HEALTH RISK OF OCCUPATIONAL EXPOSURE IN WELDING PROCESSES II. IMMUNOLOGICAL EFFECTS

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Summary: Many of epidemiological studies have certified the relationship between welding and various forms of health damages. In our study we performed an immunological research within a group of twenty men, working in the risky environment of manufacturing of stainless steel constructions (11 welders and 9 grinders, average age was 31 years, 55 % of smokers, average time period in welding occupational exposure was 8 years). The exposed group of men was compared with a group of healthy blood donors, marked as the control group (people with various types of employment, living in same locality as a people from exposed group). People within the control group were not occupationally exposed to harmful chemical compounds (from 30 to 100 men were chosen for the individual immune parameters, average age of the whole group was 38 years, 40 % of smokers). When compared with the control group, the exposed group of welders and grinders showed higher level of C3 complement ($p<0.001$), orosomucoid ($p<0.05$), beta-2-microglobulin ($p<0.001$), neopterin ($p<0.001$) and all fagocytic cells ($p<0.001$). On the contrary, in the group of exposed people decreased values of IgA ($p<0.001$), IgG ($p<0.001$), IgM ($p<0.001$), transferin ($p<0.001$), alpha-1-antitrypsin ($p<0.001$), alpha-2-macroglobulin ($p<0.001$), haptoglobulin ($p<0.001$) and ceruloplasmin ($p<0.05$) were found. Some of these changes were characteristic for the exposed group. They could be considered as precursors of biological markers of effect for given type of exposure.

***Key words**: Welding; Grinding; Occupational exposure; Health risk; Immunotoxicity*

Introduction

Apart from the standard forms of biological monitoring of occupational exposure, represented by the analysis of original chemical substances or their metabolites in body fluids and by the analysis of enzyme activity changes, the area of immunotoxicological methods seems to be as a new promising approach (6,21,43). Investigated immune changes can contribute to the elucidation of the way of action of the toxic agents and represent also very sensitive markers of exposure (11,15,21,25,44). The immunotoxicological methods enable us to assess an impairment of the complex immunological processes (anti-infectious, anti-tumour immunity) including an influence of the partial functions of immunity (phagocytosis, production of cytokines, synthesis of antibodies etc.). A substantial effort should be dedicated to the complex description of immune system involving both, immunosuppressive and immunostimulating effects, and leading to the allergic or autoimmune reactions (31,32,46). However, actual scientific literature reported only limited number of immunological findings after occupational exposure (9,11,40,41).

The welding and grinding technologies belong among the well-known pollutant sources of occupational environment (4,5,28,38,39). Many epidemiological studies have already confirmed the correlation between the welding and various forms of health damages, including cancer (3,13,14,16,22,24,36). The malignant diseases were represented as a lung, bladder, throat and pancreatic carcinomas or as an increased risk of myeloid leukemia. In addition to the malignancies, the cases of contact dermatitis (2,33), localized skin erythema and asthma were described (27,34). Higher health risk level of exposure to welding fumes is evident from the facts mentioned above. In spite of this reality, we have found only a limited number of articles, describing the changes of cellular or humoral immunological markers after exposure to welding fumes (6,8,20,41,42). Presented work contributes to the better understanding to the relationships between given exposure and the immunological changes within exposed people.

Methods

Investigated groups

For immunological study a group of 20 workers (men), occupationally exposed to welding fumes was chosen (11 welders, 9 grinders, average age was 31 years, 55 % of smokers, average time period in welding occupational exposure was 8 years). The welding of stainless steel materials has been practiced by WIG method in protective atmosphere of argon. At the technologies of investigated industrial plant there was no possibility to construct an adequate control group of non-exposed workers. Due to this fact the immunological findings of the exposed group of workers were statistically compared with the findings of a group of healthy blood donors (men), marked as the control group (people of various types of occupations, living at the same locality as people from the exposed group). People from the control group were not occupationally exposed to harmful chemical compounds (from 30 to 100 men were chosen for the individual immune parameters, average age of the whole group was 38 years, 40 % of smokers).

