



# HIGHER MORTALITY RISK OF LUNGS CARCINOMA IN VINEYARD SPRAYERS

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## ABSTRACT

This study investigated mortality rate of lungs carcinoma in professional vineyard sprayers. Clinical investigation was performed in 187 professional vineyard sprayers who had been exposed to the inhalation of the particles of Bordeaux mixture for 24 years on average. Bordeaux Mixture is used for prevention against mildew attacking vineyards. The control group was composed of 187 inhabitants of the same area who did not have any contact with the mentioned substance. A cytological investigation of the sputum specimens obtained from 104 tested inhabitants was performed. The sputum specimens were stained with standard haematoxylin-eosin method and also with special method (rubeanic acid) to prove the incidence of copper granules in macrophages. The findings show a considerable statistical difference in the frequency of occurrence of lungs carcinoma between the group of vineyard sprayers smokers and the control group ( $X^2=4.77, p<0.01$ ). The risk of lungs carcinoma in the vineyard sprayers was three times higher compared to the risk of smokers in the control group, with a statistical probability of 95% in the scope from  $1.16<RR<7.78$ . Mortality risk of lungs carcinoma in the professional vineyard sprayers is significantly higher ( $p<0.01$ ) compared to the risk in the control group.

**KEY WORDS:** lungs carcinoma, vineyard sprayers

## INTRODUCTION

Pimentel and Marques described the case of pulmonary disease in vineyard workers and they named it "vineyard sprayer's lung". It is an occupational disease caused by inhalation of the copper sulphate solution neutralised by hydrated lime (1). The changes in the respiratory system are caused by inhalation of the pesticide known as Bordeaux mixture (1-2.5% copper sulphate solution) which is used for spraying vineyards as a protection against mildew (2). The roentgenogram of vineyard sprayer's lung is similar to that of alveolitis, with a tendency to develop into diffuse interstitium fibrosis. Inhalation of the particles of Bordeaux mixture for a long period causes frequent chronic respiration symptoms as cough, chest constrictions and dyspnoea, along with ventilation disorder with dominant restrictive-obstructive changes (3,4). Experimental research, carried out on the animals exposed to the inhalation of Bordeaux mixture, caused the interalveolar heaping of macrophages, formation of granulomas and multiplication of collagen, elastic and reticular fibres in the interstitium (5). Plamenac and collaborators described alveolar macrophages with copper formations in the cytoplasm found with the workers engaged in vineyard spraying (6). Pulmonary biopsy in vineyard sprayers often showed the presence of inflammatory changes of bronchi and interstitium. Taking into the consideration clinical picture of the disease appearing after longer exposition to copper sulphate, Villard found the relation between pulmonary lesions and the development of pulmonary carcinoma (2). So far professional risk of increased incidence of respiratory changes in vineyard sprayers has not been described extensively in medical literature as this type of study demands aimed cohort research through longer period. Monitoring the health in vineyard workers during a longer period encouraged us to undertake the investigation with a special focus on the mortality rate of pulmonary carcinoma in professional vineyard sprayers.

## SUBJECTS AND METHODS

The investigation was performed among the vineyard sprayers who worked in the largest vineyards in Brotnjo and Blato (51 000 hectares of vineyards) in the Herzegovina-Neretva Canton and West Herzegovina Canton, Bosnia and Herzegovina. Bordeaux mixture is used for spraying the vineyards in the whole area. All subjects were men in the age of 51.3 on average. They were divided into two groups. The first group was composed of 187 workers who were for about 24 (SD±6) years engaged

