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Decreased immune status in Nigerian workers occupationally exposed to lead

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Summary

Some immunological indices of 80 Nigerian lead workers were assessed. The mean blood lead level (BLL) in the study subjects was significantly higher than in control non-exposed subjects *($p<0.001$)*. The mean values for total globulins and C-reactive protein (CRP) were also significantly raised in the test subjects. *($P<0.001$, $P<0.01$ respectively)*. In contrast, levels of IgA and IgT were significantly depressed *($P<0.01$, $P<0.001$ respectively)*. Furthermore, a significant negative correlation was established between IgA and BLL ($r= 0.28$ $P<0.009$). Ascorbate excretion was also significantly reduced in the exposed workers when compared with control subjects. *($P<0.02$)* Multiple regression analysis established signifcant interaction between BLL and total globulin and IgA I*($P<0.01$, $P<0.01$, 0.28 and $P<0.009$ respectively)*. Principal component analysis showed that CRP, TLC, IgA and IgC have strong interactions with BLL. These data suggest depressed immune status in workers occupationally exposed to lead. This clinical state may be modulated by genetic and nutritional factors (such as ascorbate level).

The control population was made up of 50 volunteers who had never been exposed to lead. Their mean age was 36.6 (semi 1.2 range 22-58) years. Informed consent was obtrained from all 130 subjects and the ethical committee of the college of Medicine Ibadan, approved the conduct of the study. Additionally, it may predispose the subjects to increased susceptibility to infectious diseases, inflammatory disorders and cancer.

Keywords

Immunosupression, Immunotoxicology, Immuneparesis, plumbism, Immunoresponsiveness, hypersensitivity.

Résumé

Certains indices de 80 traveilleurs Nigerians exposés au plomb ont été mesurés. Le taux moyen de plomb (BLL) chez les sujets étudiés a été significativement élevé comparé aux sujets controles non exposes ($P < 0.001$). Les moyennes totale de globulines et de proteines reactive-C (CRP) ont aussi été significativement élevé chez les sujets testés ($P < 0.001$, $P < 0.01$ respectivement). Par contre, les taux d'IgG et d'IgA ont été significativement faible ($P < 0.010$, $P < 0.001$ respectivement). Le compte total de lymphocytes (TLC) a aussi été significativement reduit ($P < 0.001$). Plus encore, une correlation negative significative a été etablie entre l'IgA et la BLL ($r = -0.28$, $P < 0.009$). L'excretion de l'ascorbate a aussi été significativement reduite chez les travailleurs exposés comparé aux sujets controles ($P < 0,02$). Les analyses de regression multiple ont etablient une interaction significative entre la BLL, le taux total de globuline et d'IgA ($P < 0,001$, $P < 0,01$, $r = 0.28$ et $P < 0,009$ respectivement). Le composant principale de l'analyse a montré que la CRP, la TLC, l'IgA et l'IgG ont des interactions forte avec la BLL. Ces données suggerent un status de baisse d'immunité chez les travailleurs exposés aux plombs..

Cet etat clinique pourrait etre modulé par des facteurs genetiques et nutritionelles (telsque le taux de l'ascorbate).

Deplus, elle pourrait predisposé les sujets à une susceptibilité corissance aux maladies infectueuses, les desordres inflammatoires et des cancers.

Introduction

In recent years, there has been increasing concern about the effects of occupational and environmental pollutants on immune status [1]. Lead (Pb) is one of such pollutants. The element and its compounds have been shown to induce suppression or decreased host resistance to infectious agents in experimental models [2-4]. There is also a strong evidence that host resistance may be altered in humans on Pb exposure [5]. Among the suggested mechanisms for the interactive roles of Pb on the immune system are inactivation of antibodies [3] and interference with phagocytic activity of polymorphonuclear leucocytes [3]. However, reports and findings on this subject have not been consistent. Reigart and Garber [6] had, in their study, indicated that Pb had little effect on serum immunoglobulins in subjects chronically exposed to the element. Imunotoxicology is therefore still controversial, partly because definitive mechanisms of the action of toxicants on the immune system have not been completely elucidated.

