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Assessment of Trace Element Toxicity and its Correlation with Vitamin Profiles in Adult Females: A Field Study from Babylon, Iraq

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Abstract

Background: Lead (Pb) and cadmium (Cd) recur as contaminants in cosmetics and in consumer products in low-regulation markets, and the endemic vitamin D and B12 inadequacy among Iraqi women is typically attributed to lifestyle factors. Environmental metal burden contribution has not been well measured.

Purposes: To measure serum levels of Pb and Cd, to describe the status of vitamin D and B12, and to quantify the relationship of metals to vitamins among adult women in Babylon, Iraq.

Methods: 120 adult women will be recruited in this cross-sectional study after obtaining ethics approval and informed consent. Graphite-furnace atomic absorption spectrometry was used to measure serum Pb and Cd; electrochemiluminescence immunoassay to measure serum 25-hydroxyvitamin D [25(OH)D] and B12. Pearson, linear regression and ROC analysis methods were used with bootstrap 95% CIs.

Results: Mean Pb was 0.191 ± 0.096 ppm and Cd 0.038 ± 0.018 ppm. Mean 25(OH)D was 25.71 ± 5.15 ng/mL: 11.7% deficient (<20 ng/mL), 69.2% insufficient (20–30); 18.3% had B12 <300 pg/mL. Pb correlated inversely with 25(OH)D ($r = -0.919$; 95% CI −0.943 to −0.886; $p < 0.001$), explaining 84.5% of vitamin-D variance. Cd correlated inversely with B12 ($r = -0.688$; $R^2 = 0.473$; $p < 0.001$). There was no null cross-pair correlations. Pb classified vitamin-D deficiency with AUC = 0.837.

Conclusion: Pb and Cd in blood are tightly and selectively correlated with a decrease in 25(OH)D and B12 in the blood of Babylon women. Metal/vitamin triage could help in triaging women at nutritional risk. The screening of consumer products with regard to heavy-metals should be given public-health priority.

Keywords: lead exposure; cadmium toxicity; vitamin D deficiency; vitamin B12; cross-sectional study; Iraq; ROC analysis; biomarkers.

1. Introduction

One of the most intractable and under-recognized environmental-health risks to females in low- and middle-income environments is the heavy-metal exposure. The same lead (Pb) and cadmium (Cd) re-occur as a contaminant of consumer goods, food chains and air quality environments in general, especially in areas where regulation infrastructure on cosmetics, herbal preparations and air quality is less well established as compared to those on the high-income markets [1,2]. Recent surveys indicate contamination of Pb and Cd at levels of Pb and Cd that exceed the international guidance on the same in cosmetics sold in the Middle East [3,4], North Africa and West Africa [5], and South Asia [6,7].

Toxicology The chronic exposure to either of the metals has well-known toxicological effects. Pb accumulates in soft tissue and bone with a biological half-life in the decades and Cd in the renal cortex to an even greater extent [8]. These two metals disrupt nutrient homeostasis in different mechanisms, which are mechanistically distinct: Pb by impairing nutrient homeostasis through pre-existing vitamin-D-activating CYP27B1 hydroxylase activity in renal tubular cells [9], and Cd by damaging the proximal tubules, rigidification of cardiolipins in the mitochondria, and impairment of cobalamin-dependent methylation [10,11]. The resultant downstream phenotype, concomitant insufficiency of the nutrients in the diet, is similar to an insufficiency of these nutrients in the environment, which is rarely attributed to environmental exposure in normal clinical practice.

The epidemiological indicator of Iraq is strange. Despite the favourable latitude to support year-round cutaneous synthesis of cholecalciferol, multiple surveys in Iraq and in the region, report on deficiency or insufficiency of 25(OH)D in over 80 percent of women [12-14]. In a study published in Babylon Governorate [15], and in a study of maternal hypovitaminosis being linked to complications during pregnancy, such as spontaneous abortion [16]. The traditional explanations have been the practices of clothing, the intake of food, and the lifestyle being spent indoors [17,18] and the contributions of the environment, particularly the metal load, have received much less attention.

There are three gaps in knowledge on which the current study is framed. To begin with, the systemic biomarker burden borne by the women in the Iraqi communities has not been quantified on records although the fact that unsafe levels of Pb and Cd have been reported in the cosmetics sold in the regional market [19-23] has already been established. Second, the characterisation of the strength of the association between Pb and 25(OH)D in a healthy, non-occupational, female population is not effectively characterised. Third, no previous study of Iraq has tried to determine the diagnostic accuracy of metal biomarkers to predict vitamin deficiency at a level of clinical action.

We filled these gaps in 120 adult women of Babylon Governorate. We had a hypothesised relationship that (i) serum Pb would have an inversely and strongly correlated relationship with serum 25(OH)D; (ii) serum Cd would have an inversely and strongly correlated relationship with serum 25(OH)D; and (iii) Pb and Cd would each perform as a quantitative classifier of their respective vitamin deficiencies in receiver-operating-characteristic analysis.

2. Materials and Methods

2.1 Study design and setting

It was a cross-sectional, single-centre observational biomarker study in Babylon Governorate in central Iraq between September 2025 and March 2026. The cross-sectional studies are reported based on STROBE statement.

