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DETERMINATION OF BLOOD SERUM ONCOPROTEIN NEU AND ANTIONCOPROTEIN p-53 - MOLECULAR BIOMARKERS IN VARIOUS TYPES OF OCCUPATIONAL EXPOSURE

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Abstract. Application of various diagnostic tests for occupational cancer risk monitoring is associated with the fact that gene mutations and changes in gene expression correspond to the earliest stages of carcinogenesis, namely early stages of the promotion process. The changes in protooncogenes and suppressor genes can be detected either at the genome level, at the level of transferring the genetic information from DNA to protein, or at the level of protein synthesis controlled by genes (oncogenes or antioncogenes). In the latter instance, as the concentrations of these proteins are considerably increased, their quantities in blood serum can be determined by the immunochemical methods. In our work, blood serum p-53 and NEU proteins were determined in 32 workers exposed to asbestos and in 57 workers exposed to PAHs. The proteins were also determined in 99 patients with overt cancer and in 47 controls. The data obtained in this work show positive values of oncoprotein NEU or antioncoprotein p-53 in 17.3% to 31.8% of workers exposed to PAHs or asbestos. The percentage of positive values for the examined proteins in the patients with overt cancer ranged from 12.5% to 42.5%. It should be noted that positive values of the oncoproteins detected in the biomaterial of the persons exposed do not mean that people must necessarily develop cancer, nevertheless the elevated values should be regarded as a warning and an implication for undertaking suitable preventive steps.

INTRODUCTION

Recent molecular epidemiology works have proved that cancers, both those involving hereditary genetic defects and those resulting from the exposure to carcinogenic chemicals, develop according to the routes of similar mutations resulting finally in genome destabilization and disturbances between the processes of proliferation and differentiation. Molecular biomarkers, such as oncogenes and antioncogenes (i.e. suppressor genes) play an essential role in the development of human cancer (12).

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Oncogenes trigger transition of healthy cells into cancer cells through oncoproteins. These proteins are synthesized under the control of these genes and according to their nucleotide sequence. The oncogenes may reveal their transforming effect on the cells either by increased synthesis of those proteins or by synthesizing a mutated form of that protein. Some protooncogenes may develop point mutations when exposed to environmental carcinogens. As a result, oncoprotein with modified characteristics appears in the cell (while the speed of the synthesis is the same). This modified oncoprotein may trigger the mechanisms responsible for uncontrolled cellular growth and division. About 100 oncogenes have been identified heretofore; c-erbB-2 protein, known also as NEU or Her-2, is one of them.

Antioncogenes, or suppressor genes, belong to the family of genes which, under normal conditions, prevent or even stop carcinogenesis (5). Gene p-53 is the best known antioncogene in this family. It plays an essential role in controlling the mitosis and cell differentiation cycle, DNA replication and apoptosis. Gene p-53, as a negative growth controller, able to block the proliferation of damaged-DNA cells, triggers repair mechanisms before new mitosis cycle is started (9).

Recent research has revealed that c-erbB-2 class gene activation and p-53 suppressor gene inactivation is associated, among other things, with gene point mutations induced by carcinogens, such as polycyclic aromatic hydrocarbons, asbestos, chromium, aromatic amines (3,6–8,11).

The discovery that protooncogene activation, suppressor gene inactivation, and the associated changes in the cell oncoprotein and antioncoprotein levels can be monitored by their determination in easily available biological fluids (blood and urine) has been essential for occupational medicine. It has been also observed that the increased oncoprotein and antioncoprotein levels in biological fluids may precede by many months or even years a clinically overt form of cancer, when it is still possible to undertake more efficient preventive steps, designed especially for people occupationally exposed to carcinogens.

Our current research has been intended to assess the feasibility of oncoprotein NEU and antioncoprotein p-53 determination in blood serum of workers exposed to carcinogens and attempted to utilize those determinations in preventive health care.