in vineyard spraying. The control group was composed of 187 workers who did not get in touch with Bordeaux mixture. They all had 23 (SD ± 5) years of work experience. In this longitudinal prospective study we monitored the occurrence of lungs carcinoma in both groups, in two periods, first from 1983-1992 and second from 1993-2002. We also investigated influence of smoking on the incidence of lungs carcinoma in both groups. For these reasons each group was divided into sub groups of smokers and non-smokers. Taking into account professional exposure to pesticide, personal record of a patient, work experience, systematic clinical examination and chest roentgenograms all the subjects suspected to have lungs carcinoma were sent to pulmonary department for further diagnostic and therapeutic treatment. For each patient with confirmed diagnosis of lungs cancer further therapy was performed (surgery, radiological therapy or combined chemotherapy) and his health condition was monitored until death. We made cytological analyses of sputum specimens randomly taken from 52 vineyard sprayers as well as from the control group. The only criteria for the selection had been that none of the subject had any respiratory disease of any aetiology in his case history. Sputum specimens were obtained by morning cough on three consecutive days. Only expectorated material contained pulmonary macrophages was accepted as sputum. After fixation in 75% alcohol, the sputa were embedded in paraffin and the section were stained with haematoxylin-eosin, tested for iron by Turnbull method and for copper with rubeanic acid and benzidine.

## RESULTS

In the group of 121 vineyard sprayers non-smokers, during the investigation 12 vineyard sprayers died of lungs carcinoma (mortality rate 9.917%). During the first investigation period (1983-1992) 4 sprayers died (mortality rate 3.305%) and during the second period (1993-2002) 8 sprayers died (mortality rate 6.611%). In the control group of 121 workers non-smokers, 3 workers died of lungs carcinoma (mortality rate 2.479%). In the period from 1983-1992, 1 worker died (mortality rate 0.826%) and in the period from 1993-2002, 2 workers died (mortality rate 1.652%) in Table 1. In the group of 66 vineyard sprayers smokers 15 workers died. Five workers died in the first investigation period (mortality rate 7.5%) and 10 workers died in the second period (mortality rate 15.151%). In the control group of 66 smokers, 5 subjects died from lungs carcinoma (mortality rate 7.576%). Two subjects died in the first period of investigation (mortality rate 3.030%) and 3 workers died

| SUBJECTS                            | AGE   | NUMBER OF SUBJECTS | NUMBER OF DEAD 1983-1992 | NUMBER OF DEAD 1993-2002 | TOTAL NUMBER OF DEAD SUBJECTS 1983-2002 |
|-------------------------------------|-------|--------------------|--------------------------|--------------------------|---|
| VINEYARD SPRAYERS                   | 36-56 |                    |                          |                          |   |
| NON-SMOKING MORTALITY RATE PER 1000 |       | 121                | 4 (33.05‰)               | 8 (66.11‰)               | 12 (99.17‰)                             |
| SMOKERS MORTALITY RATE PER 1000     |       | 66                 | 5 (75.76‰)               | 10 (151.51‰)             | 15 (227.27‰)                            |
| CONTROL GROUP                       | 36-65 |                    |                          |                          |   |
| NON-SMOKING MORTALITY RATE PER 1000 |       | 121                | 1 (8.26‰)                | 2 (16.52‰)               | 3 (24.79‰)                              |
| SMOKERS MORTALITY RATE PER 1000     |       | 66                 | 2 (30.30‰)               | 3 (45.45‰)               | 5 (75.76‰)                              |

TABLE 1. Mortality of lungs carcinoma in professional vineyard sprayers

| SUBJECTS          | AGE   | NUMBER OF SUBJECTS | SPUTA WITH ABNORMAL COLUMNAR CELLS | SQUAMOUS METAPLASIA without atypia | with atypia | MACROPHAGES WITH COPPER | EOSINO-PHILIA | RESPIR. SPIRALS | SALIVA (LACK OF EXPECTORATION) |
|-------------------|-------|--------------------|------------------------------------|------------------------------------|-------------|-------------------------|---------------|-----------------|--------------------------------|
| VINEYARD SPRAYERS | 36-65 |                    |                                    |                                    |             |                         |               |                 |                                |
| NON-SMOKERS       |       | 33                 | 26 (79%)                           | 11 (33%)                           | 2 (6%)      | 23 (70%)                | 4 (12%)       | 5 (15%)         | 16 (48%)                       |
| SMOKERS           |       | 19                 | 19 (100%)                          | 7 (37%)                            | 5 (26%)     | 14 (74%)                | 2 (11%)       | 4 (21%)         | 1 (5%)                         |
| CONTROL GROUP     | 36-65 |                    |                                    |                                    |             |                         |               |                 |                                |
| NON-SMOKERS       |       | 35                 | 4 (11%)                            | 0                                  | 0           | 0                       | 1 (3%)        | 1 (3%)          | 21 (60%)                       |
| SMOKERS           |       | 17                 | 13 (76%)                           | 2 (12%)                            | 1 (6%)      | 0                       | 2 (18%)       | 3 (18%)         | 3 (18%)                        |

