Original Article

# Cytomorphological changes in the sputum after Radiation Therapy for patients with Bronchogenic Carcinoma

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## Summary:

***Background :*** *Bronchogenic carcinoma is a common malignancy in iraq and radiotherapy is one of treatment methods for this disease . This study is cytopathological study dealing with sputum samples for bronchogenic carcinoma before and after radiotherapy .*

***Objectives :*** *To find morphological changes of the malignant cells as seen in cytopathological examination of the sputum after radiotherapy to the chest .*

***Patients and Methods :*** *Twenty five patients with a recent diagnosis of bronchogenic carcinoma presented to the Institute of Radiotherapy and Nuclear Medicine for the treatment in the period between 20th july 2004 and 25th june 2005 , sputum samples were obtained from these patients before and after they received radiation therapy to the chest (850 rad from cobalt 60 machine) .The malignant cells were studied by light microscope , cytological features of the malignant cells are compared before and after irradiation . Chi square test and P value were used as statistical methods for comparison of the changes in the malignant cells .*

***Results :*** *Squamous cell carcinoma was the most common type in this study. Cytoplasmic vacuolations in the malignant cells after radiation were not significant ( p value>0.05),nuclear changes following irradiation were significant (p value<0.05) .*

***Conclusion :*** *malignant cells are affected by radiation with cytoplasmic and nuclear changes .*


***Key Words :*** *Cytomorphological changes ,Bronchogenic carcinoma , radiation*

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## Introduction:

Cigarette smoke contains 19 known carcinogens including radioisotopes from the radon decay sequence, nitrosamine, and benzopyrene (1) . Recent investigation of sidestream smoke suggests it is more dangerous than direct smoke inhalation (2) . Sputum cytology examines a sample of sputum under a microscope to determine whether abnormal cells are present . For best results, collect the sample in the morning right after waking up (3) . By examination of the sputum , it is now possible to make a diagnosis in 80% to 90% of patients with lung carcinoma (4) , it should be remembered that malignant cells present in the sputum may originate in any portion of the upper aerodigestive tract , so imaging techniques like chest x ray and CT scan of the chest are required (5) . WHO classification of the Bronchogenic carcinoma include (6)

1- Squamous cell carcinoma
a) well differentiated
b) moderately differentiated
c) poorly differentiated
2- Small cell carcinoma
a) Oat cell
b) Intermediate cell
c) Combined
3- Adenocacinoma
a) acinar
b) papillary
c) bronchoalveolar
d) mucin secreting
4- Giant cell carcinoma
5- Clear cell carcinoma

In squamous cell carcinoma the malignant cells usually exfoliate singly in the sputum with a characteristic cytoplasm as bright orangeophilic dense ,granular , refractile with glassy appearance . In small cell carcinoma the malignant cells form lines of small hyperchromatic , neoplastic cells , their size is slightly larger than small lymphocytes , they have scanty cytoplasm and molding feature. In adenocarcinoma type of malignancy the cells usually shed in acini or clusters with vacuolated cytoplasm (7).

Large cell carcinoma is a fast-growing form that grows near the surface of the lung. It is primarily a diagnosis of exclusion, and when more investigation is done, it is usually reclassified to squamous cell carcinoma or adenocarcinoma. (8)

Radiotherapy is often given together with chemotherapy, and may be used with curative intent in patients who are not eligible for surgery . (9)

**Patients , Materials and Methods :**

Early morning sputum specimens collected from twenty five patients having bronchogenic carcinoma presented to the institute of radiotherapy and nuclear medicine in Baghdad in

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the period between 20th july 2004 and 25th june 2005. These specimens were collected in plastic containers , smears prepared from them and fixed directly with alcohol (90%) and stained by the papanicolaou stain for cytological examination . These patients were referred for radiotherapy and they received 850 rad to the chest . Other specimens collected by the same method from the same patients three weeks after irradiation and slides prepared by the same method .

Papanicolaou staining method and its modifications consist of nuclear stain and two counterstains, hydration prepares the cell sample for uptake of the nuclear dye ; dehydration prepares the cell sample for uptake of counterstains. Dehydration and clearing solutions result in cellular transparency and prepare the cell samples for the final step : mounting and coverslipping. (10)

The cytoplasmic vacuolations and nuclear changes (nuclear enlargement , nuclear vacuolations and loss of nuclear chromatin ) in the malignant cells were studied .

Chi –square test and p value are used as statistical methods in this study to compare the changes in the maliganant cell before and after irradiation .

**Results :**

Table -1- shows the demographic characters of the patients in this study regarding age , sex and residence . The age of these patients ranges between 49 and 72 years , most of them ( 12 patients , 48% ) were 50-60 years , 17 patients (68%) were males and 8 patients (32%) were females , 16 patients (64%) live in urban areas while 9 patients (36%) live in rural areas .

Table -2- shows the types of the bronchogenic carcinoma (with their percentages ) as diagnosed by sputum cytology in this study , 13 patients (52%) have squamous cell carcinoma , 7 patients (28%) have adenocarcinoma , 3 (12%) patients have small cell carcinoma and 2 patients (8%)have undifferentiated large cell carcinoma . The percentage difference in the types of the bronchogenic carcinoma is significant ( p value < 0.05% ) .

