Immunohistochemical Expression of P53 in Invasive Cervical Carcinoma (A Clinicopathological Study)

Saif Janan Baythoon[1] *MSc. Path*, **Hussam Hasson Ali**[2] *MSc. Path, FICMS Path*, **Ban Jumaa Qasim** [2], *MSc. Path*.

Abstract

Background: Cervical cancer is one of the most frequent diseases in women; it comprises approximately 12% of all cancers in women worldwide. P53 is a tumor suppressor gene, functional inactivation of the P53 gene is a key event in tumorigenesis of many human malignancies, in cervical carcinoma this functional inactivation could occur either due to mutations or causes other than mutations like binding and inactivation or degradation by viral proteins.

Objective: To assess the immunohistochemical expression of P53 in invasive cervical carcinoma (squamous cells carcinoma and adenocarcinoma) and to study the correlation between P53 over-expression with clinico-pathological variants (age, grade of tumor and histological type).

Materials and methods: A total of 42 tissue samples of invasive cervical carcinoma (30 cases of squamous cell carcinoma and 12 cases of adenocarcinoma) were included in this retrospective study.

The samples were obtained from archival paraffin embedded blocks covering the years 1998 to 2005 from the histopathology files of al-Kadhimiya Teaching Hospital, Al-Ulwiya Teaching Hospital and from private laboratories. All the clinico-pathological data had been obtained from the files of these patients.

Out of 12 cases of adenocarcinoma, 8 had punch biopsy, and 4 had hysterectomies .For the 30 cases of squamous cell carcinomas, 16 patients had punch biopsy and 14 had hysterectomy.

All cases were analyzed by immunohistochemical staining with P53 tumor marker.

Results: The percentage of P53 over-expression in cervical adenocarcinoma (58.3%) was significantly higher than P53 over-expression in cervical squamous cell carcinoma (16.66%), ($P<0.05$).

P53 nuclear positivity in poorly, moderate and well-differentiated invasive cervical cancers was (50%, 18.18%, and 16.16% respectively), with no significant difference between P53 over-expression in different grades ($P>0.05$).

The percentage of P53 over-expression for the patients below the age of 50 was (32.14%) and for those equal and above 50 was (17.64%), no significant difference was found in P53 over-expression between the two age groups.

From the clinico-pathological assessment, the mean age of cervical adenocarcinoma (38.5 ±1.11 S.D. years) was significantly lower than the mean age of cervical squamous cell carcinoma (47.5±1.94 S.D. years).

No significant difference was found between the grade of the invasive cervical carcinoma and the two histological types.

Conclusion: In this study, a significant correlation has been found between P53 over-expression and the histological type of the invasive cervical carcinoma.

-Although there was no statistical correlation between P53 over-expression and the three grades of the invasive cervical carcinoma, poorly differentiated tumors showed the higher percentage of P53 over-expression.

-No significant difference was found between P53 over-expression and the age of the patient.

Key words: P53, cervical carcinoma, immunohistochemical expression.

IRAQI J MED SCI, 2008; VOL.6 (2):90-102

Introduction

Cervical carcinoma is the second leading cause of death in women worldwide.

Cervical cancer affects 13,500 women and accounts for 4,500 death annually in the United States [(1)].In Iraq, the neoplasms of the cervix uteri ranked the 6th among the commonest 10 cancers in female during the period 1976-1985, whereas during the period 1995 – 1997, it ranked the tenth within the leading cancers in females [(2,3)].From the overview of cervical

[1]Al-Kadhimiya Teaching Hospital, [2]Dept. Pathology, College of Medicine, Al Nahrain University.

Address Correspondences to: Professor Hussam Hasson Ali

Received: 4th April 2008, Accepted: 8th June 2008.

cancer situation , we found that cancers of the cervix uteri constitutes 1.4% of the total number of cancers with annual number of 113 new cases of cervical cancer reported in 1995, 1996 and 1997 respectively[3].

P53 is a tumor suppressor gene located on the short arm of chromosome 17[4].Wild "normal" type P53 protein is non- mutant , has tumor suppressor effect because of its ability to inhibit transformation [5].It has a short half-life of about 20 minutes , and can not be detected in the nucleus of most normal tissues [6].The mutant type P53 protein is much more metabolically stable than the wild type and accumulates in the nucleus and has prolonged half-life (up to 9 hours) which renders it more likely to be detected by immunohistochemical assay [7]. P53 gene is capable of modulating the expression of a variety of genes such as genes controlling cell arrest in G1/S phase, genes controlling apoptosis and genes controlling P53 protein [8], so functional inactivation makes the cell turn onto a one way street leading to malignant transformation [9]. This inactivation could occur either due to mutations in the P53 gene (which are the most frequent mutations encountered in human tumors) [10]or causes other than mutations like binding and inactivation or degradation by viral proteins[11] .

Hence this study aims to assess the immunohistochemical expression of P53 in invasive cervical carcinoma (squamous cells carcinoma and adenocarcinoma) and to study the correlation between P53 over-expression with clinico-pathological variants (age, grade and histological type).

Patients and methods

A total of 42 tissue samples of invasive cervical carcinoma (30 cases of squamous cell carcinoma and 12 cases of adenocarcinoma) were included in this retrospective study.

The samples were obtained from paraffin embedded blocks covering the years 1998 to 2005 from the histopathology files of Al-Kadhimiya Teaching Hospital, Al-Ulwiya Teaching Hospital and from private laboratories. All the clinical data had been obtained from the files of these patients. Out of 12 cases of adenocarcinomas, 8 patients had punch biopsies, and 4 had hysterectomies .For the 30 cases of squamous cell carcinomas, 16 patients had punch biopsies and 14 had hysterectomies.