In prolonged exposure to Pb, the extent of harm based on blood levels and on intra-individual physiologic differences remains difficult to predict. There is therefore a current need to ascertain the immune status of Pb workers in order to determine whether there are significant health risks in occupational exposure to Pb-induced immune dysfunction.

The increasing abundance of Pb in our environment, a normal consequence of progressive industrialization, demands that we establish good measure of human risks. It is desirable to know if risk are insignificant or are substantial in order to provide an intelligent occupational and environmental health policy.

Materials and methods

(i) *Subjects*: The study subjects were 80, all males. They were drawn from various lead-based occupations. Their mean age was 36 (SEM 0.03, range 21-66) years and were all adjudged to be clinically healthy, based on the administration of a medical and social questionnaire. Most of the subjects (93%) had

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a low level of education and had little knowledge of the risk of continued exposure to lead. The dietary evaluation of all subjects was carried out by means of a 24-hour dietary recall [7,8]. This procedure consists of a carefully elicited recollection of all foods and beverages consumed over 24 hours. The control population was made up of 50 volunteers who had never been exposed to head. Their mean age was 36.6 (Semi 1.2., range 22-58) years. Informed consent was obtain from all 130 subjects and the ethnical committee of the college of medicine Ibadan, approved the conduct of the study.

(ii) *Methods:* 15 ml venous blood sample and a random urine sample were collected from each of the 130 subjects. Whole blood lead concentration was determined by Atomic Absorption Specttroscopy using the modified method of Hesse [9]. Plasma total protein, albumin and globulin were measured and calculated using standard methods [10]. C-reative protein (CRP) was determined using the Serascan C-reactive protein kit (Hycor Biomedical Inc. Chapman Ave, Garden Grove, California). Immunoglobulin A,G and M (IgA, IgG, IgM) levels were measured by the method of Mancini et al. [11] using Biomedical Accuplate RID Test System (Hycor Biomedical In. Von Karmen Ave, Irvine.

Total lymphocyte count (TLC) was assessed by first performing total leukocyte count, then total lymphocyte count was carried out by finding our the proportion of each type of white cell in a thin blood film stained with Giemsa's stain (differential count). Two hundred cells were examined for each subject, classified, and the results were expressed, first as percentage of total leucocyte count, then actual number of lymphocytes (absolute number of lymphocytes) per mm^3 according to the method of Kirkshaw [12]. The methods are reasonably precise and accurate with a standard deviation (S.D.) of 10 and a coefficient of variation C.V. of 10% [13]. Ascorbate excretion rate was determined in freshly voided urine using the standard 2,6 dichlorophenol indophenol method [14]. Urinary creatinine was determined by standard Jaffe reaction to standardize ascorbate excretion rate.

Statistical methods included student's 't' test for unpaired data, Pearson's product moment formula, single and multiple regression analysis and principal component analysis. Principal component is considered to be an exploratory technique that may be useful in gaining a better understanding of the interrelationships among highly interrelated variable. It is performed to determine whether a set of components will be linear functions of the original data. The first two or three principal components explain most of the variations in the original data. It has been described by Afifi and Clark [15] as a fine statistical tool. The dispersion of data was represented by standard error of mean (SEM).

Results

Dietary recall studies showed that both exposed and unexpected control subjects were on similar diets (Table 6).

Table 1: Blood lead level and urinary ascorbate level in lead workers and controls

Variables	Lead workers	Controls	T	P
Blood lead level(ug./dL	56.3 ± 0.95	30.4 ± 1.4	18.91	< 0.001
Urinary ascorbate (mg/100 mg creatinine)	9.0 ± 1.62	14.3 ± 1.23	2.57	< 0.02

Values are means ± S.E.M.

Table 2: Immunological indices in lead workers and controls.

