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# CHRONIC LEAD POISONING, RENAL FUNCTION AND IMMUNE RESPONSE

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The aim of this study was to investigate possible correlations between chronic, recurrent lead poisoning, renal function and immune response. The study involved 74 patients with a history of at least one lead poisoning. Fifty-three patients were occupationally poisoned, and 21 were poisoned accidentally after consumption of alcohol beverages or food from lead glazed pottery. In all patients glomerular filtration rate (GFR) was determined by measuring creatinine and DTPA clearances, and T- and B-lymphocytes were assessed as indicators of cellular and humoral immunity. A significant negative correlation was found between the number of past lead poisonings indicating increased lead body burden, and both creatinine and DTPA clearances. There was a significant positive correlation between the number of poisonings and the percentage of B-lymphocytes ($r=0.31$; $P<0.05$), and no correlation at all with the T-lymphocyte count. Our results show that chronic, recurrent lead poisoning with a consequently increasing lead body burden can cause an impairment in renal function and a concomitant stimulation of humoral immunity.

*Key terms*:
alimentary exposure, B-lymphocytes, creatinine clearance, DTPA clearance, kidney, occupational exposure, T-lymphocytes

For centuries lead has been known to be inherently toxic to animals and humans. The principal target organ for toxic lead effects, besides the haemopoietic and nervous systems, is the kidney. During the past decade there has been an increasing evidence that lead can modulate immunity in animals (1-4). Most animal studies have demonstrated the immunosuppressive effect of lead (5, 6). However,

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there are some indices that immune abnormalities leading to self-reactivity may be related to accumulation of environmental lead (3). *Lawrence* (1) and *McCabe and Lawrence* (3) demonstrated a direct stimulating effect of lead on humoral immunity. This effect was partly mediated through a direct interaction with B-cells causing their differentiation into antibody-producing cells.

The present-day knowledge of the lead effects on the immune system in humans is incomplete due to the paucity of relevant human studies. Several main factors appear to be responsible for difficulties encountered in the assessment of immunotoxic processes in humans. First, the immune system is a highly complex and sophisticated organ system that operates at several levels; it interacts with the nervous and endocrine systems, and is highly sensitive to the effects of xenobiotics (7). Second, there is a network of immune processes related to multiple organ cell types. Those differ in sensitivity to external effects at various stages in their generative cell cycles (8). The immune system also comprises a number of alternative pathways and reserve processes that may be involved in the reaction to a stimulus.

The effect of chemicals on the immune system can be measured by intermediate and/or endpoint biomarkers. Those are used to estimate the exposure level, the internal dose of a toxic agent, the biologically effective dose, to identify an early phase of the disease (altered structure and function) as well as the disease endpoint (9). However, interpretation of such findings is difficult because there is a little information on the degree to which such parameters need to be modified in order to cause increased risk of disease. Furthermore, susceptibility

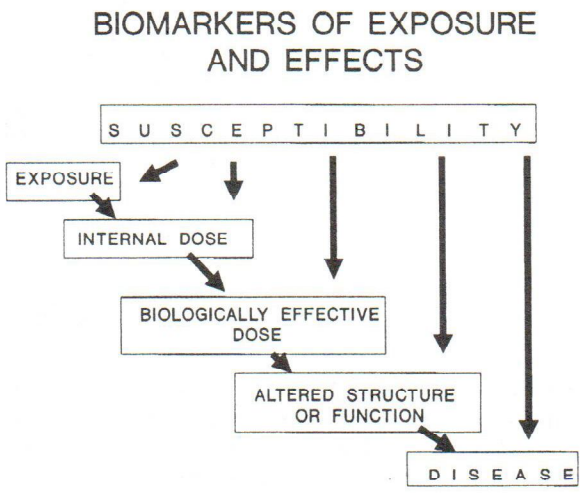


Figure 1 *Biomarkers of exposure, biomarkers of effects and biomarkers of host susceptibility reflect an interaction between the toxic agent and the biological system, including the susceptibility of an organism as an important factor in the pathway from exposure to disease*

of an organism that does not fit directly in the spectrum of exposure to disease, plays an important role in every step along the way and acts as a major determinant of whether or not disease will occur (Figure 1) (9).

