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Carcinomas epidermóides do pulmão na doença vibroacústica

Respiratory squamous cell carcinomas in vibroacoustic disease

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Resumo

Enquadramento: Em 1987, observou-se durante a autópsia de um doente com doença vibroacústica (VAD) dois tumores: Um carcinoma de células renais e um glioma maligno cerebral. Desde 1987, tem-se vigiado o aparecimento de tumores em doentes com a VAD. Até à data, num universo de 945 indivíduos, há 46 casos de tumores malignos, dos quais 11 são múltiplos. Dos 11 casos de tumores do aparelho respiratório, todos eram carcinomas epidermóides (CE). O presente estudo aborda as características morfológicas destes tumores. **Métodos:** Foram recolhidos fragmentos destes tumores (biópsia endoscópica ou cirúrgica) de 11 doentes do sexo masculino (idade média: 58±9 anos, 3 não fumadores): 2 na glote e 9 no pulmão. Dos 3 não fumadores, 2 tinham tumores

Abstract

Background: In 1987, the autopsy of a vibroacoustic disease (VAD) patient disclosed two tumours: a renal cell carcinoma and a malignant glioma in the brain. Since 1987, malignancy in VAD patients has been under close surveillance. To date, in a universe of 945 individuals, there are 46 cases of malignancies, of which 11 are multiple. Of the 11 cases of respiratory tract tumours, all were squamous cell carcinomas (SqCC). This report focuses on the morphological features of these tumours. **Methods:** Tumour fragments were collected (endoscopic biopsy or surgery) from 11 male VAD patients (ave. age: 58±9 years, 3 non-smokers): 2 in glottis and 9 in the lung. In the 3 non-smokers, 2 had lung tumours and 1 had a glottis tumour. All were employed as or retired aircraft technicians,

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do pulmão e 1 tinha tumor da glote. Todos eram trabalhadores ou reformados da indústria aeronáutica, pilotos militares ou de linhas comerciais. Foram fixados fragmentos para microscopia óptica e electrónica. Para os estudos imuno-histoquímicos usou-se coloração com cromagranina e sinaptofisina. **Resultados:** Todos os tumores pulmonares se localizaram no brônquio do lobo superior direito, e a sua histologia era de tumores epidermóides pouco diferenciados. A pesquisa com marcadores neuroendócrinos foi negativa. Nove doentes faleceram. Os 2 sobreviventes são grandes fumadores (> 2 maços/dia). A média de idade de aparecimento do tumor em pilotos de helicóptero estava abaixo dos 50 anos, enquanto para os outros dois grupos profissionais era superior a 50. Os hábitos tabágicos não tiveram influência na progressão e desfecho dos casos. **Conclusões:** O CE constitui aproximadamente 40% dos tumores pulmonares na população em geral. Face aos actuais resultados, torna-se muito importante especificar o tipo histológico exacto do tumor em todos os estudos estatísticos. Não surpreende a idade mais jovem de início dos tumores em pilotos de helicóptero, atendendo aos resultados dos estudos de mutagénese em modelos animais e em trabalhadores expostos a RBF: a frequência de trocas de cromátides irmãs em pilotos de helicóptero está aumentada, bem como em modelos animais expostos a ruído e vibrações simultâneos, simulando o ambiente no *cockpit* de helicópteros.

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Palavras-chave: Ruído de baixa frequência, infra-sons, pilotos de helicóptero, cancro do pulmão, carcinoma epidermóide, trocas de cromátides irmãs.

military or commercial pilots. Fragments were fixed either for light and electron microscopy. Immunohistochemistry studies used chromagranine and synaptophysine staining. **Results:** All lung tumours were located in the upper right lobe bronchi and were histologically poorly differentiated SqCC (Figs. 1, 2). The search with neuroendocrine markers was negative. The average age of tumour onset in helicopter pilots was below 50 years old while for the other professional groups it was above 50. Nine patients are deceased. The 2 surviving patients are heavy smokers (> 2 packs/day). Smoking habits had no influence on tumour outcome and progression. **Discussion:** Epidemiological studies indicate that squamous cell carcinomas account for approximately 40% of all lung tumours in men. It seems to be highly relevant that all VAD patient respiratory tract tumours are squamous cell carcinomas. It is not surprising that helicopter pilots are the ones who are affected the earliest because previous studies have shown that helicopter pilots exhibited the highest values for the frequency of sister chromatid exchanges. Generally, epidemiological tumor studies do not take histological tumor type into account, but given the results herein, it would seem of the utmost importance to begin specifiying the exact histological type of tumor in all statistical studies.

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Key-words: Low frequency noise, infrasound, helicopter pilots, lung cancer, squamous cell carcinoma, sister chromatid exchanges.

Introduction

The genotoxicity of low frequency noise (LFN) (≤500 Hz, including infrasound) exposure has been an unexplored field of science. In fact, the genotoxicity of acoustical phenomena in general has been assumed to be non-existent. In Portugal, LFN-induced pathology has been the object of study since 1980. Initially, the pathological features identified in a group of aircraft technicians were restricted to the realm of neurophsyiology[1-4] and cognition[5], since the most evident clinical signs were related to central nervous system dysfunction[6]. However, in a 1987 autopsy of a deceased patient, the extent of LFN-induced pathology was uncovered; both the cardiovascular and respiratory systems were also compromised in LFN-exposed individuals[7]. By 1998, vibroacoustic disease (VAD) had been defined as a systemic pathology caused by long-term exposure to LFN[8,9].

