Environmental Risk Factors of Systemic Lupus Erythematosus: A Case Control Study

Rania H. Refai (✉ hph.rattia@alexu.edu.eg)

Alexandria University

Mohammed F.Barghout

Alexandria University

Anna N. Abou-Raya

Alexandria University

Mamdouh H. Abdou

Alexandria University

Research Article

Keywords: SLE, environmental risk factors, lead, cadmium, zinc, passive smoking, sunlight.

Posted Date: February 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1269968/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: Systemic Lupus Erythematosus (SLE) was suggested to be a condition of multifactorial etiology. Several genetic and environmental factors were suggested to be implicated in its pathogenesis. Recognizing these factors that trigger the disease onset and progression is pivotal to help taking measures to reduce the individual's risk.

Methods: The main objective of this study was to examine how exposure to selected environmental factors was associated with SLE risk.

This case control study was conducted at the Rheumatology outpatient clinic of Alexandria Main University Hospital, in Alexandria, Egypt. The study sample consisted of 29 female SLE patients, and 27 healthy controls, who matched the cases on age and parity. Data were collected by a structured interviewing questionnaire, additionally; blood levels of lead, cadmium, and zinc of all participants were assessed by flame atomic absorption spectrometry. SPSS version 21 was used for data entry and analysis.

Results: The findings of this study add to the evidence that SLE can be environmentally induced. The multivariate stepwise logistic regression model revealed that five factors showed significant association with SLE, namely living near agricultural areas, passive smoking, blood lead levels ≥ 0.075 mg/L, and exposure to sunlight (OR =58.556, 95% CI =1.897-1807.759, OR =24.116, 95%CI =1.763-329.799, OR =18.981, 95%CI =1.228-293.364, OR =9.549, 95%CI =1.299-70.224 respectively). Whereas walking or doing exercise were significantly protective to SLE ($P = 0.006$).

Conclusion: Efforts should be made to decrease the environmental pollution and reduce the effect of environmental risk factors on disease occurrence.

Background

Systemic Lupus Erythematosus (SLE) is a chronic rheumatic autoimmune disorder that could be manifested by many symptoms and complications. It is a multi-system disease that may involve nearly any organ resulting in serious organ complications and even death [1, 2]. It predominantly affects women in the child-bearing ages (15-44 years old) [3].

SLE was suggested to be a condition of multifactorial etiology; including genetics, hormonal, environmental exposures, age, race, gender, and socioeconomic factors. It is currently known that different environmental factors could trigger SLE onset and flares in genetically susceptible individuals [4, 5]. Owing to the increasing prevalence and overall SLE burden, efforts have been made to recognize these genetic and non-genetic factors [6].

The role of the environment was more prominent by the fact that SLE concordance among identical monozygotic twins is far below 40-50% and in dizygotic twins is around 5% [7]. The contribution of environmental factors to SLE risk has been evaluated to constitute 56% [8].

Smoking, silica dust, UV radiation, infections, stress, air pollution, pesticides, and heavy metals are the major environmental risk factors having some evidence for association with SLE [9].

Heavy metals

Heavy metals are derived from natural as well as anthropogenic sources. In recent years, heavy metal pollution has become a significant health issue, and it has been on the rise in the developing population; due to the increase in their use in agriculture, technology, and industry. Continuous exposure to low levels of these toxic trace elements may result in bioaccumulation and produce a wide variety of biological effects on human beings [10].

Lead and cadmium are known to pose serious risks to human health. Toxicity of these agents is evidenced by being identified in the top 10 environmental hazards by the Agency for Toxic Substances and Disease Registry [11].

Environmental sources of lead include air, food, beverages, and ink. Clay pots or dishes and ceramic or crystal glassware that contain lead may also contribute to food contamination [12]. Inhalation of airborne dusts containing lead and ingestion through food or water contaminated by lead are considered the most probable routes of exposure [13]. In addition, active and passive smoking were found to be associated with increased blood lead levels [14].

Lead is used in the production of lead-acid batteries, ammunitions, and X-rays shielding devices. Old deteriorating household paints, and lead use in some traditional medicines and cosmetics can also be a source of lead exposure [15].

Cadmium is present in cigarette smoke, air, food, and water. The major industrial uses of cadmium include the production of alloys, pigments, and batteries. It can enter human bodies through inhalation, ingestion and dermal contact [16].

