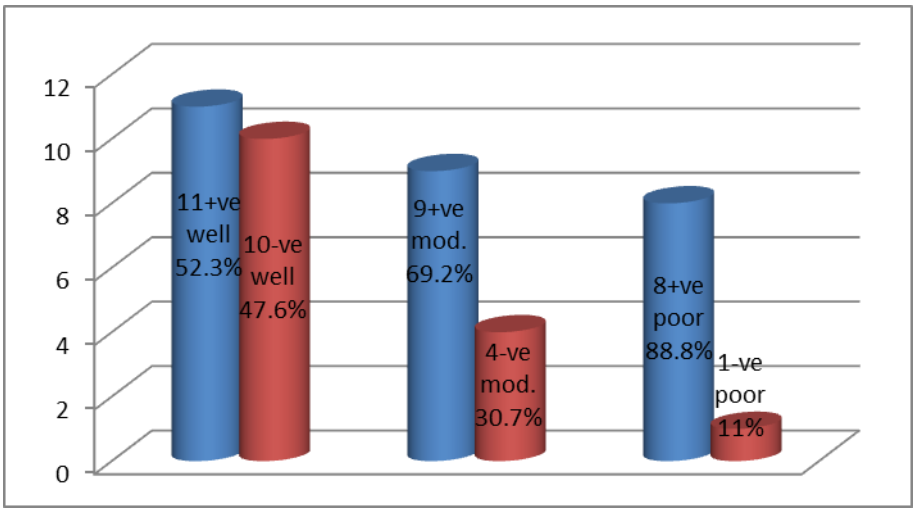

Figure 5: Distribution of p53 according to the tumor grade of gastric cancer patients

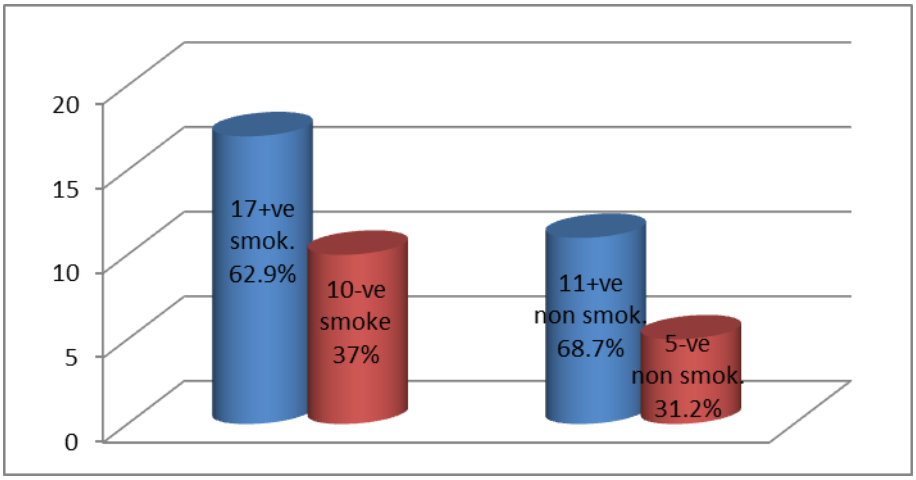

Figure 6: Distribution of p53 according to the smoking gastric cancer patients

DISCUSSION

Mutation of **P_{53}** acts as a dominant oncogenic agent, its thought to be that **P_{53}** overexpression is generally refers to pattern of mutation **P_{53}**, so, it was varying with gastric carcinoma geographical origin, depends on different etiologic agents.[17] Overexpression of **P_{53}** protein previously detected in 26-65% of stomach tumors.[18] In this research, **P_{53}** tumor suppressor gene were detected in 28 out of 43 cases (65%) of all gastric cancer biopsies. It is higher than study of[19] who reported (36.9%) of positive overexpression **P_{53}** protein in gastric cancer cases, and compatible with[20] who found that 66% of gastric carcinoma patients were expressed positive **P_{53}** reaction. The patients predominant age over 50 years in this study, it was approach to[20] who reported that the peak and increase occurrence in seventh decade of life and agree with[22] which their patients age actually over 50 years of old , male : female ratio in our study was 2 : 1, it is similar to[23] who recorded the same ratio between the gender, well differentiated carcinoma acupide the major type of cases, this was disagreed with[24] who reported that major cases comes from moderately differentiation, and falling in stage III of the tumor, in contrast with our study that the most cases were falling in stages I and II.

Regarding to the patients age, gender, grade, stage and tobacco smoking, statistically there were highly significant association with **P_{53}** protein, it is consisting with[25] who reported significant correlation between**P_{53}** over expression and tumor grade, but disagree with[19] and[26] who found there were no significant association between **P_{53}** expression and age, gender, histological stage and grade, another researcher recorded highly incidence of **P_{53}** with gastric cancer.[27]

We can conclude from this study that **P_{53}** high level and highly significant association with gastric carcinoma patients and their general aspect and its good prognostic of clinical evaluation of patients with different part of gastric cancer.