MATERIALS AND METHODS

Three groups of patients were studied: group 1 – comprised cancerous patients, group 2 – patients occupationally exposed to carcinogens, and group 3 – healthy people without a history of occupational exposure to carcinogens. The first stage of our study included examinations of patients with pulmonary, urinary bladder, and breast cancers. Two first cancers are frequently induced by occupational exposure, *inter alia*, to asbestos, PAH or aromatic amines, while up to now no relationship has been observed to occur between occupational exposure and the increased incidence of breast cancer in women. The second stage was devoted to determine blood serum p-53 and NEU proteins in groups of workers exposed to asbestos or PAHs. The asbestos-exposed workers were employed in the manufacture of asbestos/cement boards and insulation products. Those exposed to PAH were splitted into two subgroups: (a) workers of the electrolysis department in an aluminium plant (metallurgists), and (b) workers performing road and bridge repair works (roadmen).

The determinations were also performed in the group of healthy people not occupationally exposed to any carcinogens. Information on the age, and smoking habits was collected from all patients. In the exposed groups, duration of employment under the exposure was also recorded.

Blood serum NEU and p-53 concentrations were determined in the test groups. The method of blood serum NEU level determination was based on the immunoenzyme technique using three antibodies. The first antibodies, i.e. monoclonal mouse antibodies, were attached to the solid phase bottom of the well and 100 μl pre-diluted blood serum was added to the well. The samples were incubated for 18 h, and antibody-NEU oncoprotein was formed. Detector rabbit antibodies were added to the complex, which bind in proportion to the quantity of the NEU oncoprotein quantity present in the serum. The quantity of the oncoprotein-bound detector antibodies was determined by their ability to become bound to goat antibodies vs. horse-radish peroxidase-labelled rabbit antibodies. Horse-radish peroxidase catalyses conversion of chromogenic o-phenylendiamine into a coloured product determined by spectrophotometry. Immunoenzymatic method was also used for protein p-53 determination; two (mouse monoclonal and mouse detector) antibodies were used, and horse-radish peroxidase-labelled streptoavidin was applied instead of the goat antibodies. Oncogene Science Inc. (USA) reagent kits were used in our work to determine the two proteins.

In the control group not occupationally exposed to any harmful agents, NEU protein was determined in 47 people, and the cut off concentration value was determined as 95 percentile, which was 4200 HNU/ml or 210 fm/ml. Blood serum p-53 protein was determined in 36 people of the control group, and the cut-off concentration was set at 92 pg/ml. As already stated, the second group comprised cancerous patients; the determinations were performed in the patients with lung cancer (n = 40), urinary bladder cancer (n = 21) and breast cancer (n = 38). In the exposed group, the determinations were performed in 32 workers exposed to asbestos, and in 57 workers exposed to PAHs.

RESULTS AND DISCUSSION

Immunochemical determinations of blood serum oncogene proteins were performed in two basic groups, i.e. in patients with overt cancer and in people occupationally exposed to carcinogens. Table 1 shows the percentage of the positive results in patients with overt cancer. Test protein concentration values above the value corresponding to 95th percentile of the concentration determined for the control group were assumed to be positive for this group.

Table 1. The number and percentage of positive protein NEU and p-53 results in patients with overt cancer

Cancer	Protein NEU		Protein p-53	
	No.	%	No.	%
Breast cancer	38	18.4	24	12.5
Bladder cancer	21	33.3	21	28.6
Lung cancer	40	42.5	20	25.0

The mean age of cancerous patients was similar for all cancer types: 60.6 years for breast cancer, 65.3 years for bladder cancer and 62.0 years for lung cancer patients. The highest concentrations of the oncogene proteins were found in lung cancer: 643.8 pg/ml for p-53 and 13461 HNU/ml for the NEU protein. It should be noted that, for oncoprotein NEU, the highest percentage of the patients with blood serum concentration of this protein above the cut off was found in those with lung cancer. The percentage was 42.5%, while the lowest, 18.4%, was found in persons with breast cancer. In the group of bladder cancer patients, every third patient had an elevated blood serum oncoprotein level. Fig. 1 presents the percentage of patients with oncoprotein NEU values above the cut-off in patients with different cancer sites and in the healthy people. The results of p-53 determinations in cancerous patients were somewhat different. For this protein, the highest percentage of abnormal results was found in the group of patients with bladder cancer (28.6%). It should be noted, however, that in the group of patients with lung cancer, in which the highest total abnormal values of p-53 protein concentrations were noted in 25 of the patients examined, the highest blood serum concentrations of oncogene proteins were detected both for p-53 and NEU.