Experimental studies of lead and cadmium exposure in rodent models proposed that metals may play a causative role in SLE [17, 18, 19]. There is relatively little data pertaining to lead or cadmium exposure with the risk of SLE in humans; exposure to stained or leaded glass as a hobby was found to be more common among SLE cases than controls [20].

Trace elements such as zinc play a crucial role in growth and development of all organisms [21]. Zinc is the second most abundant trace metal in the human body after iron, but zinc cannot be stored and has to be taken up via food daily to guarantee sufficient supply. However, it was stated that zinc excess as well as zinc deficiency may result in severe disturbances in immune cell numbers and activities leading to immune dysfunction [22].

A study by Sahebari et al. [23] found that serum Zn values were lower in SLE patients than healthy age and sex-matched controls. On the other hand, Zinc-deficient diets retarded autoantibody production and enhanced survival in mice [24]. Moreover, zinc deprivation in a strain of hybrid New Zealand mice (NZB/NZW) was found to cause a marked decrease in lymphoproliferation, anti-dsDNA titer and glomerulonephritis [25]. In their review, Constantin et al. [26] advised to restrict consumption of some minerals such as zinc and sodium.

As data about the association of environmental risk factors with SLE in Egypt are lacking and the incidence is increasing. The present study was proposed to shade the light on the association of some environmental exposures with SLE risk, in order to determine the extent to which SLE is environmentally induced. This would help to take measures to reduce the individual's risk.

Methods

This case control study was conducted at the Rheumatology outpatient clinic of Alexandria Main University Hospital. The Inclusion criteria were patients diagnosed with idiopathic SLE according to SLICC criteria [27]. The controls included healthy subjects who accompanied the SLE patients in their visit to the Rheumatology outpatient clinic.

Exclusion criteria included (1) male SLE patients; (2) drug-induced Lupus; (3) overlap syndrome as lupus and rheumatoid arthritis; (4) any other rheumatic diseases; (5) coexisting morbidity not related to SLE, e.g. diabetes, hypertension; (6) cancer; (7) dementia or psychosis; (8) intake of nutritional supplements in the six months prior to the blood collection.

Sample size

Based on a previous case control study, the mean of serum Zinc among systemic lupus erythematosus (SLE) was 700.61 ± 135.91 and among controls was 860.45 ± 123.74 , using an alpha error of 0.05 and power 98% [28]. The minimum required sample size was estimated to be 46 adults, 23 for each group, which was increased to 56 adults, 29 cases and 27 controls. The sample size was calculated using G. Power software.

Data collection methods and tools:

1. A pre-designed structured interviewing questionnaire was used to collect data from cases and controls. It included personal and socio-demographic data, data about occupation, data about lifestyle factors, the medical history, the smoking history including exposure to passive smoking, and some possible indoor environmental risk factors.
2. **Laboratory investigation:**

A blood sample (5 ml) was drawn from each participant to measure blood levels of lead, cadmium, and zinc. The samples were transferred into heparinized collection tubes. Cadmium, lead, and zinc were extracted from the blood samples using the conventional wet acid digestion method using concentrated nitric acid (HNO_3). A blank using deionized water instead of blood was done for each batch of analysis for comparison [12]. The digested samples were filtered and were subjected to elemental analysis using flame atomic absorption spectrophotometer (Shimadzu model AA-6650) at the central laboratory of the High Institute of Public Health (HIPH), University of Alexandria.

Statistical design

SPSS version 21 was used for data entry and analysis. Qualitative variables were described through number of cases and percentages of cases. For quantitative continuous variables, tests of normality were done. Mean and standard deviation ($\text{Mean} \pm \text{SD}$) were calculated if the variable follows normal distribution, and median and interquartile range ($\text{Median} \pm \text{IQR}$) if it does not follow normal distribution. "Pearson's Chi-square test (χ^2)" was used to calculate significant differences between cases and controls for the categorical data. If the assumptions were violated, "Fisher's exact test" (if 2×2 table) or "Monte Carlo test" (if $m \times n$ table) were used.