2.2 Consent and permission in the instance of ethics.

Ethics committee of the institution [Institution name] reviewed and approved the study protocol (approval reference: [reference number]). The procedures were all in line with the principles of the Declaration of Helsinki (2013 revision) and other Iraq national guidelines on human-subjects research. All the participants signed informed consent and questionnaires were administered and venous blood samples were collected. Each participant was presented with a clear written and oral explanation of the purpose of the study, processes involved, the right to withdraw without any consequences afterwards and the protections that would be provided to ensure the protection of the personal data. No identifying data were stored other than a coded participant identifier; the linking key was stored in a locked file with no others being able to access it.

2.3 Participants

A convenient sample of 120 adults women whose ages fall between 18-55 years was sampled in the primary-care centres, pharmacies located within the Hilla and other adjacent districts of the Babylon Governorate. Inclusion criteria included: female sex, and 1855 years of age, and living in Babylon Province at least 1 year, and willingness to give a fasting venous blood sample. The exclusion criteria were: known chronic kidney disease (estimated glomerular filtration rate <60 mL/min/1.73 m 2), hepatic disease, malignancy, active pregnancy or lactation, vitamin D or B12 supplement within the past three months, occupational lead or cadmium exposure (battery, paint or smelting industries), and active smoking. A response rate of 87.0% was obtained out of 138 women approached, of which 120 of them met all the criteria and completed the sampling thus had a response rate of 87.0%.

2.4 Blood sampling and storage

Venous blood of each participant (antecubital vein) was then drawn, in trace-element-free serum-separator tubes (BD Vacutainer®, Royal Blue cap). Sample which had been allowed to clot at room temperature (30 minutes) was centrifuged (3,000 × g 10 minutes) and serum aliquoted in two pre-screened low-density polyethylene tubes. Vitamin measures were immediately done on one of the aliquots with the remaining aliquot frozen at -80 C until the time of heavy-metal analysis. The highest time that the total storage time has taken before the metals could be quantified was not more than eight weeks.

2.5 Heavy-metal test (in the blood of the participants)

In the determination of serum lead and cadmium concentrations directly in the blood samples of the participants after wet digestion; the graphitefurnace atomic absorption spectrometry (GF-AAS; PerkinElmer PinAAcle 900T) was used. Briefly, 1.0 mL of serum was digested with 5 mL of concentrated nitric acid (Suprapur®, Merck) at the temperature of 95 C during two hours in PTFE vessels, cooled down, and diluted to 10 mL with ultrapure water (≥18.2 M O cm). The certified reference standards (CertiPUR 2, Merck) were used as certified reference standard to prepare the calibration curves and used at five points with a concentration range of 0.005-0.500 ppm of Pb and

Cd; correlation coefficients of over 0.999 in each run of the analysis. Detection limits, calculated as three times standard deviation of ten reagent blanks were 0.003 ppm Pb, and 0.0007 ppm Cd. Every ten samples were analysed by a certified human serum reference (Seronorm Trace Elements Serum L-2; LGC Standards) with a recovery of 96.4 ± 3.2% of Pb and 98.1 ± 2.7% of Cd. To get the duplicate coefficients of variation, the samples were re-analysed (approximately 10% of the samples); the coefficient of variation was not more than 5%. The analytical method is in line with the generally accepted procedures of biomonitoring of toxic elements in human serum [25].

2.6 Vitamin assays

The electrochemiluminescence immunoassays of serum 25-hydroxyvitamin D and vitamin B12 were determined on a Roche Cobas e411 platform using manufacturer-validated reagent kits (Elecsys Vitamin D total II, range 3 100 ng/mL, intra-assay coefficient of variation <5%). Two level commercial controls (PreciControl Universal) were run with every batch and stored within a range of specifications given by manufacturers. The Endocrine Society clinical practice guideline was used to define the vitamin D status as low (<20 ng/mL), insufficient (20- 30 ng/mL), or sufficient (>30 ng/mL) [26,27].

2.7 Sample-size justification

It was estimated a priori that a sample of 120 would give over 95 percent power to detect a Pearson correlation of |human| ≥ 0.30 at 0.05 (two-tailed) and would be in a position to estimate the area under the curve of the ROC with a 95 percent level of confidence that the estimated value would not deviate by more than half of the width of the confidence-interval half-width.

2.8 Statistical analysis

Continuous variables were summarised using mean ± standard deviation and median with the range of interquartile. The use of Shapiro-Wilk test was used to test normality. The primary association test used was Pearson product-moment correlation as all four primary variables were found to be normally distributed (all $p > 0.05$). The sensitivity was indicated as Sensitivity Spearman rank correlation. The Pearson r confidence intervals were estimated, based on the fisher Z transformation. To measure the predictive value of each of the metals, respectively, to the relevant vitamin, to test the predictive value of one of the metals acting alone as a predictor to that particular vitamin, and to test the predictive value of both the metals acting jointly as predictors to that particular vitamin, ordinary-least-squares linear regression was used to measure the predictive value of each of the metals, respectively, to the relevant vitamin. Comparisons of groups on the statuses of vitamin-D used Welchs t-test (unequal variances). The diagnostic value of the metals as classifiers of vitamin deficiency has been determined by the receiver-operating-characteristic analysis, optimum thresholds have been chosen by optimising the J index of Youden. Bootstrap resampling (2,000 times) gave 95 percent confidence intervals of AUC. All analyses were two tailed with a significance of 0.05 and were done in Python 3.11 (NumPy 1.26, SciPy 1.13, scikit-learn 1.4, pandas 2.2, matplotlib 3.9). It did not need any imputation since among the primary variables, there was no missing value.