Table -3- shows the effects of radiation on the morphology of the malignant cells by study of specific cytological features before and after irradiation. These features include

A- Cytoplasmic changes : cytoplasmic vacuolations were seen in the malignant cells of 13 cases (52%) before irradiation and increased to be found in the malignant cell of 20 cases (80%)after irradiation,this difference is not significant(p value>0.05%) .

B- Nuclear changes include three points :

1- nuclear vacuolations were seen in the malignant cells of 9 cases (36%) before irradiation and increased to be found in the malignant cell of 21cases (84%) after irradiation , this difference is significant ( p value < 0.05% ) .

2- nuclear enlargement was seen in the malignant cells of 3 cases (12%) before irradiation and increased to be found in the malignant cell of 17 cases (68%) after irradiation , this difference is significant ( p value < 0.05% ) .

3- loss of nuclear chromatin was seen in the malignant cells of 6 cases (24%) before irradiation and increased to be found in the malignant cell of 19 cases (76%) after irradiation , this difference is significant ( p value < 0.05% ) .

Figure -1- is histogram of the cytoplasmic and nuclear changes before and after irradiation in the malignant cells .

Figure -2- and figure -3- show microscopical appearance of the squamous cell carcinoma in the sputum cytopathology using papanicolaou stain before and after irradiation respectively (Pap × 400) .

Figure -4- shows microscopical appearance of the adenocarcinoma in the sputum cytopathology using papanicolaou stain before irradiation (Pap × 400) .

Figure -5-shows microscopical appearance of the small cell carcinoma in the sputum cytopathology using papanicolaou stain after irradiation (Pap × 400) .

| Age/year | No. of patients | % |
|---|---|---|
| <50 | 2 | 8 |
| 50-60 | 12 | 48 |
| 61-70 | 10 | 40 |
| >70 | 1 | 4 |

**A**

| Sex | No. of patients | % |
|---|---|---|
| Male | 17 | 68 |
| Female | 8 | 32 |

**B**

| Residence | No. of patients | % |
|---|---|---|
| Rural | 9 | 36 |
| Urban | 16 | 64 |

**C**

**Table(1) Shows the demographic characters of the patients:**

A- **Age of the patients**
B- **Sex of the patients**
C- **Residence of the patients**

| Types of the Bronchogenic Carcinoma | No. of patients | % |
|---|---|---|
| Squamous cell carcinoma | 13 | 52 |
| Adeno carcinoma | 7 | 28 |
| Small cell carcinoma | 3 | 12 |
| Undifferentiated Large cell carcinoma | 2 | 8 |
| Chi-square | 2.705 | |
| P-valu | P<0.05 Significant | |

**Table(2) Shows types of Bronchogenic carcinoma as diagnosed by sputum cytopathology in the study**

| Types of the cells | Cytoplasmic vacuolations | Nuclear vacuolations | Nuclear enlargement | Loss of the nuclear chromatin |
|---|---|---|---|---|
| Maligmant cells (before radiation) | 13(52%) | 9(36%) | 3(12%) | 6(24%) |
| Maligmant cells (after radiation) | 20(80%) | 21(84%) | 17(68%) | 19(76%) |
| Chi-square | 1.091 | 4.033 | 8.450 | 5.760 |
| P-value | P>0.05 | P<0.05 | P<0.05 | P<0.05 |
| Significance | Not significant | Significant | Significant | Significant |