The diagnosis was confirmed by review of two freshly prepared hematoxylin and eosin- stained slides. Two slides had been prepared to be stained immunohistochemically with P53 monoclonal antibody. To determine the signal specificity, negative control slides were included.

In the first run, the negative control slides included sequential omission of reactive component in the test; the primary (monoclonal) antibody, the secondary antibody (the biotinylated link), the conjugate and the substrate. Then, in each immunohistochemistry run, the negative control slides were obtained by omitting the primary antibody and, this was undertaken under identical test conditions [12].

Sections from a breast cancer patient that were known to be immunoreactive for P53 antibodies were used as positive controls for P53 and run with each batch stained [12].

Positive P53 results give nuclear brownish color, granular or homogenous, without any cytoplasmic or artifactual staining as in analytic cells or hemorrhage [13]. The results of P53 positivity in each individual case were analyzed according to the following variables:

1. Semi-quantitative assessment: A minimum of 100 tumor cells were scored. Those cervical tissues with P53 immunostaining in at least 5% of the cell nuclei were considered to have P53 over-expression [14].

Scoring was done at X40 objective as follows [14]:

-Negative (score 0): None of the cells revealed positivity for P53 marker.

-Weak or mild: Staining [(5 %-< 10%) positive of tumor cells] (score +1)

-Moderate: Staining (<25%) (Score +2)

-Strong: Staining (>25 %-< 50%) (Score +3)

-Highly strong: (over 50%) (Score +4)

2. Pattern of staining: The pattern was classified into Diffuse, Regional and Focal. It was considered Diffuse if the P53 positive cells were distributed through almost all the section as homogenous distribution, while considered Regional if more than one area of section showed large number of P53 positive cells, and it was considered Focal if P53 positive cells were localized in only one area of the section [15].

3. Intensity of the staining: The intensity of the brownish coloration was graded as strong, moderate or weak .It is strong if the brownish coloration is detected very clearly even at low power (100) and moderate if it was detected with difficulty at low magnification while if P53 positivity could only be detected at high magnification (1000) it was considered weak [16].

Statistical analysis was done using student t-test were P value of <0.05 was considered significant.

Results

Forty two cases of cervical cancer were included in this study; 30 cases of squamous cell carcinoma their age ranges from (30-65) years with a mean age of (47.50±1.94 S.D.) years. And 12 cases of adenocarcinoma their age ranges from (32-45) years with a mean age of (38.50±1.11 S.D.) years. From the descriptive analysis, there was a significant difference between the mean age of the two histological types; the mean age of adenocarcinoma was significantly lower than that of the squamous cell carcinoma (P<0.0.5). (Figure1)

Histopathologically, there were 30 cases of squamous cell carcinoma: 4(13.33%) cases were well –differentiated, 17(56.66%) cases were moderately –differentiated and 9 (30%) cases were poorly differentiated, and 12 cases of adenocarcinomas: 2(16.66%) cases were well –differentiated, 5(41.66%) cases were moderately –differentiated and 5(41.66%) cases were poorly –differentiated adenocarcinomas, from the descriptive analysis shown in (Table 1) there was no significant difference between the grade of the tumor and the two histological types. (P>0.05)(Figure 2)

Immunohistochemical staining of P53 showed granular brown nuclear staining in positive cases, from the total 42 cases of invasive cervical carcinoma;30 (71.43%) cases were found to be negative immunohistochemical expression of P53 with a (score 0), while 12 (28.57%) cases showed a positive over-expression of P53, 5(11.91%) cases were considered as score 1, 3(7.14%) cases were considered as score 2, 2(4.76%) cases were considered as score 3 and 2(4.76%) cases were considered as score 4 as shown in (Table 2).

The pattern of nuclear immunostaining of the 12 positive cases was in 4 (33.33%) cases having a diffuse pattern, 2 (16.67%) cases having a Regional pattern, and 6(50%) cases having a Focal pattern as shown in (Figures 3 and 4).

The intensity of nuclear immunostaining of the 12 P53 positive cases was Weak in 3(25%) cases, Moderate in 5(41.67%) cases and Strong in 4 (33.33 cases) as shown in (Figures 5 and 6).

There was no significant difference between the age of the patients and P53 over-expression (P>0.05). Also there was no significant difference between the P53 over-expression and the three grades of invasive cervical carcinoma (P>0.05), however poorly differentiated tumors showed high percentage of P53 over – expression as shown in (Table 3).

Immunohistochemical analysis of P53 over-expression in relation to the histological type of invasive cervical carcinoma revealed that P53 over-expression was detected in 5(16.66%) cases out of the 30 cases of squamous cell carcinoma and 7 (58.3%) cases out of 12 cases of adenocarcinoma. A significant correlation was found between the over-expression of P53 and the histological type. The over-expression of P53 in adenocarcinoma was significantly higher than P53 over-expression in squamous cell carcinoma (P<0.05) (Table 4).