Differences between the means of the two groups were examined using independent t test for the continuous, normally distributed variables. The Mann-Whitney-Wilcoxon non-parametric test (U), for the continuous, non-normally distributed variables. Odds Ratio (OR) was calculated to measure SLE risk [29, 30]. Increasing trends in risk of SLE concerning some risk factors were tested using "Chi-square for trend". Pearson's correlation coefficient was used to test the association between quantitative variables [31].

A multivariate stepwise logistic regression analysis was used to see if there were significant associations between specific exposures and SLE and to adjust for some potential confounders. Nagelkerke R^2 was calculated to tell the amount of variation in SLE risk which is explained by the model.

Ethics approval and consent to participate

Approval of the Ethics Committee of the High Institute of Public Health for conducting the research was obtained. Approval for conducting the study at the rheumatology outpatient clinic of Alexandria Main University Hospital was obtained from the hospital outpatient clinics director after a formal written request for that.

A written informed consent was taken from all study participants after explanation of the purpose and benefits of the research. Anonymity and confidentiality were ensured. All methods were performed in accordance with the relevant guidelines and regulations.

Results And Discussion

Estimation of SLE risk in relation to some socio-demographic characteristics

Analysis of data regarding the socio-demographic characteristics revealed that patients and controls were similar in terms of demographic background, no statistical significant difference between cases and controls except for the education level, the occupation, and the residence near agricultural areas (table 1).

Regarding the education level, there was a significant difference between cases and controls ($P < 0.05$). The percentage of illiterate cases was (31%) and constituted more than double their percentage in the control group (14.8%). Also, the percentage of cases with higher education was only (3.4%), which is very low compared to controls (14.8%). The findings showed that females with SLE who were in the primary or preparatory educational level were significantly more likely to be at risk of SLE (OR =14.67, 95%CI =1.16-185.23) compared to controls.

This assumes that lower education level - which is an indicator of socioeconomic status - may constitute a risk for SLE, which is similar to other studies that showed the association between lower education level and SLE onset and flare [32].

With regard to occupation, there was a statistically significant difference between cases and controls ($P < 0.05$), the majority of cases were housewives (86.2%) compared to (33.3%) in the controls group.

The distribution of the study sample according to the residence area revealed that 48.3% of cases reported living near agricultural areas compared to 7.4% of controls, with 11.67 times more SLE risk (OR =11.67, 95%CI =2.32-58.6) compared to those not living near these areas. This may be attributed to some environmental exposures such as exposure to sunlight and pesticides used in agriculture, in addition to lower socioeconomic status, lower educational level, and poverty.

Table 1: SLE risk estimation in relation to some sociodemographic characteristics and residence area (Rheumatology clinic, Alexandria University Hospital, 2020).

Item		Cases (n=29)		Controls (n=27)		Test of Significance	Odds Ratio OR (95%CI)
		N	(%)	N	(%)	(P-value)	
Socio-demographic characteristics	Age						
	20-	9	31	12	44.4	$\chi^2=1.07$ (P=0.3)	0.563 (0.188-1.67)
	35+ (Ref)	20	69	15	66.6		1
	Mean $\pm$ SD	37.48 $\pm$ 8.378		37.81 $\pm$ 9.872		t = - 0.136 (P =0.892)	
	Marital status						
	Married	27	93.1	23	85.2	0.414 [□]	2.34 (0.39-14.01)
	Single/ Divorced/ Widow (Ref)	2	6.9	4	14.8		1
	Level of education						
	Illiterate	9	31	4	14.8	0.010**	9 (0.75-108.31)
	Primary/ Preparatory						
Work	Secondary/ Above intermediate	11	37.9	3	11.1		14.67 (1.16-185.23)
	University/ graduate (Ref)	8	27.6	16	59.3		2 (0.19-20.97)
	Post	1	3.4	4	14.8		
							1