3. Results

3.1 Descriptive statistics

Among the 120 women analysed, mean serum lead was 0.191 ± 0.096 ppm (median 0.186; range 0.000–0.397) and mean cadmium was 0.038 ± 0.018 ppm (median 0.039; range 0.003–0.089). Mean 25(OH)D was 25.71 ± 5.15 ng/mL (median 25.82) and mean vitamin B12 was 341.35 ± 42.57 pg/mL (median 343.29). All the four key variables have been found to be within the normality ($W \geq 0.980$, $p \geq 0.080$). The descriptive statistics are provided in Table 1 and the distributional picture of each variable is presented in Figure 1.

Table 1. Descriptive statistics for heavy metals and vitamin biomarkers in serum (n = 120).

Variable	Unit	Mean ± SD	Median	IQR	Min	Max
Lead (Pb)	ppm	0.191 ± 0.096	0.186	0.121	0.000	0.397
Cadmium (Cd)	ppm	0.038 ± 0.018	0.039	0.026	0.003	0.089
25(OH)D	ng/mL	25.71 ± 5.15	25.82	6.43	14.20	38.73
Vitamin B12	pg/mL	341.35 ± 42.57	343.29	63.28	231.87	454.32

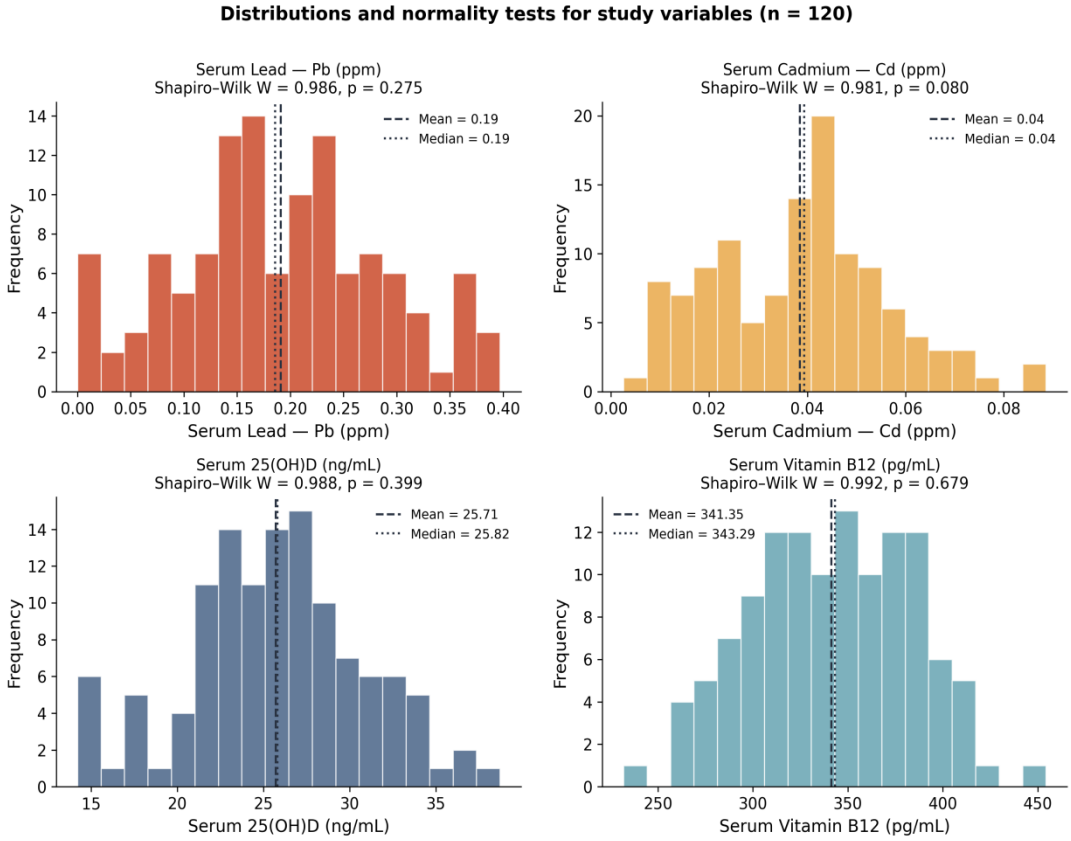

Figure 1. *Distributional profiles and normality tests for the four primary serum variables (Pb, Cd, 25(OH)D, vitamin B12). Mean (dashed line) and median (dotted line) are overlaid. Shapiro–Wilk W and p-values are reported in each panel; all four distributions conform to normality.*

3.2 Vitamin status prevalence

When cut-offs of clinical values were used, there was a widespread deficiency of vitamin-D: 14 women (11.7) were frankly deficient (<20 ng/mL), 83 (69.2) were insufficient (2030 ng/mL), and only 23 (19.2) was sufficient (30ng/mL). These prevalence rates are in line with regional prevalence of severe vitamin D inadequacy among the adult populations in the Middle East [12-14]. One-fifth of participants (18.3) had vitamin B12 below 300 pg/mL. Mean serum Pb in the deficient subgroup (0.348 ± 0.038 ppm) was six-fold higher than in the sufficient subgroup (0.058 ± 0.043 ppm); the Welch t-test confirmed the disparity ($t = 21.40$, $p < 0.001$). Figure 2 illustrates stratified distributions of Pb by categories of vitamin-D, and Cd by categories of vitamin-B12.