Immune parameters	Lead workers (n = 80)	Controls (n = 50)	T	P
Total lymphocyte count/ mm^3	2157 ± 63	2515 ± 115	2.74	< 0.01
Total globulin (g/dL)	3.73 ± 0.05	3.20 ± 0.07	6.84	<0.001
IgA (mg/dL)	143.79 ± 6.76	187.51± 14.2	2.62	<0.01
IgG (mg/dL)	1187.73 ± 65.33	1997.33 ± 108.33	6.79	<0.0001
IgM (mg/dL)	190.87 ± 11.76	215.43 ± 12.66	1.25	>0.05
CRP (mg/dL)	0.60 ± 0.03	0.50 ± 0.03	2.56	<0.01

Values are means ± S.E.M.
n = number of subjects

Table 3: Correlation between blood lead levels and IgA in lead workers

Blood lead level versus IgA	n	r	P
BLL Vs IgA	80	-0 28	<0.009

Table 4: Multiple regression characteristic in lead workers and controls (combined, n = 130).

Parameters	P
Total globulin	<0.01
IgA	<0.01

Table 5: Principal component analysis

	Prin. 1	Princ. 2	Prin. 3
IgA	0.004385	0 021249*	0.013958
IgG	0.036065	0.233100*	0.969987*
IgM	0.004245	0.011562	.008751
TLC	0.998096*	-0.0054524*	-024729
CRP	-014010	-002525	-049430*
Eigenvectors	3751773	1311887	621962
Percent total variation	64 (%)	23(%)	11(%)

Significant principal component ratios are asterisked.

Table 6: Dietary intake in lead workers and controls.

Protein intake	High	Moderate	Low
Lead workers	10(12.5)	30(37.5)	40(50.0)
Controls	7(14.0)	16(32.0)	27(54.0)
Milk/Dairy products intake			
Lead workers	10(12.5)	20(25.0)	50(62.5)
Controls	7(14.0)	13(26.0)	30(60.0)
Fat/oil intake			
Lead workers	11(13.8)	42(52.5)	27(33.8)
Controls	7(14.0)	26(52.0)	17(34.0)

Number in parentheses represent percentage (%)

The mean blood lead level (BLL) in test subjects was significantly higher than in controls *(P<0.001,* Table 1). Total globulins and CRP levels were also significantly higher in the exposed test subjects than in non-exposed control subjects (P<0.01, P<0.01 respectively, Table 2).

In contrast, TLC, IgA, IgG were all significantly depressed in lead workers *(P<0.01, P<0.01, P<0.001,* respectively, Table 2). Furthermore, IgA showed a significantly negative correlation with BLL r = 0.28, *P*<0.009, Table 3). Ascorbate excretion rate was significantly lower in Pb workers than in controls *(P<0.02,* Table 1). Multiple regression analysis established statistically significant interactions between BLL and total globulins *(P<0.01)* and IgA *(P<0.01)* (Table 4). On the other hand, principal component analysis showed that TLC, IgA and IgG all have strong interactions with BLL (Table 5).

Discussion

The elevated BLL in Pb workers was not surprising. Lead workers are well known to carry a higher Pb burden than the rest of the population [16]. Evaluation of immune status in the selected subjects provides useful information. Total lymphocytes have roles in both cell-mediated immunity (CMI) as well as humoral immunity (HMI) and are indeed more strongly indicative of alterations in CMI [17]. The significantly lower TLC therefore suggests a very strong association with depressed CMI (Table 2). In adult humans, the principal lymphocytes in the peripheral blood are the T lymphocytes, thus the suggestion of depressed CMI appears strongly plausible. Similar findings had been reported by various other investigators [1,18-19].

Others have failed to demonstrate any indication of immunosuppressive effect on CMI in Pb workers and among suggestions for such a discrepancy are route of exposure and presence of mercaptoethanol in reaction media in experimental models. [1,20].