Malignancy in VAD patients has been under study since the 1987 autopsy, where the deceased individual disclosed two tumours: a renal cell carcinoma in a renal cyst and a malignant glioma. The cause of death had been myocardial infarct, and no suspicion of malignancy was ever documented[7]. Until 1996, in a universe of 945 individuals, there were 41 known cases of malignancies, of which nine were multiple. They occurred in hollow organs or cavities, such as bladder, intestine, kidney, and lung. In the CNS, all tumours in these patients were malignant gliomas, where it is speculated that the skull functions as yet another cavity[9].

A genotoxic assay that measures the frequency of sister chromatid exchanges was applied to both LFN-exposed human[10,11] and animal[12] models. In both, the frequency of sister chromatid exchanges was statistically significantly increased, indicating that LFN can indeed be considered a genotoxic agent.

Within the context of VAD studies, the respiratory tract of LFN-exposed Wistar rats was studied using scanning and transmission electron microscopy[13]. Displasia and metaplasia of the tracheal epithelium was a frequent finding, especially after 4000 cumulative hours of exposure[13,14]. To date, no tumours have been identified in the LFN-exposed rat populations, most probably because rat life span is too short for tumoral development.

Thus far, 11 cases of respiratory tract tumours have been documented in VAD patients. This report will discuss the peculiar similarities among these cases, as well as the morphological features of these tumours.

Methods

Tumoral fragments obtained through endoscopic biopsy or surgery were collected from 11 male VAD patients (ave. age: 58 ± 9 years): 2 from the glottis and 9 from the lung. Of the 3 non-smokers, 2 had lung tumours and 1 had a glottis tumour. All subjects were employed as or retired helicopter pilots (N=5), fixed-wing aircraft pilots (N=4), and aircraft technicians (N=2). Fragments were immediately fixed either for light or electron microscopy.

Specimens for light microscopy were fixed in 10% buffered formalin, sectioned and prepared for histological observation using standard methods. The sections were stained with hematoxylin-eosin, Masson trichrome solution, chronotrop aniline blue, and PAS.

Specimens for electron microscopy were placed in a solution of 3% gluteraldehyde in

0.1 M phosphate buffer, pH 7.2 and then washed with several changes of 5% sucrose in 0.1 M phosphate buffer, pH 7.2, for ultrastructural studies.

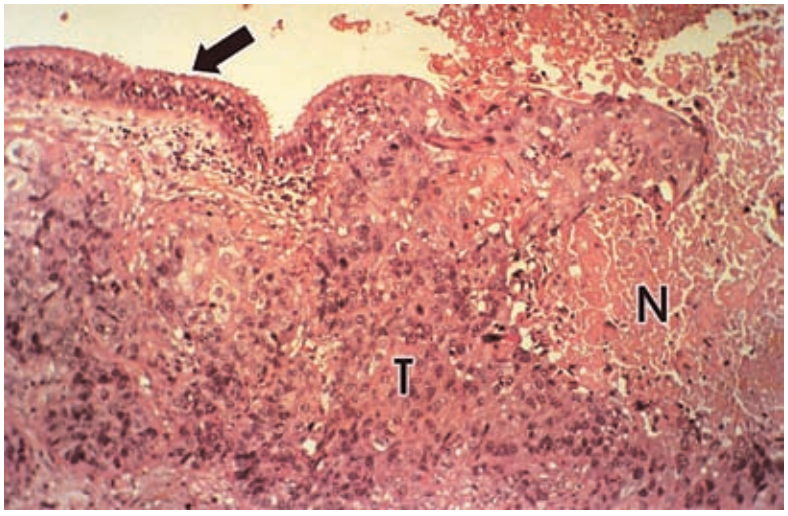

Fig. 1 – (Light microscopy x100) Bronchial tumor. From left to right: bronchial mucosa is almost normal with evident cilia (arrow); transition to tumor (T) and then necrotic tumor (N), deep in the bronchial wall. The structure is of a squamous cell carcinoma.