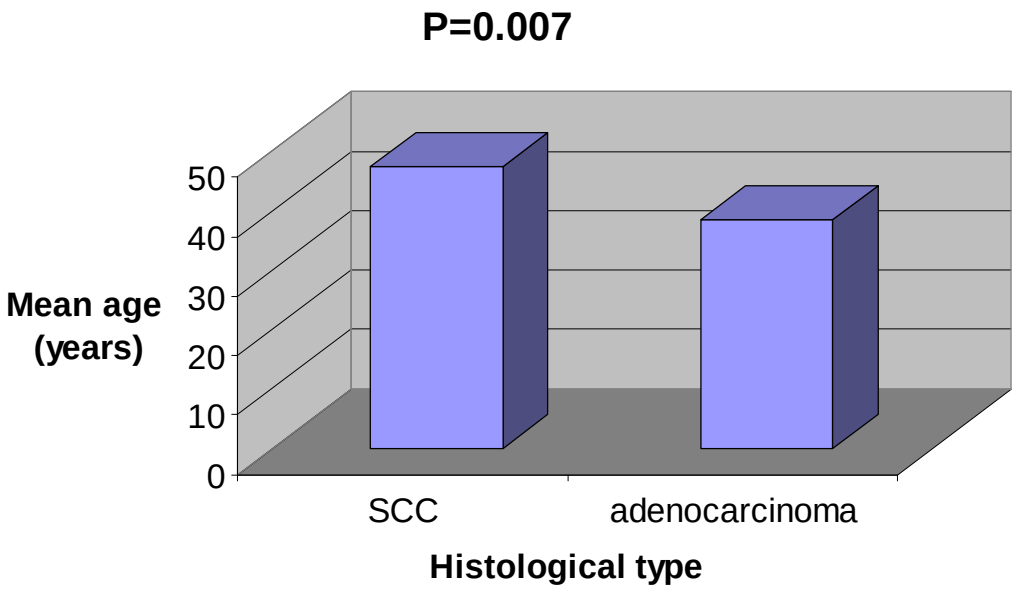
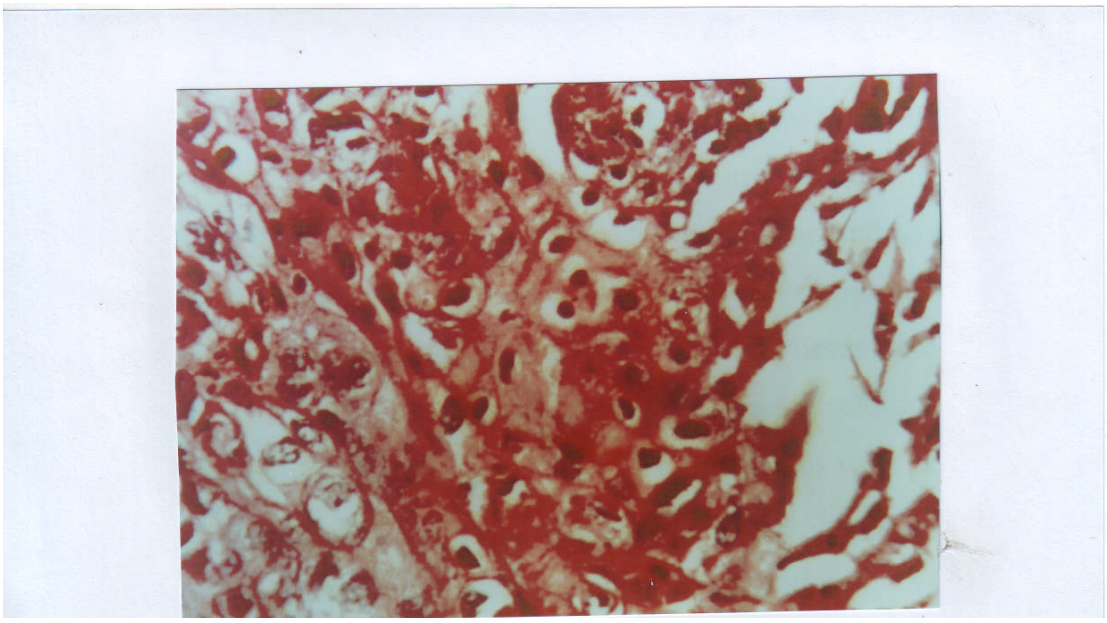


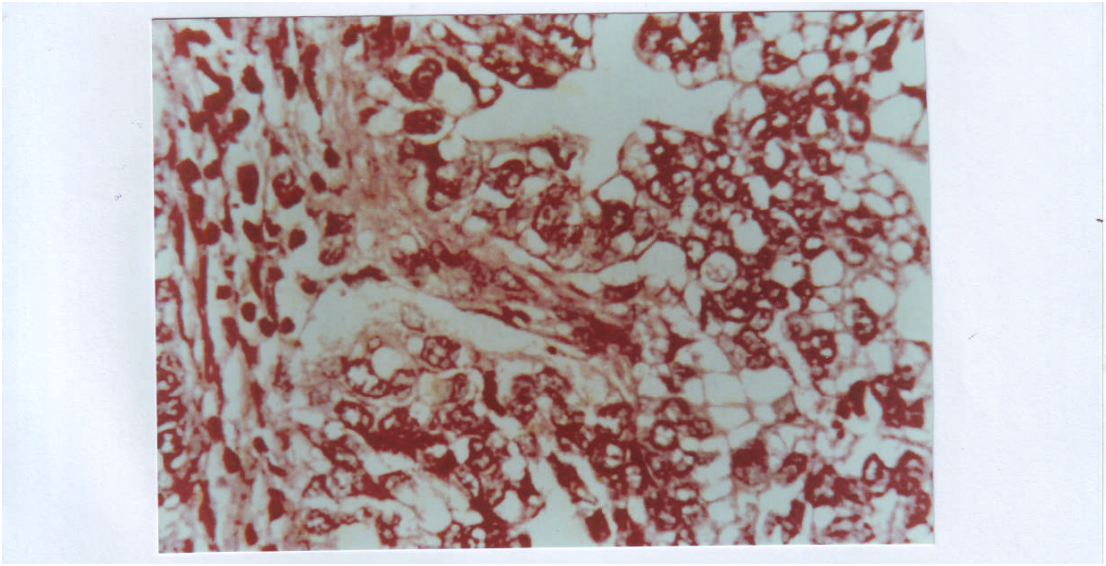
Figure 1: The relation between the age of the patients and the histological type of the invasive cervical carcinoma.