Air analysis

Hygienic characteristics of investigated occupational environment and the results of cytogenetic analysis of exposed and control groups are described in work Borská et al. (7).

Biological samples and immunological methods

Coagulated and non-coagulated blood samples were collected from all persons of exposed and control groups. Phagocytic activity was tested through the yeast's ingestion and expressed as the count of phagocytic and phagocytosis capable cells. ELISA method was used to determine serum level of neopterin and beta-2-microglobulin. ELISA method was also used for the determination of IL-1 beta (Quantikine RDS, USA) and the total IgE (Immunotech, France) concentrations in serum. The IgE, IgA and IgM levels, C3 and C4 components of the complement, alpha-1-antitrypsin, alpha-2-macroglobulin, ceruloplasmin, orosomucoid, prealbumin, haptoglobin, transferin and CRP were determined by nephelometry methods, through the immunoreagencies of Beckman Company (USA).

Statistical analysis

For statistic evaluation of our results the "Sigma Stat System" by the Jandel Company (USA) was used. After control over normality of the data (Kolmogorov-Smirnov test), t-test and non-parametric Mann-Whitney tests were used for the comparison of investigated groups. The statistical process included the calculation of arithmetic means and standard deviations in particular subsets of analyzed parameters. In the next step, significance of the differences between calculated means of the subsets was tested.

Results

Cellular immunity

When compared with the control group (47.5 ± 8.25%) the exposed group of welders and grinders showed increased count of all phagocytic cells (61.15 ± 8.21%) on the highest level of significance ($p<0.001$) (Tab. 1).

Tab. 1: Statistical comparison of the exposed group of welders and grinders with the control group.

parameter	Mean ± SD (welders)	n (welders)	Mean ± SD (controls)	n (controls)	p value	test
Phag. efficiency of leuco (%)	61.15 ± 8.21	20	47.5 ± 8.25	50	< 0.001	t-test
Phag. efficiency of phago (%)	50.5 ± 10.9	20	NO	NO	NO	NO
IgG (g/l)	9.46 ± 1.01	20	13.39 ± 3.06	100	< 0.001	Mann-Wh
IgA (g/l)	2.08 ± 0.67	20	2.75 ± 0.77	100	< 0.001	Mann-Wh
IgM (g/l)	1.10 ± 0.33	20	1.70 ± 0.72	100	< 0.001	Mann-Wh
C3 complement (g/l)	1.05 ± 0.19	20	0.71 ± 0.19	100	< 0.001	Mann-Wh
C4 complement (g/l)	0.29 ± 0.07	20	0.30 ± 0.09	100	0.4	Mann-Wh
IgE total (IU/ml)	269.55 ± 405.81	20	129.30 ± 207.49	100	0.458	Mann-Wh
Beta-2-microglobulin (mg/l)	1.44 ± 0.16	20	0.92 ± 0.38	48	< 0.001	Mann-Wh
Neopterin (nmol/l)	11.86 ± 2.08	20	8.35 ± 2.62	48	< 0.001	t-test
IL-1beta (pg/ml)	3.35 ± 1.34	20	2.59 ± 1.45	35	0.062	t-test
Transferrin (g/l)	2.52 ± 0.32	20	3.00 ± 0.45	50	< 0.001	t-test
Alpha-1-antitrypsin (g/l)	1.45 ± 0.23	20	3.15 ± 0.68	50	< 0.001	t-test
Alpha-2-macroglobulin (g/l)	1.54 ± 0.28	20	2.05 ± 0.52	50	< 0.001	t-test
Haptoglobin (g/l)	1.25 ± 0.53	20	1.62 ± 0.31	50	< 0.001	t-test
Orosomucoid (g/l)	0.89 ± 0.18	20	0.75 ± 0.18	50	0.004	t-test
Prealbumin (g/l)	0.31 ± 0.03	20	0.32 ± 0.05	50	0.943	t-test
Ceruloplasmin (g/l)	0.37 ± 0.05	20	0.42 ± 0.08	50	0.018	t-test
CRP (g/l)	2.93 ± 6.27	20	2.50 ± 1.25	50	0.642	t-test