Contrary to expectation, the concentration of total globulins was found to be significantly elevated *(P<0.001, Table 2).* It is not clear if this represents a polyclonal response to the presence of Pb. Interestingly, CRP level was also significantly elevated in the test subjects *(P<0.01, Table 2).* CRP is an acute phase reactant. The increase in total globulin may therefore be suggestive of an acute inflammatory response to the presence of Pb. Two of the principal immunoglobulins responsible for HMI, IgA and IgG, were significantly depressed in Pb workers. These data are therefore suggestive of HMI immunosuppression, probably an immunotoxic effect of Pb. The elevated total globulin level and concomitant decrease in the immunoglubulins suggest the need to always evaluate individual immunoglublins in order to establish immunosuppression. The humoral immuno suppressiveness being suggested by these data is consistent with the findings of previous investigators [20,21]. The present study represents the first report of Pb-induced imunosuppression in Pb workers in this environment.

It should not be surprising that IgM level was not significantly depressed. IgM is normally synthesized in significant amounts in the primary phase of immune response, while IgG and IgA are only abundantly produced in the secondary phase. Occupational lead toxicity is a chronic disorder arising through the cumulative effect of lead. Thus it is to be expected that the Igs secreted abundantly in the secondary phrase that will be more severely depressed.

Conflicting and confusing data which suggest enhanced immunoresponsiveness (hypersensitivity) instead of immuno suppression in Pb workers have also been reported [22,23]. Explanations for such inconsistencies are yet to be clearly elucidated.

However, possible mechanisms such as genetic and nutritional factors have been suggested to explain the observed discrepancy in immune response to Pb [20]. It has been argued that response to lead exposure may be genetically determined. Thus, it is possible that Pb workers from this environment are genetically susceptible to the immunosuppressive effect of Pb This implies the need to discern any regional patterns in reports of Pb-induced immune response to confirm or exclude genetic predisposition as a modulating factor. The other alternative interactive factor suggested is nutritional status; this also requires further comment

In the present study, dietary intake was similar between control and test subject (Table 6) Therefore, any alteration in the immune status may be Pb-induced It is well documented that many nutrients are required in adequate amounts for optimum immune response [24]. The mobilization of nutrients to counteract the toxic effects of Pb in its principal target sites such as the bone, CNS and the kidney may diminish the bioavailability of these nutrients in other anatomic sites such as the lymphoid system, resulting immune paresis. In this report, ascorbate excretion rate was lower in Pb workers than in controls, probably due to increased metabolism to counteract the toxicant. Ascorbate plays important roles in immune function [25].

The health implications of immunosuppression should be well recognised. In this environment, it likely to increase susceptibility to infectious diseases. The depression in immune status is probably the immunological basis of a previous observation that Pb workers have more colds and influenza infections per year than the rest of the population [26, 27]

Deficiencies and disturbances in immune function not only increase the risk of contracting allergies and infectious diseases but also auto-immune diseases, arthritis and cancer [26]. In addition, the changes that follow the ageing process occur earlier if the immune system is already weakened [26].

Thus, the data in this report may have far-reaching health implications; consistent confirmation of findings in this report should therefore be given proper and sufficient attention.

