

Clinical note

Trace element profile in pemphigus foliaceus and in pemphigus vulgaris patients from Southeastern Brazil

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ABSTRACT

Pemphigus foliaceus (PF) and pemphigus vulgaris (PV) are autoimmune bullous diseases; they are endemic in the northeastern region of the state of São Paulo, Southeastern Brazil. Patients' copper (Cu), zinc (Zn), and selenium (Se) metabolic deficiencies have already been associated with PV pathogenesis in the Middle East but not in Brazil. Lead (Pb), Cu, Zn, and Se concentrations were determined in whole blood or serum samples obtained from 56 PV patients, 62 PF patients, and 135 healthy controls from the endemic region and compared. The PV patients had higher (above the reference values) Pb and lower Zn levels as compared to controls. Both the PV and the PF patients presented decreased Se levels as compared to controls. The PV, PF, and control groups did not differ in terms of Cu concentration. These results indicate that high Pb blood levels are related with environmental contamination and consequently with PV pathogenesis. Moreover, Zn and Se depletion, observed in the PV patients and in the PV and the PF patients, respectively, may result from metabolic consumption verified in chronic diseases.

1. Introduction

Exposure to trace elements may disrupt immune system homeostasis and might be linked to autoimmune diseases [1,2]. Exposure effects depend on the extent of exposure and on the trace element route, dose contamination, and chemical form [3].

Immunomodulation is related to trace element deficiency or to environmental contamination by lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), and selenium (Se) [4–7]. For instance, Zn, Cu, and Se are essential to immune system integrity, but high levels of these elements may harm the organism [8,9]. The immunomodulatory role played by Pb may increase expression of MHC class II molecules [10,11], exacerbate production of IL-4 and TNF cytokines, and drive immune response to a Th2 pattern [12–14].

Pemphigus is a group of autoimmune bullous diseases characterized by IgG autoantibody production against desmoglein (Dsg), which causes intraepidermal acantholytic blisters. The two main clinical forms

of pemphigus are pemphigus vulgaris (PV), which can affect mucous membranes and the skin through anti-Dsg3 and anti-Dsg1 production, and pemphigus foliaceus (PF), in which anti-Dsg1 production exclusively affects the skin [15–17]. Albeit unclear, pemphigus pathogenesis has been linked to a combination of genetic [18–24] and environmental factors, such as stress, virus, medications, radiotherapy, UV radiation, nutritional elements, neoplasm, infectious agents, and, more recently, blackfly and sandfly bites [15,19,22,24–31], contributing to an individual's susceptibility to the disease. PF is endemic in the northeastern region of the state of São Paulo, an area where the concentration of pemphigus patients is high. In the last decades, the number of PV cases diagnosed in this area where PF is endemic has surpassed the PF incidence [28,32].

The trace elements Hg, Se, Cu, and Zn have been linked to pemphigus pathogenesis [1,33–35]. Moreover, Zn, Se, and Cu deficiencies have been associated with enhanced oxidative stress in PV patients [33].

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Table 1
Demographic and clinical data of the studied groups.

		Pemphigus vulgaris (n = 56)	Pemphigus foliaceus (n = 62)	Controls (n = 135)	P-value
Mean age ± SD (years)		52.8 ± 15.4 ^a	42.8 ± 17.1 ^b	42.6 ± 16.7 ^{c*}	^{a,b} 0.004 ^{a,c} 0.0004
Gender n (%)	Man	20 (35.7)	25 (40.3)	52 (38.5)	0.87
	Woman	36 (64.3)	37 (59.7)	83 (61.5)	
Treatment on sample's collection date	Yes	38 (70.4)	42 (73.7)	N.A.	0.057
	No started	4 (7.4)	5 (8.8)		
	No > 60 days	12 (22.2)	10 (17.5)	N.A.	
Pemphigus clinical activity on sample's collection date	Yes	32 (59.3)	33 (59.3)	N.A.	0.116
	No	23 (40.7)	24 (40.7)		

N.A. = not applicable; SD = standard deviation; *based on n = 131 (4 missing data);

Taking advantage of the fact that both clinical forms of pemphigus are endemic in our region, we took an interest in verifying trace element concentrations in pemphigus patients in order to compare them in PV patients, PF patients, and controls living in the same region.

2. Material and methods

This study was approved by the local Research Ethics Committee (# 423/2011).