	Not working	25	86.2	9	33.3	$\chi^2=16.388$ (P=0.001)*	12.5 (3.32-47)
	Working (Ref)	4	13.8	18	66.7		1
Residence Area	Heavy Traffic						
	Yes	10	34.5	12	44.4	$\chi^2=0.582$ (P =0.446)	0.66 (0.22-1.93)
	No (Ref)	19	65.5	15	55.6		1
	Petrol station						
	Yes	0	0	2	7.4	0.228 [□]	0.17 (0.01-3.77)
	No (Ref)	29	100	25	92.6		1
	Rubbish						
	Yes	6	20.7	2	7.4	0.254 [□]	3.26 (0.6-17.81)
	No (Ref)	23	79.3	25	92.6		1
	Factory						
	Yes	5	17.2	2	7.4	0.424 [□]	2.6 (0.46-14.73)
	No (Ref)	24	82.2	25	92.6		1
	Agriculture						
	Yes	14	48.3	2	7.4	$\chi^2=11.443$ (p =0.001)*	11.67(2.32-58.6)
	No (Ref)	15	51.7	25	92.6		1
	Wireless station						
	Yes	0	0	2	7.4	0.228 [□]	0.17 (0.01-3.77)
	No (Ref)	29	100	25	92.6		1

Ref is reference, X^2 = Chi-square, + Monte Carlo, χ^2 Fisher, t = t-test, * P <0.05.

Estimation of SLE risk in relation to some lifestyle factors

Lack of physical activity, exposure to domestic animals, or to sun light showed significant results (table 2). As regards walking and physical activity, a high proportion of SLE patients (89.7%) don't like to walk or perform any physical activity compared to (40.7%) in the control group. Sedentary lifestyle increased SLE risk by 12.61 folds (OR =12.61, 95%CI =3.05-52.17). This result is in accordance with a cross sectional study that showed that a high proportion of SLE patients were physically inactive with a long daily sedentary time [33].

Dealing with domestic animals was another risk factor that was tested in the current study; the results showed that 58.6% of cases were exposed to animals more than controls (29.6%). This may be because 48.3% of cases live in rural areas compared to 7.4% of controls.

There is a statistically significant increase in SLE risk when dealing with animals or birds (OR =3.36, 95%CI =1.11-10.19). Most of the animals were farm animals (sheep, chicken). This was in the same line with a case control study in Southern Sweden that reported a statistically significant difference between cases and controls regarding close contact with sheep [34]. This was not in the same line with the results of a recent study which supported the idea that exposures related to childhood farm residence and livestock farming may decrease susceptibility to developing SLE and that contact with livestock may confer protection against SLE [35].

Results about UV radiation exposure and SLE risk showed a statistically significant increase of SLE risk (OR =13.714, 95%CI =3.768-49.920). This data supports the role of sun exposure as a trigger for SLE [34, 36, 37].

Estimation of SLE risk in relation to some indoor environmental risk factors

The risk values for the association between some indoor environmental exposures and SLE were statistically not significant except for the type of drinking water, the filled tube cooker as the fuel used at home, and passive smoking (table 2), 62.1% of SLE patients use tap water for drinking compared to 22.2% of controls, meanwhile, the majority of controls (74.1%) use filtered water compared to 37.9% of cases. The use of filtered water for drinking was assumed to have protective effects against SLE.

A significant difference (X^2 =16.021, P =0.001) between cases and controls with regard to fuel used at home, where 79.3% of cases reported the use of gas cylinders compared to 25.9% of controls. The use of gas cylinders constituted 10.95 times more risk to SLE than the use of natural gas pipes (OR =10.95 , 95%CI =3.16-38.01).

Regarding passive smoking at home, which was assessed by the number of active smokers who were residing in the same house, (62.1%) of cases were exposed to Environmental Tobacco Smoke (ETS) versus (29.6%) of controls, there was a statistically significant difference between cases and controls with regard to exposure to ETS (OR =3.886, 95% CI =1.273-11.861).

Table 2: SLE risk estimation in relation to some lifestyle factors and some indoor environmental risk factors (Rheumatology clinic, Alexandria University Hospital, 2020).