Although additional direct evidence linking lead exposure to autoimmune disease is lacking, it should be noted that major organ systems overtly affected by lead (kidney, central nervous system and haemopoietic system) are sites of well-recognized autoimmune diseases (3). Slow and gradual evolution of chronic lead nephropathy and a different individual response to a toxic agent indicate that immunological mechanism(s) may be involved in its development. The aim of this study was to investigate the correlation between chronic, recurrent lead poisonings, renal function and immunological parameters.

## SUBJECTS AND METHODS

Investigation of the late effects of lead on the kidneys was carried out in 74 subjects in whom excessive lead exposure had caused one or even several episodes of symptomatic lead poisoning. Fifty-three of the 74 were poisoned due to occupational lead exposure and 21 were intoxicated by the consumption of alcohol beverages or food from lead glazed pottery. The subjects were chosen randomly from the register of lead poisoned patients who were treated in the Institute for Medical Research and Occupational Health in Zagreb over a period of more than 37 years (1951-1989). They all had characteristic clinical symptoms of acute lead poisoning (colic or diffuse abdominal pain, constipation and anaemia) followed with a feeling of weakness, nausea and headache. The diagnosis was confirmed by increased biological indices of lead absorption (erythrocyte protoporphyrin concentration >1.6 mmol/LE, delta-aminolaevulinic acid dehydratase activity ≤10 U/LE, and basophilic stippling <3 x $10^8$ E, in the earlier period, and blood lead ≥ 3 mmol/L; 62 mg/100 ml from the seventies). Since our patients returned to the same exposure after recovering, in some of them episodes of lead poisoning repeated. Thus, out of 74 lead poisoned patients 55 were poisoned once, 10 twice, five three times, three of them four times and one patient was poisoned even five times.

The mean age of occupationally poisoned subjects was 48 years (range 25-72) and the mean duration of exposure was 13 years, ranging from several months to 53 years. Most of the workers were poisoned during employment in a glass factory, storage-battery industry, lead glazed ceramics production, and some were construction workers and painters. The mean age of the group poisoned via the alimentary canal was 53 years (range 36-65). The use of lead glazed pottery being common in their households for many years, it was not possible to estimate the exact duration of lead exposure.

All subjects with a history or clinical evidence of previous and/or present renal disease of other aetiology, immunologic disturbance or subjects treated with some immunosuppressive drugs were excluded from the study.

Creatinine in serum and urine was determined by means of a VP automatic analyser (Abbott, USA). The glomerular filtration rate (GFR) was evaluated by measuring creatinine clearance, corrected to body surface area (ml/min/1.73 $m^2$) (10), and DTPA ($^{99m}$Tc-diethylenetriaminepenta-acetic acid) clearance (11). The percentage of T- and B-lymphocytes in blood as indicators of cellular and humoral immunity was also estimated (12).

Statistical analysis of the data was done by using the linear regression (CSS-Statistica Release 3.1, StatSoft, 1991 package).

## RESULTS AND DISCUSSION

Regression analysis showed a significant negative correlation between the number of lead poisonings in the past and creatinine clearance ($r=-0.32$; $P<0.01$) (Figure 2). Similar correlation was seen between past lead poisonings and DTPA clearance ($r=-0.30$, $P<0.05$), (Figure 3). Variable "number of lead poisonings" was used as a variable of chronic, cumulative lead exposure and increased lead body burden. Namely, it was assumed that with every further episode of symptomatic lead poisoning due to excessive lead exposure, an accumulation of lead in the patient's organism increased affecting adversely the kidney as a target organ. Our results showed a decrease of creatinine clearance and DTPA clearance as a measure of renal function (glomerular filtration rate) with an increased number of lead poisoning episodes. The number of poisonings was not related to age ($r=-0.15$,

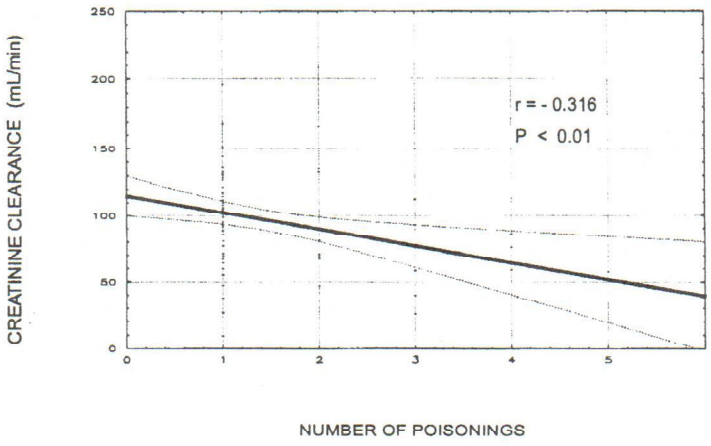


Figure 2 *Correlation between the number of past lead poisonings and creatinine clearance as an indicator of renal function*