Table 1: Distribution of cases according to the grade of invasive cervical carcinoma.

		Histological Type	
		SCC	Adenocarcinoma
Grade	Well	4	2
	Moderate	17	5
	Poor	9	5
	Total	30	12
	P-value	0.675 (non-significant)	

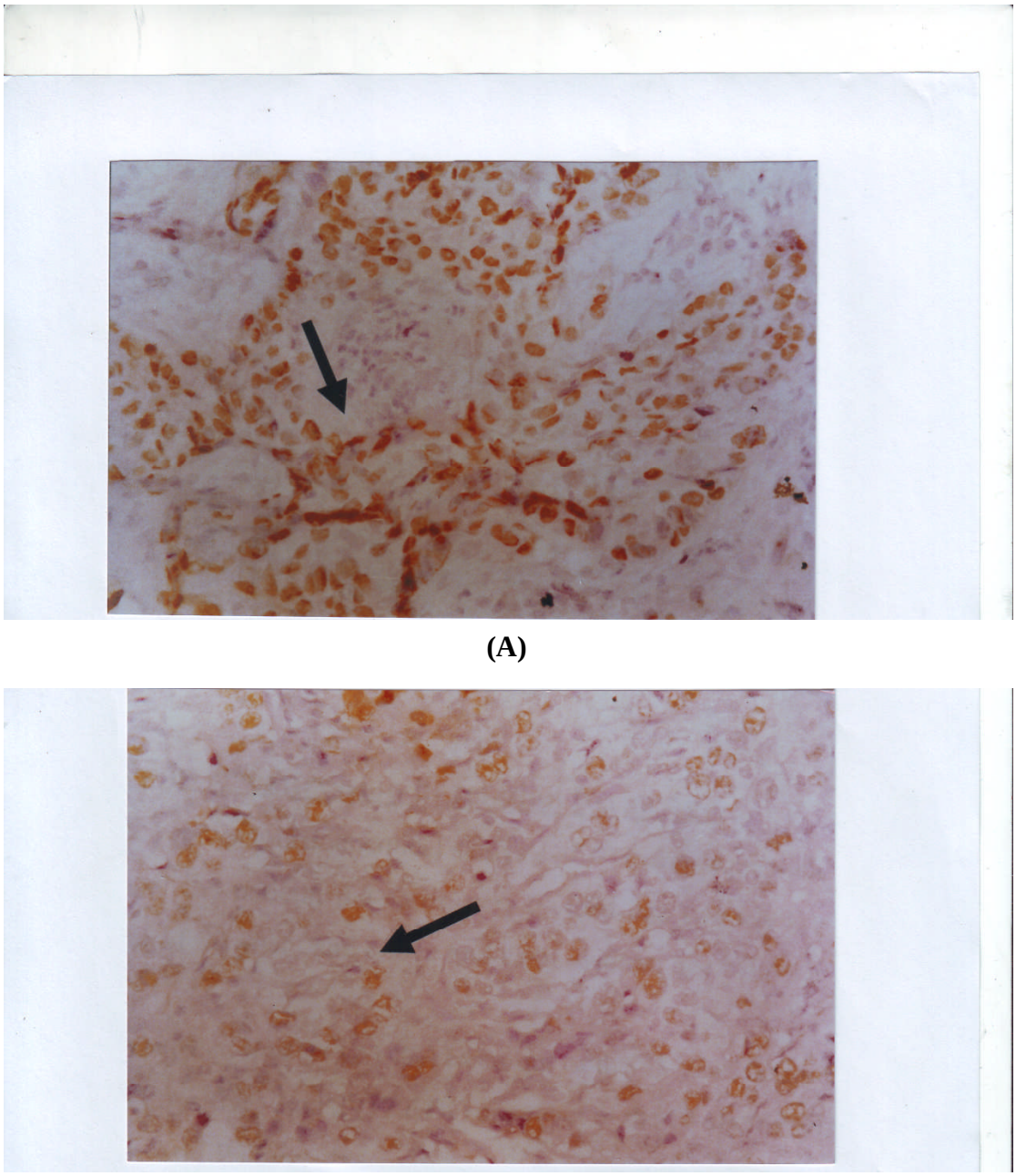


(A)



(B)

Figure 2: Histopathological section of (H&E, 400) [A] Squamous cell carcinoma of the cervix showing nests of squamous tumor cells. [B] Adenocarcinoma of the cervix showing moderately-differentiated glands.



(A)

(B)

Figure 3: Immunohistochemical staining of P53 (200) in cervical squamous cell carcinoma. Staining by DAB chromogen (brownish color) counterstained with Mayer's hematoxylin [A] Squamous cell carcinoma with a regional pattern of P53 nuclear staining (dark arrow) [B] Adenocarcinoma with a diffuse pattern of P53 nuclear staining (dark arrow)

Table 2: Distribution of P53 expression according to Sophia scoring system, pattern and intensity of staining.