TABLE 2. Incidence of cytological findings in professional vineyard sprayers' sputa

in the second period (mortality rate 45.45‰). Mortality rate of vineyard sprayers smokers was significantly higher (227.27‰) than in the control group (75.76‰). The results of monitoring show that there is a significant statistical difference in respect to the lungs carcinoma rate ( $X^2= 4.55, p<0.05$ ) between the investigated group of workers engaged in vineyard spraying non-smokers and the control group of the workers non-smokers. There is a significant statistical difference in the height of relative risk of incidence of lungs carcinoma in the vineyard sprayers non-smokers compared to the control group ( $X^2=4.55, p<0.05$ ). Relative risk of lungs cancer occurrence in the workers non-smokers engaged in vineyard spraying with Bordeaux Mixture is on average four times higher ( $1.16<RR<13.82$ ) than in the control group. The results show that there are significant statistical differences in respect to the occurrence of lungs carcinoma between the group of vineyard sprayers smokers and the control group ( $X^2=4.77, p<0.01$ ). The risk of

lungs carcinoma was three times higher in the vineyard sprayers than in the control group of smokers with a statistical probability of 95% in the range  $1.16<RR<7.78$ .

#### RESULTS OF CYTOLOGICAL INVESTIGATIONS

We made a cytological analysis of sputum specimens obtained from 52 randomly selected vineyard sprayers. We also analysed the same number of sputum specimens obtained from the control group in Table 2. We investigated 52 vineyard sprayers between the ages 36-65, 33 of them were non-smokers and 19 smokers. The table shows increased sputum expectoration in both groups of vineyard sprayers non-smokers and smokers compared to the control group. Microscopic examination of the sputum in the non-smokers group showed the following abnormalities: abnormal columnar (irritated) cells in 26 (79%) cases; squamous metaplasia of respiratory epithelium was found in 13 cases – in 11 cases (33%) without atypical, in 2 (6%) cases with

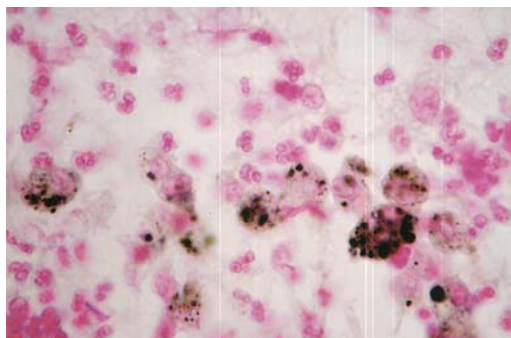


FIGURE 1. Copper granules were noted in the cytoplasm of the macrophages (halcophages). (HE, 400X)

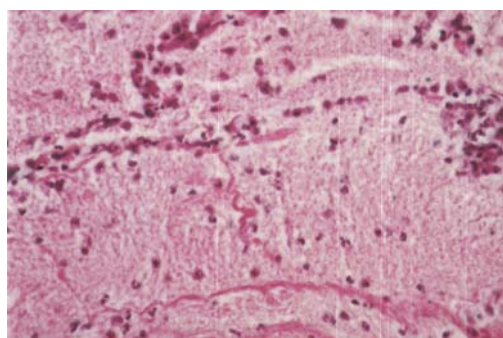


FIGURE 2. There are eosinophilia and respiratory spirals in the sputum of smokers. (HE, 250X)