References

1. Faith RE, Luster MI, Kinnel CA. Effect of chronic developmental lead exposure on cell mediated immune functions. Clin. Exp. Immunol 1979 35: 413-420
2. Hemphil FE, Kaeberle ML, Buek WB. Lead suppression of mouse resistance to *Salmonella typhimurium*. Science 1971; 172: 1031-1032.
3. DeBruin A. Certain biological effects of lead upon the animal organism. Arch Environ Health 1971; 23: 249-264.s
4. Exon JH, Koller LK, Kervliet BI. Lead-cadmium interaction effects on viral-induced mortality and tissue residues in mice. Arch Environ Health 1979; 34; 469-475.
5. Sachs, HK. Intercurrent infections in lead poisoning. Amer J. Dis Childh 1978; 32: 315-316.
6. Reigart JR, Garber CD. Evaluation of the humoral immune response of children with low level lead exposure. Bull Environ ContamToxicol 1976; 16: 112-117.
7. Young E.A, Wesser E. Nutritional assessment and therapy. In: Internal Medicine, Diagnosis and Therapy. Stein JH (ed) 2nd edn. Prentice-Hall International Inc, East Norwalk Connect. 1991; 254-275.
8. Thompson FE, Byers T. Dietary assessment resource manual J. Nutr. 1994; 124; 2245S-2317S
9. Hessel DW. A simple and rapid quantitative determination of lead in blood. At Abs Newsletter 1968; 750-755
10. Reinhold JG. Standard methods in clinical chemistry. Reiner, Me (ed) Vol. 1 Acad. Press, New York. P. 88.
11. Mancini G, Carbonora AO, Heremans, JF Immuno-chemical diffusion. Immunochemistry 1965; 2: 235-254.
12. Kirkshaw Y. Haematology. In Clive CJC, Peel, RN, James KR, Kirkshaw, Y. Basic medical laboratory technology. Pitman Medical Publishing Co. Ltd., England, 1975, p.225.
13. Dacie, JU, LEWIS SM. Practical Haematology. Edingburgh; Churchill-Livingstone, 1975. P.48.
14. Harris LJ, Ray SD Determination of ascorbic acid in urine, method using titration with 2, 6-dichlorophenol indophenol. Lancet, 1935; 71; 462.
15. Afifi AA, Clarks V. Computer aided multivariate analysis, 2nd edition, Chapman and Hall, 1990. London, pp 7, 371-394.
16. Kim. Y, Harada, K Ohmori, S Lee BK, Mivra, H, Veda A. Evaluation of lead exposure in workers at lead-acid battery in Korea: With focus on activity of erythrocyte pyrimidine 5-nucleotidase (p.5N). Occup. Environ. Med. 1195; 52:484-488
17. Fakhir S. Ahmad P, Faridi, MMA, Ratta, A. Cell mediated immune responses in malnourished host. J. Trop. Pediat 1989; 35: 175-178.
18. Castillo-Mendez A, Rodriqez-Diaz T, Leon-Lobeck A. Gravalosa-Cruz AJ. Influence of lead exposure on the concentration of immunoglobulins and immune cellular functions in humans . Rev. Aller-Mex .1991; 38: 69-72.
19. Castillo-Menzez A, Rodriquez DT. Leon-Lobeck a. Effect of occupational lead exposure on the immunoglobulin concentration and cellular imunne function in man. Rev. Allerg 1993; 40: 95-97.
20. Dean, JH, Murray MJ. Toxic responses of the immune system. In: Casarett and Doull's Toxicology: The basic science of poisons. Amdur MO, Doull J, Klassen CD (EDS) 4TH edn. Pergamon Press 1991; New York pp 282-333.
21. Jaremin B. Immunological humoral responsiveness in men occupationally exposed to lead. Bull Inst. Trop Med. Gdmia 1990; 4: 27-36.
22. Borella, P, Bargellini, A. Effects of trace elements on immune system: results in cultured human lymphocytes. Trace Elem Electroly Health Dis 1993; 7: 231-233.
23. Borella P, Giardino A. Lead and cadmium at very low doses after in vitro immune response of human lymphocytes. Environ Res. 1991; 55; 165-167.
24. Stites DP, Terr AI. Physiologic and environmental influence on the immune system. In: Basic Human Immunology. 1991. Prentice-Hall International Inc. p. 193.
25. West WL, Knight, EM, Edwards, CH. Maternal low level lead and pregnancy outcomes J. Nutr 1994:124: 9815-986S
26. Tolonen M. Vitamins and Minerals in Health and Nutrition, Ellis Horwoord, New York, 1990 pp. 11-45, 46-68.
27. Ewers U, Stiller-Winkler B, Idel. H. Serum immunoglobulin, complement C3 and salivary IgA level in lead workers. Environ Res 1982: 29: 351-357.