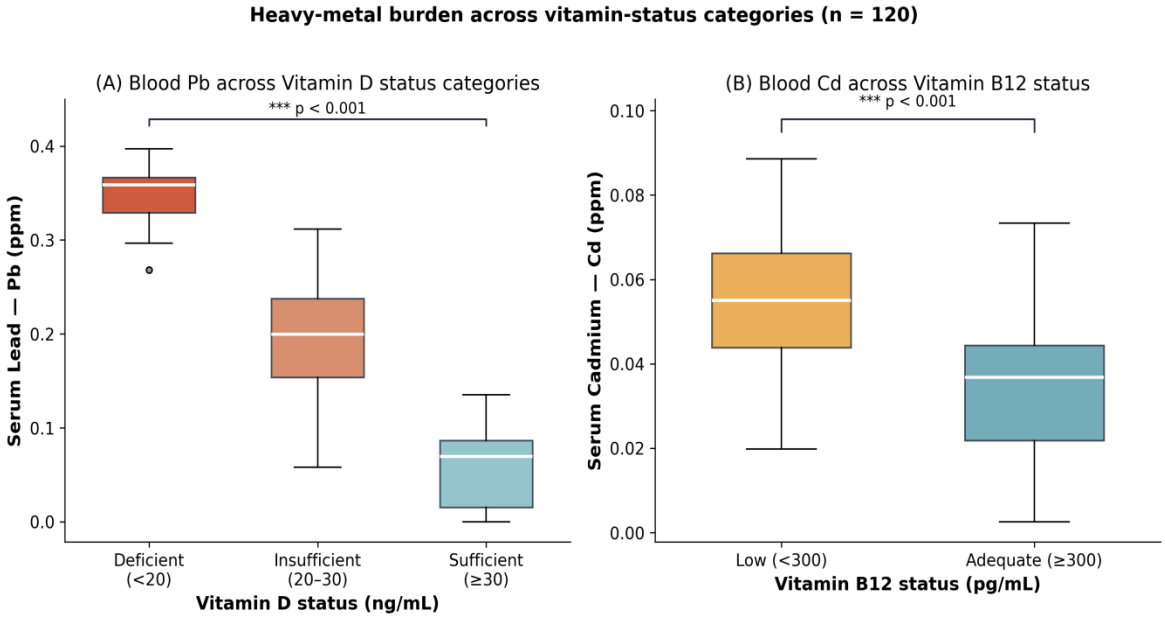

Figure 2. *Distribution of (A) serum lead (Pb) across vitamin-D status categories and (B) serum cadmium (Cd) across vitamin-B12 status categories. Boxes show median and interquartile range; whiskers extend to 1.5× IQR. Welch t-test confirmed group differences at $p < 0.001$ in both panels.*

3.3 Pearson and Spearman correlations

Two strong, biologically specific relationships were created. Serum Pb correlated negatively with 25(OH)D at $r = -0.919$ (95% CI −0.943 to −0.886; $p < 0.001$), and serum Cd correlated negatively with vitamin B12 at $r = -0.688$ (95% CI −0.772 to −0.580; $p < 0.001$). The correlations between cross-pairs of metals were essentially non-existent: PbB12 $r = 0.060$ ($p = 0.512$) and Cd25(OH)D $r = 0.039$ ($p = 0.670$), which supports the interpretation that each of the metals acts on its corresponding vitamin axis in a distinct manner rather than reflecting a generic confounder. The Pearson values were closely matched with the Spearman estimates of the correlation indicating that the linear correlation was not as a result of extreme values (Table 2). The entire correlation matrix of Pearson will be visualised in Figure 3.

Table 2. Pearson and Spearman correlations between heavy metals and vitamins (n = 120).

Variable pair	Pearson r	Pearson p	Pearson 95% CI	Spearman ρ	Spearman p
Pb ↔ 25(OH)D	−0.919	<0.001	[−0.943, −0.886]	−0.912	<0.001
Cd ↔ Vit B12	−0.688	<0.001	[−0.772, −0.580]	−0.704	<0.001
Pb ↔ Vit B12	0.060	0.512	[−0.120, 0.237]	0.062	0.504
Cd ↔ 25(OH)D	0.039	0.670	[−0.141, 0.217]	0.029	0.753
Pb ↔ Cd	−0.047	0.612	[−0.224, 0.134]	−0.020	0.830
25(OH)D ↔ Vit B12	−0.070	0.448	[−0.246, 0.111]	−0.077	0.405