When analyzing the frequency of positive results of oncoprotein NEU and antioncoprotein p-53 concentrations vs. smoking habits, a higher incidence of positive results was noted only for patients with lung cancer. In the patients with urinary bladder or breast cancer, smoking was not found to affect the incidence of positive blood serum values of the proteins studied.

As a next step, we analyzed the behaviour of oncogene proteins in people occupationally exposed to carcinogens. As already stated, the tests were performed in people exposed to PAH and asbestos. Table 2 shows the percentage of positive results of blood serum p-53 and NEU determinations.

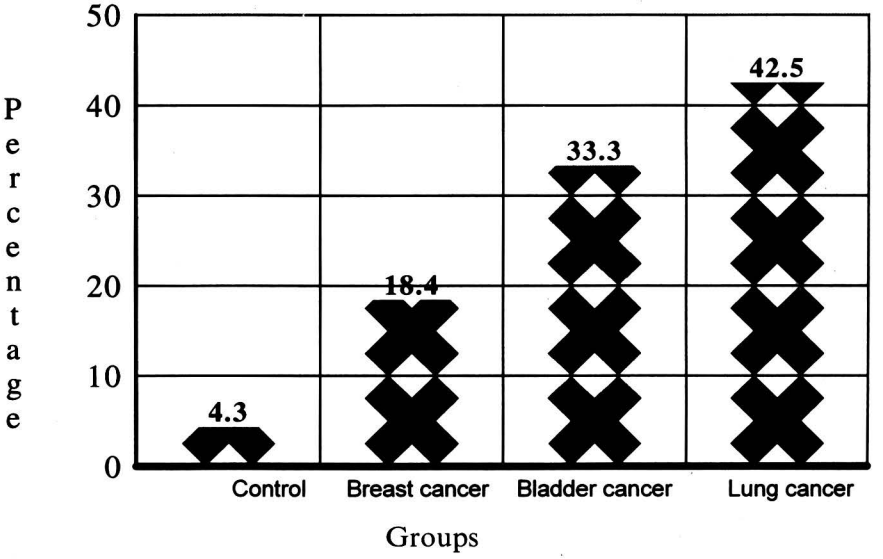

Fig. 1. The percentage of patients with protein NEU blood serum concentration above the 'cut off'.

Table 2. The number and percentage of positive blood serum NEU and p-53 results in workers exposed to asbestos and PAHs

Groups examined	Blood serum NEU		Blood serum p-53	
	No.	%	No.	%
Asbestos-exposed workers	22	31.8	32	28.1
PAH-exposed workers	57	21.0	52	17.3

Mean age of the asbestos-exposed workers was 50.0 ± 7.2 years; mean duration of asbestos-exposed employment was 19.1 ± 4.9 years; 78% people in this group were tobacco smokers. The largest quantity of protein NEU detected was 5900 HNU/ml, the corresponding value for p-53 was 119 pg/ml.

Mean age of the PAH-exposed workers was 44.5 ± 7.8 years; mean duration of PAH-exposed employment was 17.1 ± 9.5 years. Tobacco smokers made 79% of the group. The largest quantity of protein NEU detected was 5475 HNU/ml, the corresponding value for p-53 was 338 pg/ml. As mentioned before, the PAH-exposed group comprised two subgroups which differed in their incidence of positive oncogene protein concentration results. Among the roadmen, there were 28.1% of positive results for oncoprotein NEU and 14.8% for antioncoprotein p-53, while in the group of metallurgists, the respective values were 4.0% (NEU) and 12.0% (p-53). Table 3 summarises data concerning this group of workers.

Table 3. Characteristics of the PAH-exposed workers

Groups examined	No.	Age	Exposure (years)	Smokers (%)	Positive results of proteins (%)	
					NEU	p-53
Metallurgists	25	46.8 ± 4.4	22.8 ± 4.6	76	4.0	12.0
Roadmen	32	42.7 ± 9.4	12.5 ± 9.9	89	28.1	14.8

The data show clearly that the incidence of oncogene proteins was considerably higher in the roadmen, who were in direct contact with hot pitch released fumes. It is also worth stressing here that in this particular group the highest protein p-53 values were detected (337.9 pg/ml in a 52 year-old worker employed for 31 years, and 293.8 pg/ml in a 39 year-old worker with a 13-year history of PAH-exposed employment).