atypical metaplastic cells; in 23 (70%) cases copper granules were noted in the cytoplasm of the macrophages (halcophages) (Figure 1); eosinophils were found in 4 (12%) cases; respiratory spirals in 5 (15%) cases. In the sputum of smokers the following findings were noted: abnormal (irritated) columnar cells were found in 19 (100%) cases; squamous metaplasia was found in 12 cases, in 7 (37%) cases there were no signs of atypia, while in 5 (26%) cases atypical metaplastic cells were found; halcophages were found in 14 (74%) cases; eosinophilia in 2 (11%) cases; respiratory spirals in 4 (21%) cases. (Figure 2) The control group was composed of 52 subjects in the age range 36-65. There were 35 smokers and 17 non-smokers. The following findings were evident in the group of non-smokers: abnormal (irritated) cylindrical cells in 4 (11%) cases; eosinophilia and respiratory spirals were found in 1 case (3%). Squamous metaplasia and halcophages were not evident within the group. In the group of smokers the following findings were evident: 15 (88%) subjects had abnormal (irritated) cylindrical cells; squamous metaplasia was found in 3 cases, in 2 cases (12%) without atypical and in 1 case (6%) with atypical metaplastic cells; eosinophilia was found in 2 (12%) cases; respiratory spirals were noted in 3 (18%) cases; chalcoses were not found in this group of subjects.

## DISCUSSION

Morphological, clinical-radiological and functional changes in the lungs of vineyard sprayers are well known. Besides general symptoms, the patients also had the symptoms related to the respiratory system, in the beginning interpreted as a consequence of development of chronic bronchitis or tuberculosis, with hae-

matological findings almost within normal limits. Koch bacillus was not noted in the consecutive sputum that the patients produced and their tuberculin tests were negative. The applied therapy that included antibiotics and antitubercotics did not result with a complete regression of the disease. In some patients the radiological picture showed the presence of tumorous lungshadows, which was the indication for biopsy. In these cases pathohistological findings included: the desquamation of pneumocytes, formation of the predominantly histiocytic granulomas in the septa, individual and/or multiplied that gradually developed into fibrohyaline nodules similar to those found in silicosis (1,2, 5). There are the quotations in medical literature about the effects of Bordeaux mixture on the respiratory system that include changes in the pulmonary interstitium and bronchial epithelium. Numerous focuses of non-specific bronchopneumonia, with inflammatory cells and macrophages indicated the affection of bronchial parenchyma, which was proved by the experiments on animals (3,5). At present some authors are of the opinion that copper is the aethiological agent responsible for changes in the interstitium, i.e. functional changes that occur in the cases of longer exposure to its inhalation (4,5). Cytological changes of the respiratory tract found in the sputum analysis are the proof of severe lesions of cylindrical epithelium in professional vineyard sprayers. A high percentage of the sputum specimens contained desquamated, abnormal columnar bronchial cells that showed a whole spectrum of degenerative changes from non-specific irritation to severe cellular degenerative changes of ciliocytophoria type. The occurrence of numerous respiratory spirals, squamous metaplasia of the cells in the bronchial epithelium and atypical cells

indicated that long-term inhalation of the pesticide of copper sulphate might increase the risk of bronchogenic carcinoma (6,7). This study revealed enhanced expectoration of sputum in vineyard sprayers, which indicates the irritation effects of inhaled substance (8,9). The investigation of cytological changes in respiratory epithelium of the sputum specimens showed considerable deviations compared to normal sputum specimens. More frequent occurrence of squamous metaplasia and abnor-

mal cylindrical cells in non-smokers and smokers group of vineyard sprayers compared to the control group indicates chronic irritation effect of used substance-copper, which was constantly evident within pulmonary macrophages in the sputum of vineyard sprayers (10,11). There are quotations in medical literature on higher risk of lungs carcinoma caused by long exposure to the inhalation of the granules of copper sulphate (6,12,13), which is in accord with the findings of our investigation.

## CONCLUSION

Our results show a considerably higher mortality of lungs carcinoma in professional workers engaged in vineyard spraying compared to those who were not exposed to the inhalation of mentioned substance. This also applies to the group of vineyard sprayers non-smokers. In this group the tobacco smoke is excluded as a possible aetiological factor responsible for lungs carcinoma.

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