REFERENCES

1. Bozzetti F., Marubini E., Bonfanti G., etal. (1991): Subtotal versus total gastrectomy for gastric cancer: Five years survival rates in a multicenter randomized Italian trrol. Italian Gastrointestestinal tumor study group. Ann Surg, 230: 170-178.
2. World health organization (2009).
3. Schwartz G. (1996): Invasion and metastasis in gastric cancer: invitro and invivo models with clinical consideration Semin Oncol, 23: 316-324.
4. Green D., Ponce de Leon S., Leon-Rodriguez E., etal. (2002): Adeno carcinoma of the stomach univariate and multivariate analysis of factors associated with survival. AmjOncol, 25: 84-89.
5. Gore R. (1997): Gastrointestinal cancer RadiolCLIN North Am, 35: 295-310.
6. Jemal A., Siegel R., Ward E., etal. (2007): Cancer statistic, CA cancer J CLIN, 57(i): 43-66.
7. Bertuccio P., Chatena S., Levi F., etal. (2009): Recent patterns in gastric cancer: A global overview Int. J. of cancer, 125(3): 666-673.
8. Fenoglio CM, Wang J. Stemmermann GN, Offsinger AN (2003): TP_{53} and gastric carcinoma: A review. Human mutation, 21: 258-270.
9. Kastan MB, Ouyekwere O., Sindransky D., Vogelstein B., Craig RW. (1991): Participation ofP_{53} protein in the cellular response to DNA damage. Cancer Res, 51: 6304-6311.
10. Balogh GA., Maili DM., Corte MM., Ron Corac P., and Nardi H. (2006): MutationP_{53} protein is serum could be used as a molecular marker in human breast cancer. Intern. J. Oncol, 28: 695-2.
11. Hollstein M., Sidransky D., Vogelstein B., and Harris C. (1991):P_{53} mutation in human cancer. Science, 49: 253.
12. Levine AJ., Momand J., and Finaly CA. (1991): TheP_{53} tumor suppressor gene. Nature. London, 35:453-456.
13. Tamura G., Kihan T., Nomura K., Terada M., Sugimura T., and Hiroyoshi S., (1996): Detection of frequentP_{53} gene mutations in primary gastric cancer by cell sorting and

polymerase chain reaction single-strand conformation polymorphism analysis. Cancer Res, 51: 3056-3058.

14. Nakai H., Misawa S., Toguchida J., Yandel DW. And Ishizaki K. (1992): FrequentP_{53} gene mutation in blast crisis of chronic myelogenous leukemia especially in myloid crisis harboring loss of chromosome 17p. Cancer Res, 52: 6588.
15. Du M., Peng H., Singh N., Isaacspn PG., and Pan L. (1995): The accumulation ofP_{53} abnormalities is associated with progression of mucosa-associated lymphoid tissue. Lymphoma Blood, 86: 4587-4593.
16. SAS: Statistical Analysis System (2012): Users guide statistical. Version 9. 1st ed. SAS Inst. Inc. Cary. N.C. USA.
17. Uchino S., Noguchi M., Hirota T., Itabashi M., Saito I., Kobayashi M., etal. (1992): High incidence of ###### accumulation ofP_{53} protein in gastric cancer. JPN J. CLIN Oncol, 22: 225-231.
18. Gomyo Y., Ikeda M., Osaki M., Tateba S., Tsujitani S., Ikeguchi M., etal. (1997): Expression ofP_{21} (waf1/Cip1/Sdi1) but notP_{53} protein is a factor in the survival of patients with advanced gastric carcinoma cancer, 79: 2067-2072.
19. Ramin A., Abbas AK., Taghi A., Vachiodeh K. (2008): Relationship betweenP_{53} expression and gastric in Cardia and Antrum. Arch Iranian Med, 11(5): 502-506.
20. Brito MJ., Williams GT., Thompson H., Filipe MU. (1994): Expression ofP_{53} in early (T1) gastric carcinoma and precancerous adjacent mucosa. Gut, 35: 1697-1700.
21. Gore R. (1997): Gastrointestinal cancer. Radiol CLIN North Am, 35: 295-310.
22. Schwartz G. (1996): Invasion and metastasis in gastric cancer: Invitro and invivo models with clinical consideration. Semin Oncol, 23: 316-324.
23. Hurlimann J., Saraga EP. (1994): Expression of P_{53} protein in gastric carcinomas: Association with histologic type and prognosis. Am J. Surgpathol, 18: 1247-53.
24. Hermiz R., Hussain A., and Qasim B. (2008): Immunohistochemical expression ofP_{53} in gastric carcinoma (A clinicopathological study), 6(2): 77-89.
25. Al Badri B. and Ali G. (2011):P_{53}expression in gastric dysplasia and carcinoma in Erbil city. J. Fac. Med. Baghdad, 53(2).
26. Martin HM., Filipe M., Morris RW., Lare PP. Silvestr F. (1992):P_{53} expression and prognosis in gastric carcinoma. Int. J. Cancer, 50: 859-862.
27. Lee KE., Lee HJ., Kim YH., Yu HT., Yang HK., Kim WH., etal. (2003): Prognostic significance ofP_{53} nm 23, PCNA and C-CrbB-2 in gastric cancer. JPN J. CLIN Oncol, 33: 173-179.