An attempt to apply oncoproteins and antioncoproteins found in biological fluids (blood, saliva, urine) as molecular markers of early changes in the cellular genome has been made by a team of American researchers headed by Brandt-Rauf (1,3), and the number of investigators interested in new methods continues to grow (6,7,9,11). Oncoproteins and antioncoproteins have been determined, for example, in exposures to polychlorinated biphenyls, asbestos, and chromium. The determinations performed in a group of 16 workers exposed to polychlorinated biphenyls revealed the presence of oncoproteins in three of them (3). During a retrospective study (2) involving 46 asbestos-exposed workers (blood serum samples collected from these workers were stored in a refrigerator) lung cancer was found in 9 of them, and at least one oncoprotein was detected in 7 blood serum samples stored in a blood bank for minimum two years.

In the work by Hanaka Yamano (7), protein p-53 was detected in 19 out of 31 chromium-exposed workers. All these works show that the determination of oncogene proteins in blood serum of people exposed to carcinogens may prove useful.

CONCLUSIONS

The results of our study indicated that:

1. The elevated (above the limit value) concentrations of oncoprotein NEU and antioncoprotein p-53 were present in 31.8% and 28.1% of asbestos-exposed workers, respectively.

2. In PAH-exposed workers there were 21.0% of abnormal results for NEU and 17.3% for p-53.

3. In the groups of workers exposed to carcinogens specified above, we failed to detect a relationship between the incidence of abnormal values of oncogene proteins in blood serum and the duration of the exposure, or smoking habits.

To sum up, it is worth noting that positive values of the oncogenes examined (oncoprotein NEU and antioncoprotein p-53) were found in 17.3% to 31.8% of workers exposed to PAHs or asbestos. Our results are expected to stimulate health services to start and continue targeted prophylaxis, which is essential in cancer prevention.

REFERENCES

1. Brandt-Rauf PW. New markers for monitoring occupational cancer: the example of oncogene proteins. J Occup Med 30: 399–404, 1988.
2. Brandt-Rauf PW, Smith S, Hemminki K et al. Serum oncoproteins and growth factors in asbestosis and silicosis patients. Int J Cancer 50: 881–885, 1992.
3. Brandt-Rauf PW. Niman HL. Serum screening for oncogene proteins in workers exposed to PCBs. Br J Ind Med 45: 689–695, 1989.
4. Brandt-Rauf PW, Smith SJ, Perera FP. Molecular epidemiology and environmental carcinogenesis of the lung. Probl Respi Care 3: 44–61, 1990.
5. Berbeć H. Gen p-53 in humans. Post Biochem 40: 6–11, 1994.
6. Fontanini G, Fiore L, Bigini D, Vignati, Calvo S et al. Levels of p-53 antigen in the serum of non-small cell lung cancer patients correlate with positive p-53 immunohistochemistry on tumor sections, tumor necrosis and nodal involvement. Int J Oncol 5: 553–558, 1994.
7. Hanaoka T, Yamano Y, Katsumo N, Kagawa J, Ishizu S. Elevated serum levels of pantropic p-53 proteins in chromium workers. Scand J Work Environ Health 23: 37–40, 1997.
8. Hemminki K, Partanen R, Koskinen H, Smith S, Carney W, Brandt-Rauf P. Serum p-53 protein in patients with asbestosis. Chest 109 (3 Suppl); 22–26, 1996.
9. Indulski JA, Lutz W. Molecular epidemiology: Cancer risk assessment using biomarkers for detecting early health effects in individuals exposed to occupational and environmental carcinogens. Rev Environ Health 12: 179–190, 1997.
10. Lutz W, Krajewska B. Oncoproteins and antioncoproteins of blood serum as biomarkers of early health effects induced by occupational and environmental carcinogens. Med Pr 47: 511–518, 1996 (in Polish).
11. Partanen R, Koskinen H, Oksa P et al. Serum oncoproteins in asbestosis patients. Clin Chem 41: 1844–1847, 1995.
12. Weinberg RA. Oncogenes, antioncogenes and the molecular bases of multistep carcinogenesis. Cancer Res 49: 3713–3721, 1989.

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