Blood samples were collected from 118 pemphigus patients (56 PV and 62 PF patients) and from 135 controls (pemphigus patients' relatives and neighbors) living in the prevalent area. Table 1 shows the patients' demographic, treatment, and clinical activity data. Sample preparation for Pb, Cu, and Zn determination was conducted according to the standard procedures followed by the Sector of Metals of the Pediatric Laboratory of the University Hospital, Ribeirão Preto Medical School, University of São Paulo, Brazil. Whole blood was collected in a 5-mL sodium heparin tube free of trace elements for Pb analyses. Then, ~ 1.2 mL of whole blood was transferred to a polyethylene tube to which 100 µL of Triton® X-100 (for lysis of red blood cells) was added, and the tube was kept at -20 °C until analyses. After 1:10 dilution, Atomic Absorption Spectrophotometry with Zeeman Graphite Furnace (GFAAS) (Varian Equipment, ZEEMAN 640-Z) was used to measure Pb concentrations, and Pb was extracted in a precipitant solution. The laboratory is certified for lead PEP-Pb IAL (Blood Proficiency Testing for lead by Program Institution Adolfo Lutz in São Paulo). The reference value (RV) of 5 to 10 µg.dL⁻¹ was adopted for Pb concentration [36]. The limit of detection (LOD) was 0.82 µg.dL⁻¹ for Pb.

For Cu, Zn, and Se analyses, serum samples were collected in a 10-mL navy blue cap tube with clot activator free of trace elements. For the three elements, the tubes were centrifuged at 2500 rpm for 10 min, and 1.2 mL of the serum was transferred to the polyethylene tube and kept in a freezer until the measurements were conducted. Before Cu and Zn were measured, the serum samples were diluted 1:2 and 1:5, respectively, in ultrapure deionized water (Milli-Q system). Flame Atomic Absorption Spectrophotometry (FAAS) (Varian AAS-55B) was used to determine the Zn and Cu concentrations. The methodologies and the normal range values were certified according to the Controllab Proficiency Program-Toxicology module I, Rio de Janeiro. RV values of 70 and 140 µg.dL⁻¹ and of 50 and 120 µg.dL⁻¹ were adopted for Cu and Zn [37] concentrations, respectively. The LODs were as 7.0 and 0.62 µg.dL⁻¹ for Cu and Zn, respectively.

Se samples were diluted 1:20 in 0.01% (v/v) Triton® X-100, 0.5% (v/v) nitric acid containing 10 µg.L⁻¹ rhodium as internal standard. The Se concentration was determined in the Department of Clinical and Toxicological Analysis and Bromatology, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin Elmer Elan DRC II). To verify the accuracy and precision of the proposed method, the reference material QMEQAS05S07 human serum from L'Institut National de Santé Publique du Québec (Canada) was analyzed. An RV of between 4.6 and 14.3 µg.dL⁻¹ was adopted for Se

concentration [38]. The limit of detection LOD was 0.03 µg.dL⁻¹ for Se.

Statistical analysis was performed with the aid of the SPSS 24.0 software (IBM, USA). Graphpad Prism 7.0 was used to create the illustrations. Student's t-tests or One-Way ANOVA, Mann-Whitney, or Kruskal Wallis tests were used as appropriate. Data with n > 50 were considered normally distributed according to the central limit theorem [39]. Bonferroni or Dunn test was used to analyze differences among the groups. Spearman or Pearson tests were used for correlations. Chi-square test (X²) was used for variable frequencies. P ≤ 0.05 was adopted.

3. Results

The PV patients were older than the PF patients and the controls (median age 52.8, 42.8, and 42.6 years, respectively; p = 0.0004). There were no statistical differences in terms of patient's gender (p = 0.87), patients being treated or not (p = 0.057), or clinical activity (p = 0.116) on the day the sample was collected (Table 1).

The PV patients had higher Pb values as compared to the controls (4.1 and 3.3 µg.dL⁻¹, respectively; p = 0.05) (Fig. 1A). Zn concentration was lower in the PV patients than in the controls (75.6 and 83.9 µg.dL⁻¹, respectively; p = 0.04) (Fig. 1B). Se concentration was lower in the PV and PF patients as compared to the controls (7.36, 6.75, and 9.39 µg.dL⁻¹, respectively; p = 0.007 and p < 0.0001, respectively) (Fig. 1C). Concerning Cu concentration, results were similar among the groups (p = 0.9).

The frequency of PV patients with Pb concentration above RV (5 µg.dL⁻¹) was higher as compared to the PF patients and the controls (32.1%, 23%, and 14.6%, respectively) (p = 0.0089 and p = 0.3033, respectively) (Table 2). The frequency of PV and PF patients with Pb concentration above RV was not related to disease activity (55.6% and 57.1%, respectively) (p = 0.783 and p = 0.948, respectively) or to disease duration (between one and five years) (55.6% and 42.9%, respectively) (p = 0.155 and p = 0.251, respectively) (data not shown). The full database is also provided (Table S1).