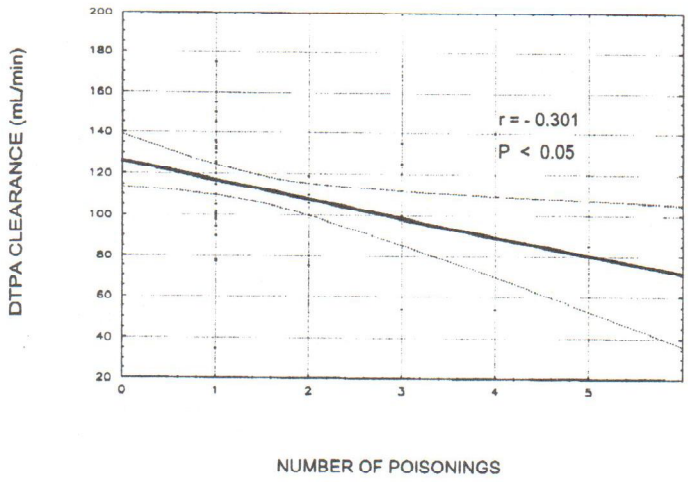


Figure 3 *Correlation between the number of past lead poisonings and DTPA clearance as an indicator of renal function*

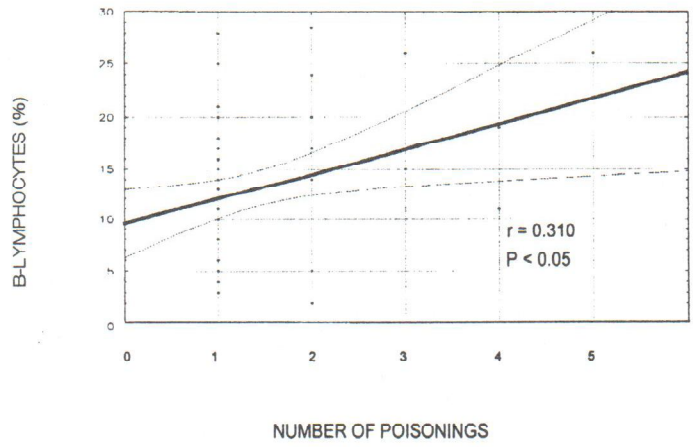


Figure 4 *Correlation between the number of past lead poisonings and the percentage of B-lymphocytes as an indicator of humoral immune response*

P=0.20), because repeated episodes of poisonings had been experienced irrelevant to the age of patients.

A significant positive correlation was seen between the number of lead poisonings and the percentage of B-lymphocytes as an indicator of humoral immunity. It was found that the number of B-lymphocytes in blood increased with a number of lead poisonings ($r=0.31$, $P<0.05$) (Figure 4). Correlation with T-lymphocytes was not found to indicate any effect on cellular immunity. There were also no

correlations between creatinine and DTPA clearances and immunological parameters.

Our results have shown that chronic, recurrent lead poisoning with consequent increasing lead body burden is associated with a decrease in a glomerular filtration rate and an increase in B-lymphocyte count in the blood. They also show that chronic lead poisoning can cause an impairment of renal function, and also a stimulation of humoral immunity (B-lymphocytes). The immunostimulating effect of lead has been previously described in some experimental studies (3, 13), indicating that lead enhanced B cell: $T_H$ cell interactions, which might lead to disrupted B cell responsiveness and autoimmunity. *Hambach and co-workers* (2) described a direct toxic effect of lead on suppressor T cells in an experiment *in vitro*. As most of the studies regarding the toxic effect of lead on the immune system have been carried out in experimental animals, only limited information is available in humans. *Coscia and co-workers* (14) demonstrated an increase in immunoglobulin concentration and lymphocyte B count in lead exposed workers. On the contrary, *Kimber and co-workers* (15) did not find any differences in serum concentration of immunoglobulin (IgG, IgA and IgM) between an exposed and a non-exposed group of workers. There was no correlation between blood lead concentrations and serum immunoglobulin level.