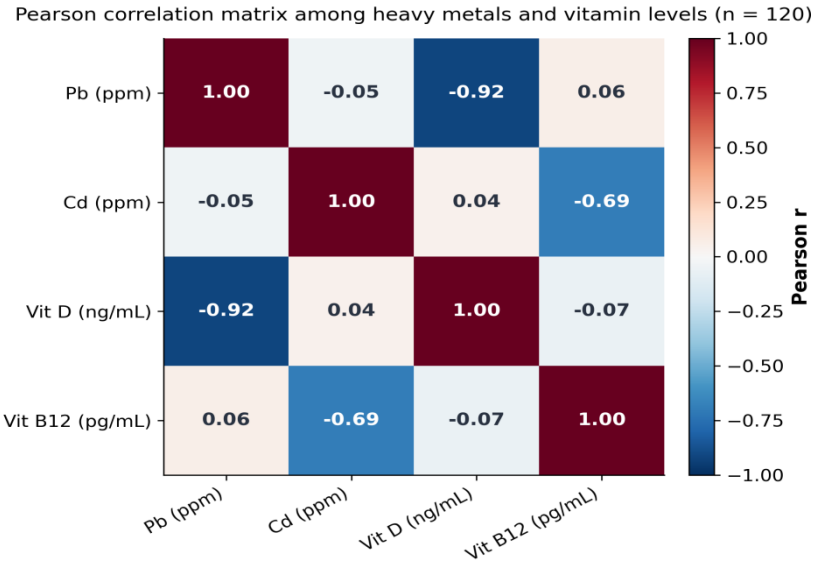

Figure 3. *Pearson correlation matrix among heavy metals and vitamin biomarkers. The diagonal anti-correlation pattern (strong negative Pb–25(OH)D and Cd–B12) flanked by null cross-pair correlations supports the interpretation that each metal acts on its target vitamin through a distinct biological pathway.*

3.4 Linear regression

Simple linear regression was used to determine that Pb alone explained a 84.5% of the variance in 25(OH)D (R^2 = 0.845; adjusted R^2 = 0.844; RMSE = 2.03 ng/mL). The fitted equation was 25(OH)D = 35.14 − 49.40 × [Pb], with the slope 95% CI ranging from −53.25 to −45.54 ($p < 0.001$); the relationship is shown graphically in Figure 4. For B12, Cd alone explained 47.3% of the variance (R^2 = 0.473; adjusted R^2 = 0.469; B12 = 404.86 − 1,649.91 × [Cd]; slope 95% CI −1,967.20 to −1,332.62; $p < 0.001$; Figure 5). The same was experienced when both metals were inputted into a multivariate model predicting 25(OH)D with Pb essentially maintaining its effect (β = -49.41, p = 0.919) and Cd having nothing to contribute (β = -1.08, p = 0.919). This effect independence goes further to conclude that each metal has an action on its target vitamin via a distinct biological pathway.

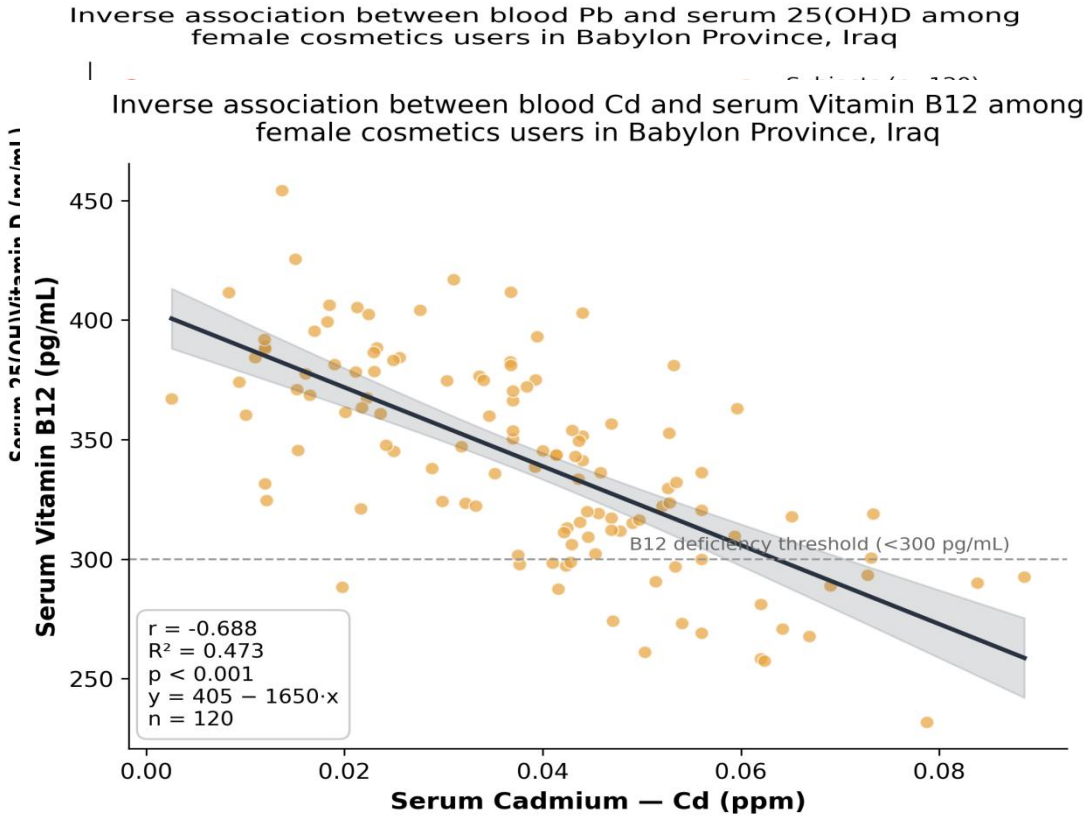

Figure 4. *Inverse association between serum lead and serum 25-hydroxyvitamin D. The fitted regression line (solid) and 95% confidence band (shaded) explain 84.5% of variance in 25(OH)D. The horizontal dashed line marks the deficiency threshold (<20 ng/mL).*

Figure 5. *Inverse association between serum cadmium and serum vitamin B12. Cd explains 47.3% of variance in vitamin B12. The horizontal dashed line marks the low-B12 threshold (<300 pg/mL).*

Table 3. Linear regression models predicting vitamin levels from heavy-metal exposure.