Scoring of P53 in all cases	0	1	2	3	4	Total
Frequency No. %	30 71.43%	5 11.91%	3 7.14%	2 4.76%	2 4.76%	42 100%
Staining in P53 positive cases	Diffuse	Regional		Focal		Total
Frequency No. %	4 33.33%	2 16.67%		6 50%		12 100%
Intensity in P53 positive cases	Weak	Moderate		Strong		Total
Frequency No. %	3 25%	5 41.67%		4 33.33%		12 100%

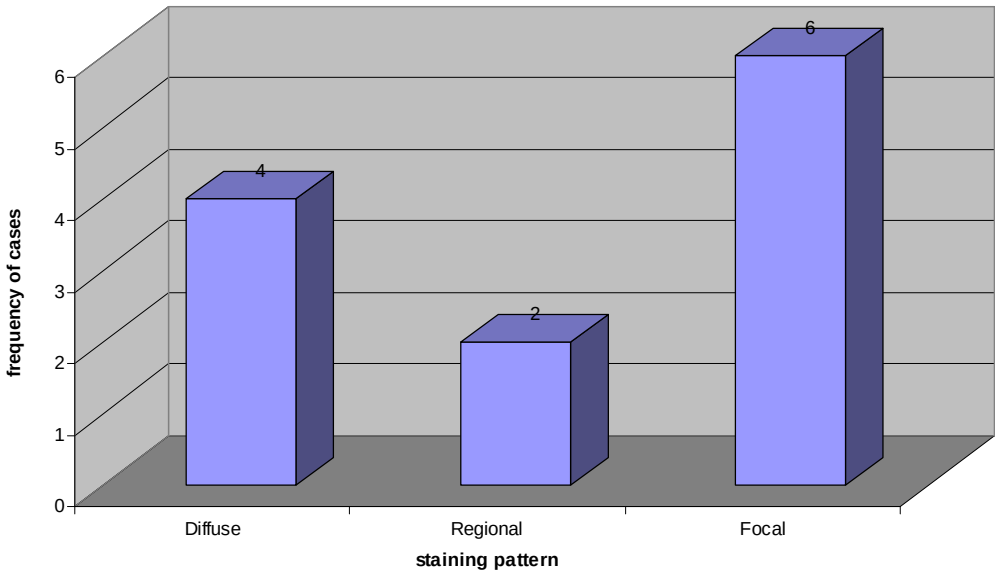
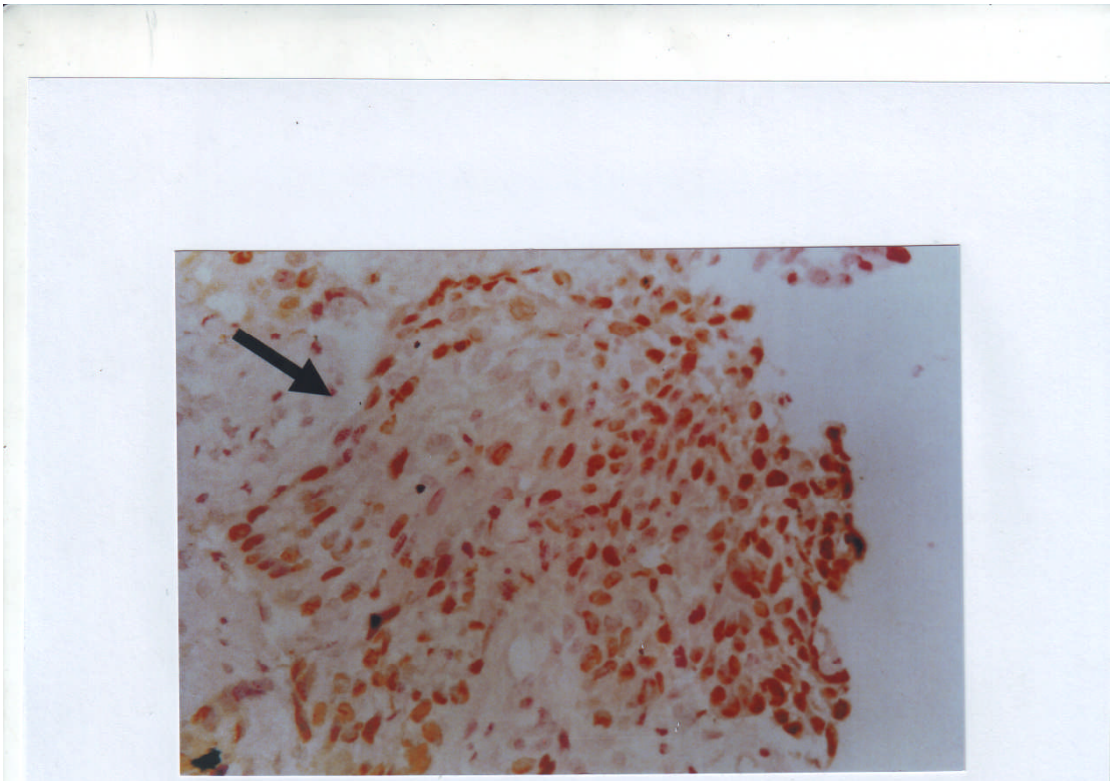
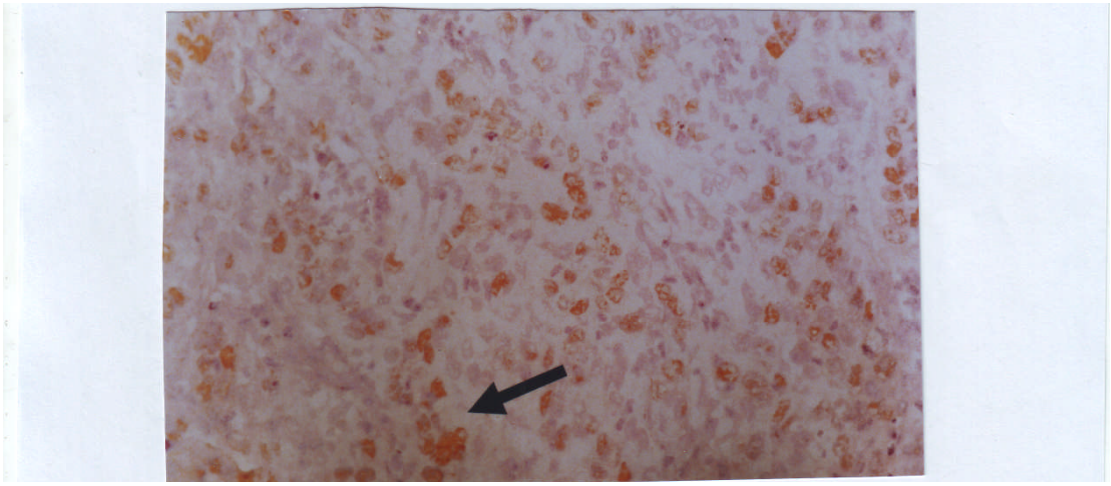