Some investigations suggest that immune mechanisms are probably involved in the development of interstitial nephritis due to chronic lead exposure. *Wedeen and co-workers* (16) found depositions of immunoglobulins IgG and IgM in renal biopsy specimens of lead exposed workers. Results of our study showed a significant correlation between the number of poisonings as an indicator of lead body burden and renal function on the one hand, and the number of poisonings and B-lymphocytes count on the other. However, a correlation between renal function and B-lymphocytes, which would suggest a direct role of immunological parameters in lead induced renal impairment, was not found. A wide array of immunological parameters has been used in an attempt to assess either descriptive or functional changes in the immune system following chemical exposure in humans. Unfortunately, these endpoints are poorly standardized and validation is incomplete so that interpretation of results is very difficult (17). Results from the experimental study of *McCabe and Lawrence* (13) suggested that lead can promote immune dysfunction characteristic of induction of an autoimmune disease. Since little is known about the aetiology of autoimmune diseases, a role of toxic agents such as lead warrants consideration.

Assessment of immunotoxicity in experimental animals is much easier than in humans, but it is rather difficult to extrapolate immunotoxic data from animals to man (9). Nevertheless, due to a high complexity of the human immune system, an explanation of contrasting and puzzling results of human studies needs to be based on many more animal studies (18). After reviewing the literature, *Fabri and De Lorenzo* (18) affirmed that lead definitely interacts with the immune system, and produces a variety of effects due to the diversity of the cell population that composed it. Due to very intricate interactions between chemicals and the organism, further studies and alternative approaches to human monitoring are necessary. Definitely more light needs to be shed on the clinical consequences

of the interaction between lead and the immune system in the context of human intoxication and long-term exposure to low doses of lead.

In conclusion, our results show that long-term lead exposure with recurrent episodes of acute lead poisoning indicating increasing lead body burden is associated with impaired renal function. Concurrent evidence of disturbed (stimulated) humoral immunity may suggest the role of the immune system in the nephrotoxic effects of lead.

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*Sažetak*

## KRONIČNO OTROVANJE OLOVOM, BUBREŽNA FUNKCIJA I IMUNOLOŠKI ODGOVOR

Pokusi na životinjama pokazali su da olovo može mijenjati imuni odgovor u životinja čak i u supkliničkim dozama. Međutim, malo je podataka o djelovanju olova na imunitet u ljudi. Procjenjivanje imunotoksičnih učinaka ksenobiotika u ljudi vrlo je teško iz više razloga. U prvom redu, imunološki sustav složen je i sofisticiran organ čije se djelovanje odvija na nekoliko razina u organizmu a sastoji se od mreže imunoloških procesa i reakcija različito osjetljivih na štetne tvari iz okoliša. Različit individualni odgovor na otrovnu tvar te postupan i često neprimjetan tijek olovne nefropatije u ljudi upućuju na moguće sudioništvo imunoloških mehanizama u njezinu nastanku. Svrha ovog rada bila je ispitati povezanost kroničnog, višestrukog otrovanja olovom, funkcije bubrega i imunološkog odgovora.
Ispitivanje je provedeno u skupini od 74 osobe jednom ili više puta otrovane olovom. Pedeset troje ispitanika bilo je profesionalno otrovano olovom, dok se preostali 21 otrovao nakon uživanja alkoholnih pića i hrane čuvane u posuđu glaziranom olovom. Svim ispitanicima izmjerena je veličina glomerularne filtracije metodom klirensa kreatinina i klirensa DTPA te postotak limfocita T i B u perifernoj krvi. Analizom regresije nađene su značajne negativne korelacije između broja prethodnih otrovanja olovom kao pokazatelja kronične, kumulativne izloženosti olovu i klirensa kreatinina, odnosno klirensa DTPA. Također je utvrđena značajna pozitivna povezanost između broja otrovanja i limfocita B, dok korelacija s limfocitima T nije nađena. Rezultati pokazuju da je kronično otrovanje olovom s posljedičnim povećanjem tjelesnog opterećenja tim metalom u svezi s oštećenjem funkcije bubrega kao i sa stimulacijom humoralnog imuniteta. Iako je ispitivanje imunotoksičnosti u ljudi vrlo složen problem, ovi rezultati upućuju na moguće djelovanje olova na imunološke mehanizme kao i na njihovu ulogu u nastanku olovne nefropatije.

*Ključne riječi:*
alimentarna izloženost, bubreg, klirens DTPA, klirens kreatinina, limfociti B, limfociti T, profesionalna izloženost

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