Pb concentration and the individual's age correlated positively when we considered the 236 pemphigus patients plus the controls (r = 0.2; p = 0.017) (Fig. 1D), but not when we compared each study group regarding age. No correlation was observed between the PF patients' gender and Pb, Zn, or Se concentration (p = 0.1, p = 0.9, and p = 0.9, respectively). The same was true for the PV group (p = 0.4, p = 0.3; and p = 0.4, respectively) (data not shown). There was no correlation between disease clinical activity and Pb, Se, or Zn concentration (p = 0.809, p = 0.299, and p = 0.711, respectively) (data not shown). Being treated or not on the day when the sample was collected did not correlate with Pb, Se, or Zn concentration (p = 0.370, p = 0.421, and p = 0.146, respectively) (data not shown). Pb, Se, Cu, and Zn concentrations and duration of pemphigus disease were not associated (p = 0.813, p = 0.238, p = 0.836, and p = 0.534, respectively) (data not shown). There was no correlation between the previous variables and the PF and PV clinical forms (data not shown).

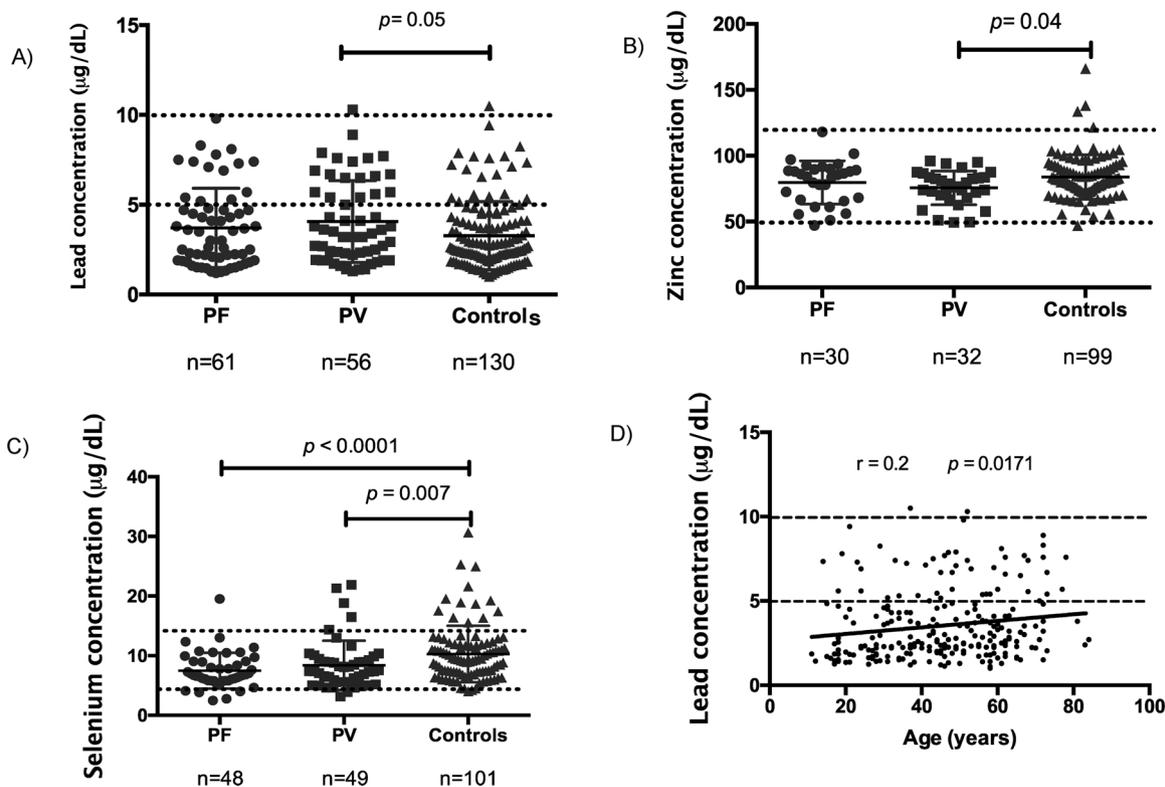


Fig. 1. A) Pb concentration in blood samples obtained from the studied groups as determined by Atomic Absorption Spectrometry. PV (pemphigus).

Table 2
Lead concentration above reference values for the studied groups.

	Lead concentration			P-value
	Pemphigus vulgaris	Pemphigus foliaceus	Controls	
Above RV (%)	18 (32.1) ^a	14 (23.0) ^b	19 (14.6) ^c	^{a,b} 0.3033 ^{b,c} 0.1581
Below RV (%)	38 (67.9)	47 (77.0)	111 (85.4)	^{a,c} 0.0089
Total (%)	56 (100)	61 (100)	130 (100)	

RV (Reference value = 5 µg/dL-10 µg/dL).