**P<0.05 Significant*

**Table(3) Effects of Radiation on the morphology of the malignant cells in the sputum cytopathology**

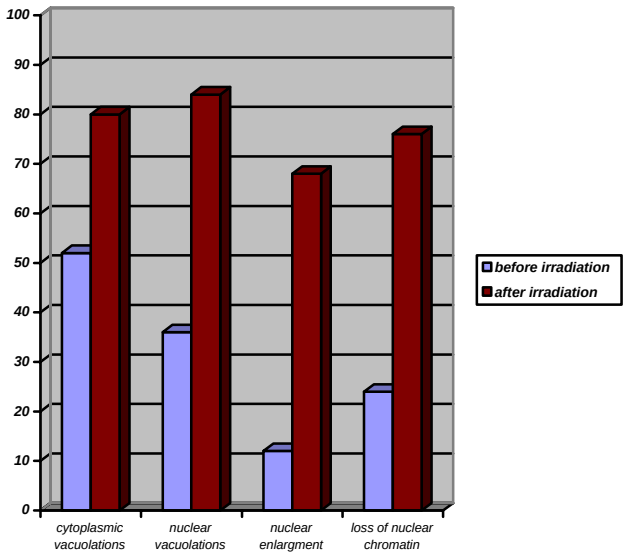


**Figure -1- shows percentages of cytological changes in the malignant cells before and after irradiatin**

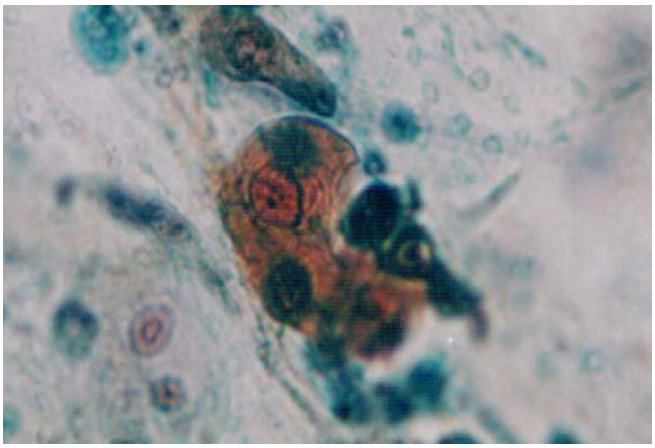

**Figure -2- shows microscopical appearance of the bronchogenic squamous cell carcinoma in the sputum cytopathology using papanicolaou stain before irradiation (Pap × 400) .**

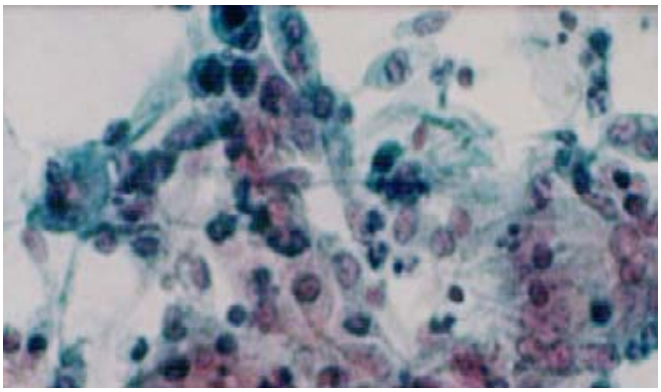

**Figure -3- shows microscopical appearance of the bronchogenic squamous cell carcinoma in the sputum cytopathology using papanicolaou stain after irradiation (Pap × 400) .**

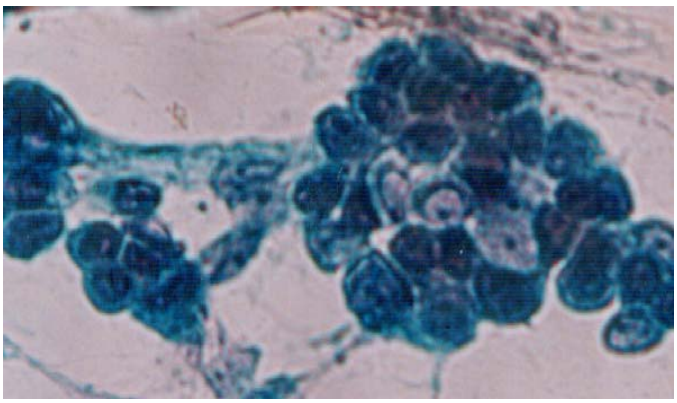

**Figure -4- shows microscopical appearance of the bronchogenic adenocarcinoma in the**

**sputum cytopathology using papanicolaou stain before irradiation (Pap × 400)**

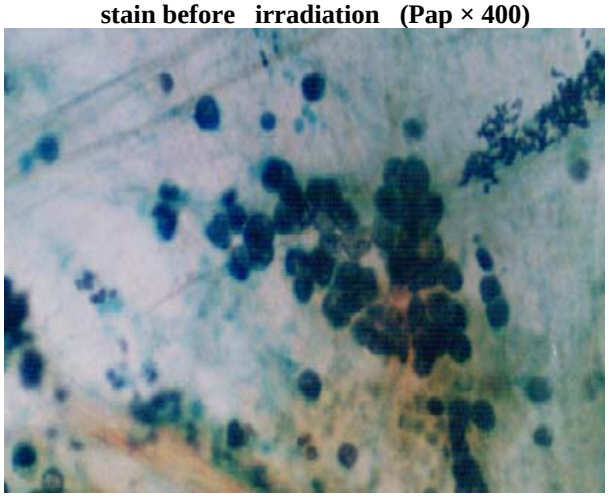

**Figure -5-shows microscopical appearance of the bronchogenic small cell carcinoma in the sputum cytopathology using papanicolaou stain after irradiation (Pap × 400) .**

**Discussion :**

The lower respiratory tract is composed of many types of tissues , some tolerate moderate doses of radiations well , whereas other tissues are relatively sensitive . The trachea and bronchi are lined with ciliated columner epithelium that is shed only with high doses of radiation , in contrast to the tracheobronchial tree , the lung is one of the structures that are more sensitive to irradiation (11)

The squamous cell carcinoma was the most common type in this study specially in old aged males where adenocarcinoma was more in females and this is comparable with Cox JD and Yesner study(1979) (12) .

This study shows that malignant cells affected by radiation and they undergo cytoplasmic and nuclear changes but with different percentages. The changes were cytoplasmic vacuolations , nuclear vacuolations , nuclear enlargement and loss of nuclear chromatin and these results were comparable with Albright-CD et. al ( 13 ) .

This study shows that the most important post radiation effect on the malignant cells is on the nuclei of these cells , this may be related to the rapid turnover and proliferation of the malignant cells .

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