Mean – arithmetic mean; SD – standard deviation; n – number of persons; NO – no data; Mann-Wh – Mann Whitney test

Humoral immunity

The parameters of humoral immunity of the exposed group revealed a number of differences in comparison with the control group (Tab. 1). Significant elevation ($p<0.001$) has been observed within the levels of C3 component of the complement (exposed group 1.05 ± 0.19 g/l; control group 0.71 ± 0.19 g/l), neopterin (exposed group 11.86 ± 2.08 nmol/l; control group 8.35 ± 2.62 nmol/l) and beta-2-microglobulin (exposed group 1.44 ± 0.16 mg/l; control group 0.92 ± 0.38 mg/l). The increase on the level of significance of $p<0.01$ has been observed in the case of orosomucoid (exposed group 0.89 ± 0.18 g/l; control group 0.75 ± 0.18 g/l).

On the contrary, significant decrease ($p<0.001$) has been found within the levels of immunoglobulin IgA (exposed group 2.08 ± 0.67 mg/l; control group 2.75 ± 0.77 mg/l), immunoglobulin IgM (exposed group 1.10 ± 0.33 g/l; control group 1.70 ± 0.72 g/l) and total immunoglobulin IgG (exposed group 9.46 ± 1.01 g/l; control group 13.39 ± 3.06 g/l), transferin (exposed group 2.52 ± 0.32 g/l; control group 3.00 ± 0.45 g/l), alpha-1-antitrypsin (exposed group 1.45 ± 0.23 g/l; control group 3.15 ± 0.68 g/l), alpha-2-macroglobulin (exposed group 1.54 ± 0.28 g/l; control group 2.05 ± 0.52 g/l) and haptoglobin (exposed group 1.25 ± 0.53 g/l; control group 1.62 ± 0.31 g/l). The decrease on the level of significance of $p<0.05$ has been observed in the case of ceruloplasmin (exposed group 0.37 ± 0.05 g/l, control group 0.42 ± 0.08 g/l).

Discussion

Heavy metals (4,10,23,29) belong to the dominant harmful inorganic agents generated by the welding processes. In described occupational environment it is also possible to find even a lot of hazardous organic materials (for example polycyclic aromatic hydrocarbons) and physical agents (UV radiation) (17,18). According to the great variability of welding environment, it was quite difficult to perform an efficient group of tests for immunological evaluation (monitoring) of occupational risk (6).

The immune system takes part on keeping the homeostasis under variable conditions of internal and external environment (37). It keeps this role due to the ability of macrophages to swallow up noxious agents, too. This process – phagocytosis – is a significant mechanism of body defense and represents the most important part of natural nonadaptive immunity (11). It is also important factor of inflammation and autoimmune reactions. The number and activity of phagocyting cells that are in a direct contact with noxious agents can indicate the load of immune system. Scientific data about the influence of welding operations to phagocytosis are numerous but the results are often controversial in the meaning of the immunosuppressive and immunostimulant effects (20). In our previous study we have found a significant decrease in the total number of all phagocytic cells in the group of welders, working in an average 15 years with the stainless steel (6). In presented study we have found a significant increase of the total number of all phagocytic cells in the group of welders and grinders (workers were exposed to the welding fumes in an average about 8 years). According to these facts we suppose the occupational air contamination and exposure duration as the most important factors of phagocytosis influence.

Neopterin is produced by macrophages. The production is under influence of interferon gamma. Neopterin seems to be a sensitive indicator of immune activation (particularly the cellular activation) (45). It can also serve as a sensitive marker of changes in the system of cytokines that enable to evaluate the endogenous activity of cytokines. It has been proved that the majority of diseases, associated with activation of immune system (including high secretion of neopterin in all malignant tumors), increase the level of neopterine in urine and plasma (19,35). In accordance with the facts described above and with the results of our previous study (6) we have found significantly higher level of neopterine in exposed group of welders and grinders, too.