Figure 4: Distribution of P53 expression in invasive cervical carcinoma according to the pattern of staining.



(A)



(B)

Figure 5: Immunohistochemical staining of P53in cervical squamous cell carcinoma. Staining by DAB chromogen (brownish color) counterstained with Mayer's hematoxylin [A] Squamous cell carcinoma with strong P53 staining (>25 %-< 50%) (200) [B] Adenocarcinoma with moderate P53 staining (<25%) of the cells revealed nuclear positivity for P53 marker (dark arrow) (400)

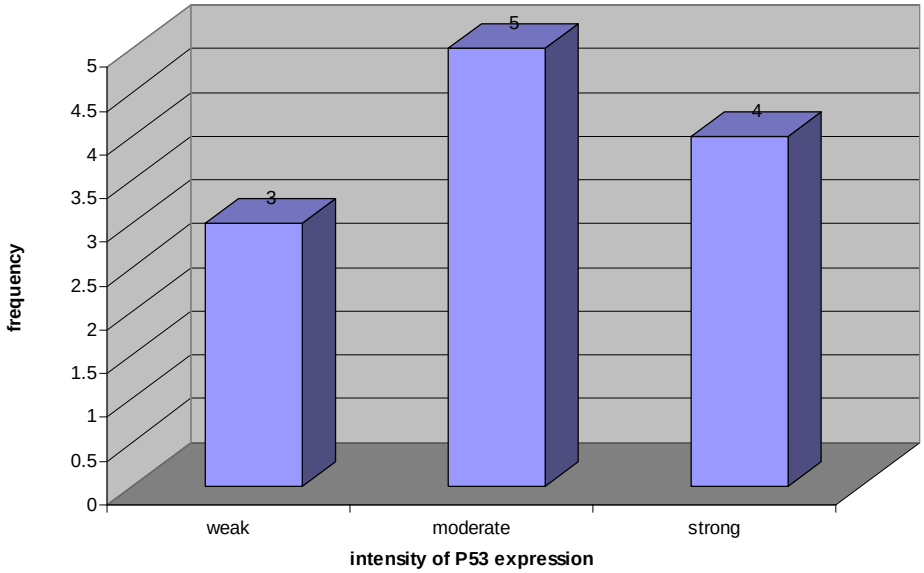


Figure 6: Distribution of P53 expression in invasive cervical carcinoma according to the intensity of staining.

Table 3: Expression of P53 in relation to the grade of invasive cervical carcinoma.

		Grade		
		Well	Moderate	Poor
P53	Positive	1 (16.66%)	4 (18.18%)	7 (50%)
	Negative	5 (83.34%)	18 (81.82%)	7 (50%)
	Total	6	22	14
	P-value	0.094 (non-significant)		

Table 4: Expression of P53 in relation to the histological type of invasive cervical carcinoma.

		Histological type	
		SCC	Adenocarcinoma
P53	Positive	5 (16.66%)	7 (58.3%)
	Negative	25 (83.34%)	5 (41.7%)
	Total	30	12
	P-value	0.02 (significant)	

Discussion

Cervical cancer is one of the most frequent diseases in women, it comprises approximately 12% of all cancers in women worldwide [17, 18].In Iraq, as in developing countries, the lower social conditions, the average age of the first intercourse, the high rate of parity and the lack of the primary care in the health system are important risk factors for the rate of cervical cancer [19].

This study showed that there is a significant difference between the mean age of invasive cervical squamous cell carcinoma and the mean age of invasive cervical adenocarcinoma ($P<0.05$) and this finding is similar to that of Abbas S.M, 2002 [20].

Concerning the relation between P53 over-expression and the age of the patients, the findings of this study were similar to that of Koyamatsu et al., 2002 [21] and Nair et al., 1999[22] which showed that there is no significant difference between P53 over-expression and the age of the patients.

Conflicting data concerning P53 over-expression in relation to the grade of invasive cervical carcinoma have been reported. Cheah and Looi, 2002 [23] showed that there is a significant correlation between P53 over-expression and the grade of the tumor, in such a way that P53 became more frequently expressed with less differentiated tumors. On the other hand, Nair et al., 1999[22] showed that there was no significant correlation between P53 over-expression and the three grades of invasive cervical carcinoma, which is similar to the findings in this study.

Although there was no significant correlation statistically between over-expression and the grade of invasive cervical carcinoma, poorly differentiated tumors showed a high percentage of P54 over-expression (50%).

The evaluation of P53 over-expression in invasive cervical carcinoma was examined in numerous studies, but the obtained results were controversial. In the majority of the studies (Abdulla A.M, 2006[19]; Kersmaekers et al., 1999[24]; Kainz et al., 1995 [25]), the frequency of the P53 over-expression in cervical squamous cell carcinoma was comparable to the results of this study.