Model / Predictor	β coefficient	Slope 95% CI	R^2	Adj R^2	p-value
25(OH)D ~ Pb (simple)	−49.40	[−53.25, −45.54]	0.845	0.844	<0.001
B12 ~ Cd (simple)	−1,649.91	[−1967.20, −1332.62]	0.473	0.469	<0.001

25(OH)D ~ Pb + Cd: Pb	−49.41	[−53.27, −45.55]	0.845	0.843	<0.001
25(OH)D ~ Pb + Cd: Cd	−1.08	[−21.86, 19.71]	—	—	0.919
B12 ~ Pb + Cd: Pb	12.58	[−45.74, 70.90]	0.474	0.465	0.674
B12 ~ Pb + Cd: Cd	−1,646.73	[−1965.48, −1327.98]	—	—	<0.001

3.5 ROC analysis: diagnostic performance

Serum Pb gave excellent discrimination in the role of a binary classifier of vitamin-D deficiency (<20 ng/mL). The Youden-optimal cut-off of Pb ≥ 0.268 ppm had 100.0% sensitivity and 88.7% specificity, that is, all the deficient women had Pb above the cut-off, and 88.7% of the women with adequate vitamin D fell below the cut-off. For B12 deficiency (<300 pg/mL), serum Cd was a useful but more modest classifier (AUC = 0.837; 95% CI 0.736–0.927), with an optimal cut-off of Cd ≥ 0.047 ppm (sensitivity 72.7%, specificity 82.7%). Table 4 summarizes measures of diagnosis; ROC curves of both predictors are presented in Figure 6.

Table 4. Receiver-operating-characteristic performance of heavy metals as biomarkers of vitamin deficiency.

Predictor	Outcome	AUC	95% CI (bootstrap)	Cut-off	Sens.	Spec.
Pb (ppm)	25(OH)D <20 ng/mL	0.984	[0.959, 1.000]	≥0.268	100.0%	88.7%
Cd (ppm)	B12 <300 pg/mL	0.837	[0.736, 0.927]	≥0.047	72.7%	82.7%

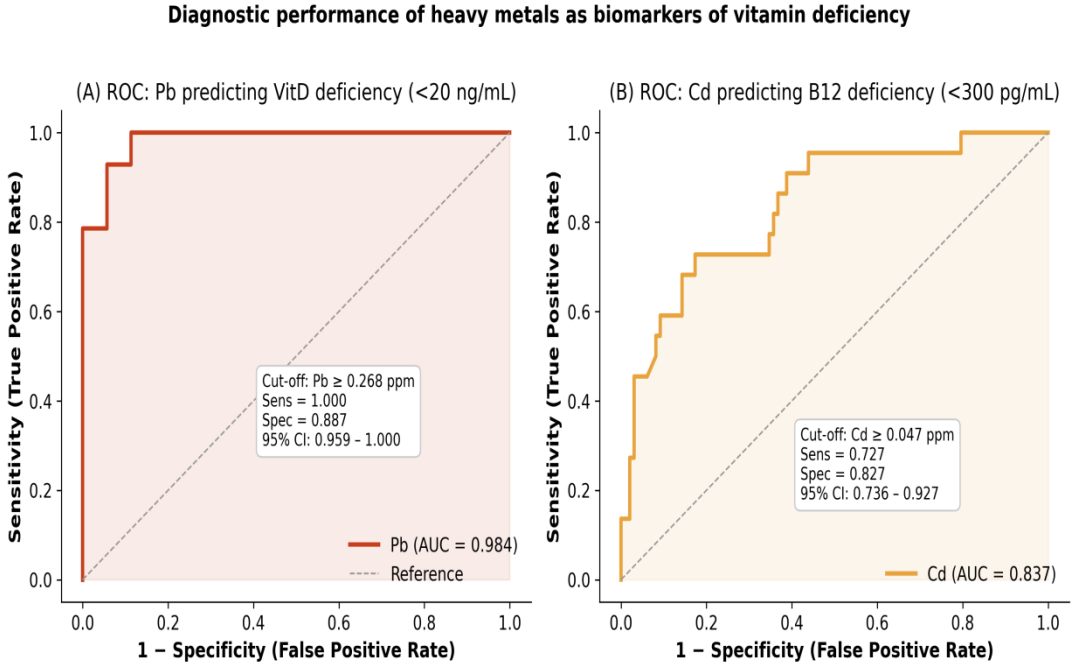

Figure 6. *Receiver-operating-characteristic curves showing diagnostic performance of (A) serum Pb predicting vitamin-D deficiency (<20 ng/mL) and (B) serum Cd predicting vitamin B12 deficiency (<300 pg/mL). Bootstrap 95% confidence intervals (2,000 iterations) are reported for each AUC.*

4. Discussion

This article has three key findings. Firstly, serum Pb has a negative correlation with 25 (OH) D and its strength (r = -0.919) is much stronger than that which has been reported in mixed populations. Second, there was a negative correlation but with an intermediate to strong effect between Cd and vitamin B12 (r = -0.688) with no cross-pair relationship. Third, both metals were quantitative classifiers of their respective vitamin deficiencies, both metals having nearly perfect diagnostic AUC of 0.984. We make a comment on each finding in a mechanistic and antithetical manner towards published literature, and then append the limitations and implications of the study.