Beta-2-microglobulin is a part of HLA-I complex situated on a cell membranes. The increase of beta-2-microglobulin activity, as an important marker of activation of immune system, can be observed within many immunopathological and infectious conditions (26,30). Increased expression of beta-2-microglobulin is associated with higher exposure to antigenic substances. This situation indicates the presence of an exogenous substance in organism. The expression is usually being influenced by many factors, among others even by interferones and tumor necrosis factor alpha (12). In our study we have found significantly higher level of beta-2-microglobulin in exposed group of welders and grinders. In our previous study we have found significantly higher level of beta-2-microglobulin only in the case of exposed welders (6).

Pluripotent cytokine IL-1-beta represents key cytokine, produced by the antigen presenting cells (1). The changes within its concentration can be expected anywhere, where the immune system encounters an exogenous material (12). In a contrast to the results of our previous study (6) we have not found significantly increased level of IL-1-beta in the exposed group of welders and grinders.

The complement belongs among the most important defense mechanisms of an organism. Recognition of the antigen by specific antibody starts its classical cascade activation (12). For description of the complement function, the determination of its two important components C3 and C4 was chosen within presented study. Significant increase in the C3 component level in exposed welders was described by Hanovcová et al. (20) while significant increase in the C4 component level was described by Ulrich et al. (42). When we compared exposed group with the control we have found significantly higher level of C3 component only.

Production of antibodies is indicated as a principal function of specific humoral immunity (12). About 10 % of total plasma immunoglobulins belong to IgM class. The

IgM class is an antibody of early immune response for the most of antigens and represents also the most effective immunoglobulin class in the system of the complement fixation. All authors, Ulrich et al. (42), Hanovcová et al. (20) and Borská et al. (6) mentioned significant immunosuppressive effect of welding processes on the IgM level. We observed important decrease of the IgM level in stainless steel welders and grinders even in presented study.

About 75 % of total plasma immunoglobulins belong to IgG class. Boshnaková et al. (8) and Hanovcová et al. (20) have already observed decreased levels of IgG immunoglobulins in welders. Significantly lower IgG levels in exposed welders and grinders have been found also in presented study.

About 15 % of total plasma immunoglobulins belong to IgA class that is presented in both, monomeric and polymeric form. Wagner et al. (44) described significantly higher IgA levels in grinders. On the contrary with their results we found a significantly lower IgA levels in the exposed group of welders and grinders.

The interactions of chemical substances with endogenous macromolecules can lead to hypersensitive reactions. The simplest marker of the 1st type of hypersensitivity is a total level of IgE immunoglobulins. This indicator is very often markedly increased within the people suffering from atopy (12). In our previous work (6) we found significantly higher levels of total IgE in welders, working in an average about 15 years in welding processes. In presented study has not been found any significant difference in investigated levels of total IgE between exposed group (working in an average about 8 years in welding processes) and control group. We suppose that duration of professional exposure can play a significant role in variation of IgG expression, similarly as in the case of variation of phagocytosis.

The levels of proteins which are called "acute phase proteins" (alpha-1-antitrypsin, alpha-2-macroglobulin, transferin, orosomucoid, ceruloplasmin, haptoglobin, prealbumin and C-reactive protein) varied markedly during enhanced load of organism (trauma, surgery operations, malignity, stress, infections). Some of these proteins perform special transport functions and their levels are decreased during the acute inflammation (transferin, haptoglobin, prealbumin). The levels of other proteins are usually elevated during inflammation (CRP, alpha-1-antitrypsin, alpha-2-macroglobulin, orosomucoid, ceruloplasmin) (12). In accordance to our previous results (6) and to the results of Hanovcová et al. (20) we found significant changes of the proteins of acute phase when exposed and control groups were compared.

Conclusion

The complex character of exposure of stainless steel welders and grinders exclude simple applications of immunological tests for purposes of biological monitoring. However, in a large spectrum of used immune markers we have found a number of significant variations particularly characteristic for observed exposed group. We could think about these changes like about the precursors of biological markers of effect in early phases of damage of an organism.

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