P53 over-expression in adenocarcinoma in this study was also comparable to other studies (Abd et al., 1999 [26]; McCluggage et al., 1997 [27]). In concordance with other studies (Cheah and Looi 2002 [23]; Abd et al., 1999 [26]; Quinn MA., 1997[28]; Nagan et al., 1997[29]), this study showed that P53 over-expression in adenocarcinoma was significantly higher than that in squamous cells carcinoma of the cervix.

Some studies (Cheah and Looi 2002 [23]; Tenti P et al., 1998[30]) showed that the higher level of P53 expression in adenocarcinoma compared to squamous cell carcinoma may be due to higher frequency of mutation in adenocarcinoma. Most mutations induce conformational changes causing over-expression of P53 protein, stabilizing it and making it detectable by immunohistochemical analysis (Zheng A et al., 1999 [31]; Berns EM et al., 1998[32];Villuendes R et al ., 1997 [33]).

It has been suggested that P53 over-expression represents a poor prognostic factor [11] .Since P53 expression in adenocarcinoma is significantly higher than that in squamous cell carcinoma of the cervix, this could contribute to the less favorable prognosis of the former than the latter [34].

The current study as other studies (Wang et al., 2004 [35]; Koyamatsu et al., 2002 [21]; Vassallo et al., 2000 [36]) was undertaken with assumption that the immunohistochemical detection of P53 with the monoclonal antibody is almost associated with the presence of the mutated forms of P53 allels, based on wild type P53 protein possessing a short half-life, ranging up to 30 minutes, hence not accumulating to immunohistochemically detectable levels, and mutant forms having longer half-lives providing for immunohistochemical detection in many instances [37] .

Such immunohistochemical detection of P53 has frequently been used as a simpler method than genetic analysis of P53 mutations. However , it is not very specific for evaluation of P53 mutations in human cancers since tissue fixation , the type of antibody and other technical factors may affect the detection [38] .In addition ,not all mutations in the P53 gene result in the increase accumulation of the protein ,frame shift and non-sense mutations can lead to expression of an altered P53 which is undetectable by the available monoclonal antibodies , or some mutations may disrupt the binding sites of the anti-p53 antibody[21].

The absence of antibody reactivity therefore does not rule out genetic alterations of the P53 in human tumors [39].

In conclusion this study showed a significant correlation between P53 over-expression and the histological type of the invasive cervical carcinoma. Although there was no statistical correlation between P53 over-expression and the three grades of the invasive cervical carcinoma, poorly-differentiated tumors showed the higher percentage of P53 over-expression. No significant difference was found between P53 over-expression and the age of the patient.

References

1. Huang CC, Kashima ML, Chen H, Shih IM, Kurman RJ, Wu TC. HPV in situ hybridization with catalyzed signal amplification and polymerase chain reaction in establishing cerebellar metastasis of cervical carcinoma. Oncology 1999; 5:587-591.

2. Iraqi Cancer Registry (1987). Results of Iraqi Cancer Registry 1976-1985.Ministry of Health. Baghdad, Iraq. P18, 35, 59.

3. Iraqi Cancer Registry (1999). Ministry of Health. Baghdad, Iraq.

4. Gebhardt MC. What's new in musculoskeletal tumor surgery. Journal of bone and joint surgery 2001; 83-A: 630-634.

5. Kressner U, Inganas M, Byding et al. Prognostic value of P53 genetic changes in colorectal cancer. J.Clin. Oncol. 1999; 17:593-599.

6. Naresh KN, Oconor GT, Soman CS et al: A study of P53 protein, proliferating cell nuclear antigen, and P21 in Hodgkin's disease at presentation and relapse. Human Pathology 1997; 28:549-555.

7. Lacombe L, Dalbagni G, Zhang ZF, et al: Over expression of P53 protein in a high-risk population of patients with superficial bladder cancer before and after bacillus calmette-guerin therapy:correlation to clinical outcome.J.Clin.Oncol.1996;14:2646-2652.

8. Ralhan R, Sandhya A, Meera M, et al. Induction of MDM2-P2 transcripts correlate with stabilized wild-type P53 in betel- and tobacco-related human oral cancer. Am.J.Pathol.2000; 157:587-596.

9. Carson D.A.; Lois A. Cancer progression and P53. Lancet 1995; 346:1009-1111.

10. Han H, Landreneau RJ, Santucci TS, et al. Prognostic value of immunohistochemical expressions of P53, HER-2/neu, and bcl-2 in stage l non-small-cell lung cancer. Human Pathology 2002; 33:105-110.

11. Graflund M, Sorbe B, Sigurdardottir S, Karlsson MG: Relation between HPV-DNA and expression of p53, bcl-2, p21^{WAM,} MIB-1, HER-2/neu and DNA ploidy in early cervical carcinoma: correlation with clinical outcome. Oncology Reports 2004; 12:169-176.

12. Miller RT (2001). Technical immunohistochemistry: achieving reliability and reproducibility of immunostaining. Propath. Laboratory,Inc.(Available online at www.propathlab.com).

13. Gascoyne RD, Krajewska M, Krajewska S, et al. Prognostic significance of Bax protein expression in diffuse aggressive Non-Hodgkin's lymphoma.blood 1997; 90:3137-3178.

14. Sophia K, Apple J, Randolph H, et al. Immunohistochemical evaluation of K-ras, P53 and HER-2/neu expression in hyperplastic, dysplastic and carcinomatous lesions of the pancreas: evidence for multistep carcinogenesis. Human Pathology 1999; 30(2): 123-130.

15. Grigioni WF, D'errico A, Bacci F, et al: Primary liver neoplasms: evaluation of proliferative index using MoAb Ki-67. J. Of Pathology 1989; 158:23-29.