4. Discussion

The systemic influence of metals on a broad range of autoimmune diseases has been studied with great enthusiasm [1,3,7,40–42]. However, when it comes to pemphigus, Hg has been the only toxic metal related to PF outbreak due to Hg contamination in mining workers in Colombia. The role of Hg in the pemphigus pathogenesis was first demonstrated by Hg binding to keratinocytes molecules. The modified structures-Hg linkage are recognized by the host immune system, in special the sulfhydryl radicals of cysteines present in cadherins proteins, including desmogleins peptides, triggering autoimmunity. [1,43]. Moreover, autoimmune response related to Hg was associated to increased concentration of pro-inflammatory cytokines, oxidative stress, activation of CD4⁺ T cells and an increment in autoantibodies production in diseases such as lupus erythematosus, rheumatoid diseases, multiple sclerosis and Kawasaki disease [44–48]. The relation between Pb contamination and pemphigus pathogenesis has not been reported yet, but Pb is known to play an immunomodulatory role that favors Th2 proliferation and consequent production of Th2 cytokines [10,14,49,50]. Th1/Th2 imbalance with increased IL-4 levels and autoantibody production is well known in both PF and PV [24,51]. Pb also competes with Ca²⁺ in various metabolic reactions, such as

mitochondrial metabolism. Because desmoglein function is Ca²⁺-dependent, Pb may underlie Dsg expression downregulation, as described in the case of Hg contamination in Colombian pemphigus [1].

In our study, the PV patients presented higher Pb serum levels as compared to the PF patients and the controls and lower Zn and Se concentrations as compared to the controls.

Given that the PV group consisted of older patients as compared to the PF and the control groups, Pb levels may have accumulated earlier and been continuously cumulative in the PV patients as a result of earlier exposure to Pb. A positive correlation between Pb concentration and patients' plus controls' ages corroborated with this analysis. For example, about 95% of Pb is stored in bones in adults. This stored Pb is endogenously released along the life of individuals with past exposure to Pb, causing delayed toxic effects that differ from the effects due to acute exposure [52,53]. Chronic exposure to low Pb doses may result in more harmful effects than acute exposure to this same metal [54]. As a late immunotoxic effect, Pb also exacerbates oxidative stress, to promote an autoimmune response linked to pemphigus pathogenesis. A similar association has been verified in autoimmune diseases like systemic lupus erythematosus and multiple sclerosis, and in cancer [4,49].

Zn, Cu, and Se depletion are related to inadequate intake and to lesion exudate and has been associated with PV in the Middle East [33,55]. Zn and Cu depletion related to increased oxidative stress has been observed in an Iranian study of PV patients [34]. Se deficiency has been detected in Iranian pemphigus patients as compared to controls [33].

Our results corroborate with previous studies regarding Zn and Se, but not Cu depletion in PV patients [33,34]. Although our study found a 9.9% reduction of Zn and Se in the PF group, the PF patients were not Zn- or Se-deficient. Zn and Se depletion are closely linked to inflammation, immune imbalance, higher oxidative stress, and less participation in antioxidant enzymes, which have been related to autoimmune diseases including diabetes mellitus, asthma, rheumatoid arthritis, psoriasis, and lupus erythematosus [34,35,56,57]. The apoptotic process associated with pemphigus pathogenesis is also related to

Zn deficiency [58]. In autoimmune diseases, such as rheumatoid arthritis, Zn and Se serum concentrations are reduced [59]. Poor nutrition is also a possible because of Zn and Cu depletion since mouth sores lead to painful eating for PV patients [33].

We must consider that environmental contamination by Pb underlying pemphigus pathogenesis is a novelty. Our findings allied to the relationship between Pb and other autoimmune diseases reinforce the need to gain a better understanding of the Pb role in PV pathogenesis. Over the last decades, the number of PV cases has increased in an area where PF is endemic, so other environmental factors, like Pb contamination, have to be borne in mind when we explain this surge in PV cases.

In conclusion, on the basis of our results we have attempted to establish a relationship between Pb contamination and PV pathogenesis. Pb contamination in chronic doses may constitute an environmental trigger, albeit not the only one, for PV pathogenesis. The fact that the PV patients evaluated here were older and presented higher Pb concentration as compared to the PF patients and the controls indicated possible continuous exposure to this element. Furthermore, Zn and Se deficiencies provided an important insight into their influence on disease recovery, suggesting that Zn and Se complements could be allied to the specific treatment.

Further studies on the relationship between trace elements and pemphigus should be carried out for better understanding of the pathogenesis of these autoimmune diseases.

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Conflict of interest

All the authors of the manuscript “Trace element serum profile in pemphigus disease in an endemic region in Southeastern Brazil” declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jtemb.2018.09.005>.

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