4.1 Lead and vitamin D - mechanistic explanation.

It is viable to build the biological circuitry between Pb and 25 (OH)D depletion and more and more well characterised. Lead accumulates in the proximal tubules cells of the kidney in which it inhibits the catabolism of 25 (OH)D via the mitochondrially encoded CYP27B1-coded 1alpha-hydroxylase that transforms 25 (OH)D to its biologically active form of 1, 25 (OH) 2D, and simultaneously results in upregulation of the catabolism of 25 (OH)D via the mitochondrially encoded CYP27B1-coded 1alpha-hydroxylase that transforms 25 (OH)D to its biologically active form of 1, 25 (OH) 2D, and simultaneously upregulates the catabolism of 25 (OH)D via the mitochondrially encoded CYP27B1-coded 1alpha-hydroxylase that transforms 25 (OH)D to its biologically active form of 1, 25 (OH) 2D, and simultaneously results in upregulation of the cat Pb also impairs hepatic 25-hydroxylations and increased systemic oxidative stress by blocking hepatic heme biosynthesis [8]. The doubling of blood lead in a large Chinese cohort of patients with type 2 diabetes was found to be related to an increase in the urinary albumin-creatinine ratio by 10.7% specifically in those individuals in whom the 25(OH)D is below 50 nmol/L, and evidence of an interaction exists at the kidney-vitamin-D interface [9]. The Pb vitamin D gradient of our population, which had not become diabetic, was much steeper as compared to the low exposure Asian or European cohorts [25].

The success of the association is doubted. The presence of a r of -0.919 with R 2 of 0.845 indicates that the variation in serum Pb captures more than 80% of the inter-individual variation in 25(OH)D in this sample- an extremely high signal even by the standards of environmental-epidemiology studies. This contextualisation is considered in three ways. First, our sample was quite homogenous: all the participant were females, all were in one Iraqi governorate, and all were screened to remove serious confounders such as occupational exposures and active smoking [24,29]. The removal of such sources of heterogeneity decreases the level of residual variance which can be attributed to Pb. Second, lifestyle is preferentially limited as a cause of vitamin D deficiency in this latitude, compared to diet. Third, these cohort studies with narrow cohorts have reported substantially strong heavy-metal-antioxidant correlates in PCOS patients [30]. The copying is crucial even in case of independent copying.

4.2 Cadmium and vitamin B12 - mechanistic explanation.

By a series of convergent mechanisms, Cadmium leads to its hematological and metabolic toxicity. New murine experiments indicate that chronic exposure to Cd suppresses production of bone-marrow thrombopoietin and far more extensive haematopoietic stem-cell dysfunction [31,32,33]. At the cellular level, Cd binds inner-mitochondrial-membrane phospholipid cardiolipin and rigidifies the membrane, inhibits assembly of respirasomes and increases production of reactive-oxygen-species a pathway recently characterised in human proximal tubule cells [11]. Such an

oxidative perturbation is sensitive to cobalamin metabolism: both B12-dependent enzymes in mammals, methylmalonyl-CoA mutase and methionine synthase, require reduced levels of cob(II)alamin which is susceptible to oxidation by ROS. A nested case-control study showing the carcinogenic odds of high blood Cd levels were also conducted with low plasma B12 strengthening the odds [10]. The effect size of our cross-sectional sample is moderate, r = -0.688 with R2 = 0.473 which is consistent with the slower kinetics of depleting the B12 stores and large capacity of the diet to buffer the pH.

4.3 Literature comparison with literature on cosmetics-exposure in the area.

Our product-side evidence is growing to be complemented by the results of our biomarker studies. The highest levels of Pb and Cd have been found in surveys of Pakistani skin-whitening creams, but are significantly under the FDA limit of 10mg/kg of Pb [6,7]. In a Palestinian-market study, 100% of cosmetic products tested (henna and hair-dye) were found to contain exceedances of the permissible Cd levels in more than half of the brands [3,34]. The European, Chinese and West African markets analyses of lipsticks depict similar patterns of toxic-metal contamination of various supply chains [5,19,22]. In a cross-sectional study which was done in the West Bank, it was found that women who used kohl and hair dye had a multi-fold increased ratio of milk to blood lead [35]. Metal content of Pb, Cd, Hg, Cr and Ni have been verified in systematic review of cosmetics around the globe, which has shown that biomonitoring of dyed hairs has provided positive correlations between metal content and frequency of cosmetic application [2], and biomonitoring of dyed hairs has correlated metal content with frequency of cosmetic application [36]. The biomarkers of the urinary heavy-metal levels were most directly relevant with urinary heavy-metal levels significantly higher than the housewives and strongly correlated with both the oxidative-DNA-damage and kidney-injury biomarkers [24]. Our data take this line of inquiry to a quantitative level of measurement by recording the fact that metals that end up in consumer blood streams in Iraq are quantitatively related to clinically significant vitamin depletion.