16. Farrugia M, Duan LJ, Reis MD, et al: Alterations of the P53 tumor suppressor gene in diffuse large cell lymphomas with translocations of the C-MYC and Bcl-2 proto-oncogenes. Blood 1994; 83:191-198.

17. Vera –Bravo R, Ocampo M, Urquiza M, Garcia JE, Rodringuez LE, Puentes A, Lopez R, et al: Human papillomavirus type 16 and 18 LI protein peptide binding to Vero and Hela cells inhibit their VLP_S binding. Int.J.Cancer 2003; 107:416-424.

18. Amrani M, Lalaoui K, El-Mzibri M, Lazo P, Belabbas MA. Molecular detection of human papillomavirus in 594 uterine cervix samples from Moroccan women (147 biopsies and 447 swabs). Journal of Clinical Virology 2003; 27:286-295.

19. Abdulla AM. A study on Human Papillomavirus: High-risk HPV E6, P53 and P16 Proteins expression and their role in cervical carcinogenesis.2006.

20. Abbas SM. Application of Immunohistochemical and Histochemical Stains in Diagnosis of Cervical Tumors. 2002.

21. Koyamatsu Y, Yokoyama M, Fukuda K, Saito T, Mastsukiima K, Ivvasaka T: A comparative analysis of human papillomavirus type 16 and 18 and expression of p53 gene and Ki-67 in cervical, vaginal and vulvar carcinomas. Gynecologic Oncology 2002; 90:547-551.

22. Nair P, Nair MIC, Jayaprakash PG, Pillai MR. Decreased programmed cell death in uterine cervix associated with high risk human papillomavirus infection. Pathology Oncology Research 1999; 5(2): 95-103.

23. Cheah PL, Looi L.M. P53 immunohistochemical expression: messages in cervical carcinogenesis. Pathology 2002; 34:326-331.

24. Kersemaekers AM, Fleuren GJ, Kenter GG, Van Den Brock LJ, Uljee SM, Hermans J,Van Den Vijver MJ. Oncogene alterations in carcinomas of the uterine cervix: overexpression of the epidermal growth factor receptor is associated with poor prognosis. Clin. Cancer Res. 1999; 5(3): 577-586.

25. Kainz C, Kohlberger P,Gitsch G, Sliutz G, Breitenecker G, Reinthaller A: Mutant p53 in patients with invasive cervical cancer stages IB to IfB. Gynecol Oncol 1995; 57(2): 212-214.

26. Abd EI, All H, and Rye A, Duvillard P: P53 immunohistochemical expression of Egyptian cervical carcinoma. Pathology Oncology Research 1999; 5(4): 280-284.

27. McCluggage G, Yoshihama Y, Kimura T, Shintani S, Alcalde RE: P53 and MDM2 expression in oral squamous cell carcinoma. Oncology 1996; 53:308-312.

28. Quinn MA: Adenocarcinoma of the cervix-Are there arguments for treatment policy? Current Opinion in Obst Gynecol 1997.

29. Negan HY, Tsao SW, Liu SS, Stanley M: Abnormal expression and mutation of P53 in cervical cancer – a study at protein, RNA and DNA levels. Genitourin. Med.1997; 73:54-58.

30. Tenti P, Romagnoli S, Silini E, Zappatore R, Giunta P, and Stella G, Carnevali L: Cervical adenocarcinoma expresses markers common to gastric, intestinal and pancreatobiliary epithelial cells. Pathol Res Pract 1998.

31. Zheng A, Castren K, Saily M, et al: P53 status of newly established acute myeloid leukemia cell lines. Br.J.Cancer 1999; 79(314): 407-415.

32. Berns EM, Klijn J, Putten W, et al: P53 protein accumulation predicts poor response to Tamoxifen therapy of patients with recurrent breast cancer. J.Clin.Oncology 1998; 16:121-127.

33. Villuendas R, Pezzella F, Gatter K, et al: P21 waf 1/cip 1 and MDM2 expression in Non -Hodgkin's lymphoma and their relationship to P53 status: A P53 +, $MDM2^-$, $P21^-$ immuno-phenotype associated with missense P53 mutations. J. of Pathol.1997; 181:51-61.

34. Andersson S, Hellstrom AC, Ren ZP, Wilander E: The carcinogenic role of oncogenic HPV and P53 gene mutation in cervical adenocarcinomas. Oncology2006; 23(1): 113-119.

35. Wang JL, Zheng BY, Li XD, Angstrom T, Lindstrom MS, Wallin KL. Predictive significance of the pi 6 INK4A,pi 4 ARE,p53 and proliferating cell nuclear antigen expression in the progression of cervical cancer. Clinical Cancer Research 2004; 10:2407-2414.

36. Vassallo J, Derchain SFM, Pinto GA, Martinez EZ, Syrjanen KJ, Andrade LAL. High risk HPV and p53 protein expression in cervical intraepithelial neoplasia. International Journal of Gynecology and Obstetrics 2000; 71:45-48.

37. Du M, Peng H, Singh N, et al. The accumulation of P53 abnormalities is associated with progression of mucosa-

associated lymphoid tissue lymphoma. Blood 1995; 86:4587-4593.

38. Penhallow J, Stiengrimsd-Ottir H, Elamin F, Warnakula-Suriya S, Faraneh F, and Johnson N, Tavassoli M. P53 alteration and HPV infections are common in oral SCC: p53 gene mutation correlate with the absence of FIPV 16-E6 DNA. International Journal of Oncology 1998; 12:59-68.

39. Denk C, Butz K, Schneider A, Duest M, Floppe-Seyler F. P53 mutations are rare events in recurrent cervical cancer. J.Mol.Med.2001; 79:283-288.