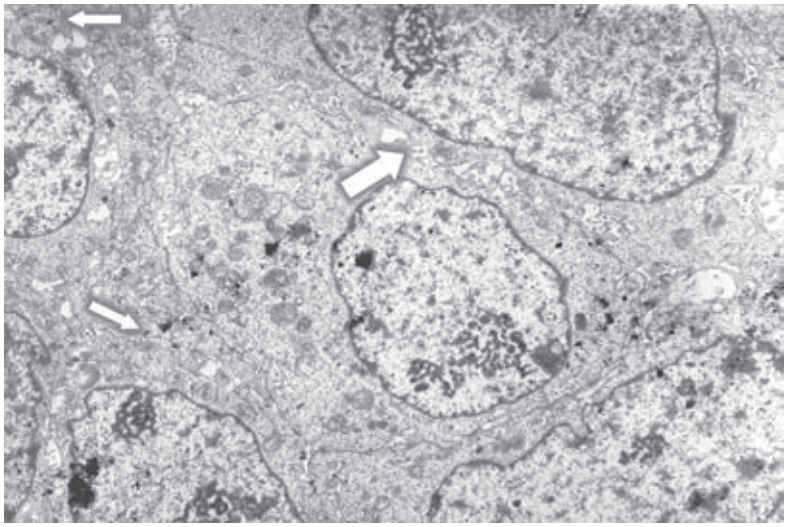

Fig. 2 – (TEM x5600) Poorly differentiated squamous cell carcinoma. Small tumor cells with desmosomes perpendicular to the cell membrane (small arrows). Tonofilaments are absent; compact aggregates of narrow cellular processes (filopodia) are seen (large arrow).

For TEM, samples were fixed at room temperature in an aldehyde mixture consisting of 4% paraformaldehyde, 1.25% glutaraldehyde, and 10mM $CaCl_2$ in 0.05 M cacodylate buffer, and pH 7.2. Specimens were washed in buffer, and post-fixed in a ferricyanide-reduced osmium solution made up of 1% potassium ferricyanide and 1% osmium tetroxide in distilled water, dehydrated through a graded ethanol series, and embedded in Epon. The samples were sectioned in an ultramicrotome (LKB, Sweden) and the thin sections stained with uranyl acetate and lead citrate. Preparations were then examined with electron microscopy (JEOL 100C, Japan).

Immunohistochemistry studies used chromagranine and synaptophysine staining.

Results

All lung tumours were located in the bronchi of the upper right lobe, and histologically, all were poorly differentiated squamous cell carcinomas. The search with neuroendocrine markers was negative in all cases.

Vast areas of necrosis was observed in all tumours (Fig.1). At the edges of the tumor, apoptotic cell death was the most common feature. Ultrastructurally, small desmosomes were visible, some perpendicular to the membranes of adjacent cells. (Fig. 2). Tonofilaments were scarce. No microvilli were identified in the intercellular processes, but microvillus-like structures (filopodia) were present (Fig. 2). This cellular pattern is typical of poorly differentiated squamous cell carcinomas.

All helicopter pilot tumours (5 cases) occurred before the age of 50 (range 44-48

years). Nine of the patients are deceased, including the 3 non-smokers. The 2 surviving patients are heavy smokers (more than 2 packs/day) who, after surgery, were retired and thus ceased to be exposed to LFN. One of the surviving patients, an airline pilot, received surgery 12 years ago, and remains under tight clinical surveillance, and still smoking heavily.

Discussion

Epidemiological studies indicate that squamous cell carcinomas account for approximately 40% of all lung tumours in men[15]. It seems to be highly relevant that all VAD patient respiratory tract tumours are squamous cell carcinomas, located in the right lobe. It is a question of time whether or not VAD patients appear with other types of respiratory tract tumours, depending on other aetiological factors for tumours to which these patients may also be exposed.

It is not surprising that the helicopter pilots are the ones who are affected the earliest since previous studies have shown that helicopter pilots exhibit the highest values for the frequency of sister chromatid exchanges[10]. This is most probably due to the synergistic effect of LFN and vibration, which is present in helicopters. In animal models, simultaneous exposure to LFN and vibration disclosed a higher frequency of sister chromatid exchanges than just LFN or just vibration alone[12].

Special attention should be paid to exposed workers over the age of 40, especially when LFN is associated (contaminated) with vibratory stress, as in the case of helicopters. Surveillance should be tight and appropriate physical examinations should occur every six months.

The fact that all lung tumours have developed in the upper right lobe might appear to be trivial. However, these results are corroborated by an experiment conducted by Ponomarkov *et al.* in 1969[16]. Here the authors explored the effects of wide-band noise at 105-155 dB on dogs . After 1.5-2 hours of exposure, the animals were sacrificed. Autopsy results revealed 3mm diameter haemorrhages in the lungs of the animals exposed to about 126 dB, and which were located under the pleura. These haemorrhages were most commonly found in the costal surface of the *upper lobe of the right lung*. There may be a biomechanical explanation for this feature: because of the position of the heart relative to the left lobe, the acoustical impedance and resonance of the left lobe will most probably be different than that of the right lobe. The difference in the acoustical properties inherent to different organ geometry most probably plays an important role in the location of the development of lung tumours in VAD patients (and haemorrhages in noise-exposed dogs), and may partially explain the apparent increased susceptibility of the right lobe.

Generally, epidemiological studies related to the incidence of cancer do not take histological tumour type into account. Given the results herein, it would seem of the utmost importance to begin specifying the exact histological type of tumour in all statistical studies. To compare the incidence of lung cancer in a group of LFN-workers with that of the general population without taking tumour-type into consideration may yield results that are, at best, misleading, and in reality, non-useful.

Conclusions

Special attention should be paid to LFN-exposed workers over the age of 40, especially when the acoustical environment is associated (contaminated) with vibratory stress, as in the case of helicopters. Surveillance should be tight and appropriate physical examinations supplied.

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