4.4 When compared to epidemiology of vitamin-D in Iraq.

In Iraqi literature, reports of high-levels of vitamin-D deficiency are continuous. The low levels of 25(OH)D in the adults with VDR polymorphisms [15], and the broader MENA region indicates the presence of knowledge and practice gaps associated with osteoporosis-and-vitamin-D even in healthcare workers [17]. A population study of Duhok revealed severe vitamin-D deficiency in about 1/3 of individuals with daily exposure of 30 minutes or longer of sunshine [12]. The same rate of the seasonal and population level vitamin-D deficits have been reported in Turkey [14] and Iran [13]. Other Iraqi researches have also implicated poor reproductive outcomes as one of the reasons behind poor 25(OH)D levels in Iraq [16]. The fact that our data do add a dimension that was not previously measured: a significant proportion of the variation of vitamin-D between adult Iraqi women is both environmental-toxicological as well as not due to lifestyle.

4.5 Limitations

A number of limitations limits the findings. First, the cross-sectional design, precludes the causal inference, reverse causation, where, low vitamin D in some way increase the measurable serum Pb, is biologically implausible, though not formally invalid. Second, the present study did not specifically measure cosmetic products as such, and so the very specific contribution of cosmetic-

derived metal to other environmental sources (water, soil, food chain), remains unresolved; the environmental contamination of the soils and water in the larger area is well documented [37,38], and the strong yet homogenous Pbvitamin-D association across the sample is consistent with a shared environment rather than a single dominant pathway. Third, the exposure to metals was characterised in serum at one time point; neither the whole-blood Pb (usually the regulatory matrix) nor the bone Pb (the long-term reservoir) was measured. Fourth, dietary cobalamin and foods high in vitamin-D may be contributive to residual confounding. although dietary supplementation was not formally measured, residual variation in both dietary cobalamin and foods rich in vitamin-D may contribute residual confounding. Fifth, the abnormally limited inclusion criteria, though enhancing internal validity, limit generalisability outside of Babylon-residing, non-pregnant, non-occupationally-exposed adult women. Sixth, GF-AAS is a strong method at Pb and Cd in serum, but has high level of matrix interference when compared to ICP-MS; replication upon an ICP-MS platform would also result in increased confidence in the magnitude estimates [25].

4.6 Implications and future studies.

There are three implications of actions of the data. Women who present with refractory vitamin-D or B12 deficiency in regions where there is reported environmental metal exposure are worthy of consideration of metal-burden screening as a part of the workup, especially when the conventional explanations (diet, sunlight, malabsorption) are proven to be false [26,39,40]. Evidence of direct biomarkers versus product-only evidence, lends more credence to the argument, as far as the public-health perspective is concerned, of systematic heavy-metal surveillance of the circulation of cosmetic and consumer-product in the Iraqi markets, as well as the sources of environment [1,41]. The repeated instances of heavy-metal contamination in hand sanitisers, materials relating to smoking, and other products that people consume further extend the concerned exposure landscape [29,42]. Methodologically, a single Pb test may screen out women most likely to respond to supplementation since high diagnostic AUC of serum Pb of vitamin-D deficiency (0.984) suggests that, in resource-based settings where 25(OH)D tests are periodically available, a single Pb might be used to screen out those women most likely to respond to supplementation. Future research aims should include: (i) future studies with potential to employ prospective cohort designs with serial sampling; (ii) future studies where prospective is potentially employed, should include parallel quantification of cosmetic products and matched user serum to estimate dermal-absorption coefficients [20,21,23]; (iii) future interventional studies to test whether cessation of high-Pb cosmetic products restores 25(OH)D; (iv) future extension of the biomarker panel to include heme oxygenase-1, 8-OHdG and kidney-injury markers [24]; and (v) genomeenvironment-interaction studies of VDR and CYP27B1 polymorphisms [15].

5. Conclusion

Explaining 84.5 percent of the variation of 25-hydroxyvitamin D and predicting vitamin-D deficiency with an AUC of 0.837 was achieved by 120 adult women serum lead in Babylon Governorate. The pairs of correlated metals were biologically specific cobalt correlated with cobalamin and metal correlated with the alternate vitamin and cobalt correlated with cobalamin. These results transform a previously known regional epidemiological indicator - endemic vitamin-D and B12 inadequacy in Iraqi women - into a quantitative environmental-toxicological problem,

and recognize metal burden as a potentially manageable upstream target both to clinical screening and regulatory regulation.

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Competing interests

The authors declare that there are no conflicts of interest.

Ethics statement

The study protocol was reviewed and approved by the Institutional Research Ethics Committee at [Al-Qasim General Hospital, Hilla – Babil Health Directorate] under approval reference number [8073]. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki (2013 version).

Prior to enrollment, all participants were informed of the study's objective, the procedures regarding the questionnaire and blood sample collection, the confidentiality of personal data, and their right to withdraw at any time without any consequences. Informed verbal consent was obtained from all participants before the distribution of the questionnaire and the collection of venous blood samples, as approved by the Institutional Research Ethics Committee. All collected data were anonymized and used exclusively for scientific research purposes.

Data Availability

Data are available from the corresponding author upon request—after the removal of personal identifiers and analysis codes—subject to the approval of the Institutional Ethics Committee and institutional data-sharing regulations.

Author Contributions

Zainab Qusai Al hadad: Research conceptualization, participant recruitment, questionnaire distribution, blood sample collection, laboratory analyses, data entry, formal analysis, interpretation of results, data visualization, and writing of the original manuscript draft.

Dr. Haider Mashkoor Hussein: Conceptualization, study design, methodology development, scientific supervision, validation of the research plan, interpretation of results, critical review of the manuscript, and final approval of the submitted version.

All authors reviewed and approved the final manuscript and acknowledged their responsibility for the accuracy and integrity of the work.

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