Lifestyle factors	Item		Cases		Controls		Test of Significance (P-value)	Odds Ratio
			(n= 29)		(n=27)			OR (95%CI)
			N	(%)	N	(%)		
	Physical activity/ Walking	Yes (Ref)	3	10.3%	16	59.3%	χ^2 =14.923 (P =0.001)*	1
		No	26	89.7%	11	40.7%		12.61 (3.05-52.17)
	Domestic animals raising	Yes	17	58.6%	8	29.6%	χ^2 =4.755 (P =0.029)*	3.36 (1.11-10.19)
		No (Ref)	12	41.4%	19	70.4%		1
	Use of kohl	Yes	9	69%	5	18.5%	χ^2 =1.168 (P =0.280)	1.980 (0.567-6.909)
			20	31%	22	81.5%		1
		No (Ref)						
	Use of hair dyes	Yes	10	34.5%	6	22.2%	χ^2 =1.030 (P =0.310)	1.842 (0.562-6.038)
		No (Ref)	19	65.5%	21	77.8%		1
	Use of lipstick	Yes	6	20.7%	5	18.5%	χ^2 =0.042 (P =0.838)	1.148 (0.306-4.309)
			23	79.3%	22	81.5%		1
		No (Ref)						
	Sun exposure	Yes	24	82.8%	7	25.9%	$18.274\chi^2$ = (P =0.001)*	13.714 (3.768-49.920)
No (Ref)		5	17.2%	20	74.1%	1		
Indoor Environmental Risk Factors	Bad water quality	Yes	23	79.3%	16	59.3%	χ^2 =2.659 (P =0.103)	2.635 (0.808-8.592)
		No (Ref)	6	20.7%	11	40.7%		1
	Drinking water pipes	New (Ref)	18	62.1%	16	59.3%	χ^2 =0.046 (P =1.000)	1
		Old	11	37.9%	11	40.7%		0.89 (0.3-2.6)
	Type of drinking water	Bottled	0	0%	1	3.7%	0.003 + *	0.12 (0-3.25)
		Filtered	11	37.9%	20	74.1%		0.18 (0.06-0.6)

	Tap water (Ref)	18	62.1%	6	22.2%		1
Type of paint	Wallpaper	1	3.4%	6	22.2%	0.152 ⁺	0.15 (0.02-1.37)
	Cement	3	10.3%	1	3.7%		2.67(0.25-28.28)
	Old	7	24.1%	4	14.8%		1.56 (0.38-6.31)
	New (Ref)	18	62.1%	16	59.3%		1
Fuel used at home	Natural gas pipes (Ref)	6	20.7%	20	74.1%	X ² =16.021 (P=0.001)*	1
	Gas cylinder	23	79.3%	7	25.9%		10.95 (3.16-38.01)
Passive smoking at home	Yes	18	62.1%	8	29.6%	X ² =5.916 (P=0.015)*	3.886 (1.273-11.861)
	No (Ref)	11	37.9%	19	70.4%		1
Degree of smoking (CPD)	No (Ref)	11	37.9%	19	70.4%	0.035 ⁺	1
	Light	12	41.4%	7	25.9%	X ² for trend=5.87 (0.01543)	2.96 (0.9-9.75)
	Heavy	6	20.7%	1	3.7%		10.36 (1.1-97.69)

Ref is reference, X²= Chi-square, + Monte Carlo, CPD the number of cigarettes smoked per day, heavy (CPD ≥ 20), light (CPD < 20), * P <0.05.

Moreover, SLE risk increased with the increase in the number of cigarettes smoked per day (the risk was 2.96 & 10.36 times for light and heavy passive smoking (OR =2.96, 95%CI =0.9-9.75; OR =10.36, 95%CI =1.1-97.69 respectively). A significant trend for risk was noticed with increased passive smoking (X² for trend=5.87, P =0.015) concluding that ETS may be an important risk factor for SLE.

ETS as a risk factor for SLE was not enormously discussed except for a cross sectional study of Brazilian SLE patients that assessed the association between smoking and SLE and confirmed that never smokers confer a 22% relative SLE risk reduction compared to ever smokers (including second hand smokers) [38].

In addition, data from a cohort of SLE patients and controls suggested that secondhand smoke during childhood may be a risk factor for SLE (OR =1.81, 95% CI =1.13 - 2.89) [39]. Whereas, a US prospective cohort study concluded that Early-life exposure to cigarette smoke due to mothers' or fathers' smoking did not increase the risk of adult-onset SLE (RR =0.9, 95%CI =0.6-1.4; RR =1.0, 95% CI =0.8-1.3 respectively) [40].

Estimation of SLE risk in relation to a family history of any auto-immune disease

The study found a statistically significant difference between cases and controls regarding the family history of any auto immune disease as illustrated in (table 3). The highest proportion of the study patients (96.6%) had negative family history of SLE. This supports the great contribution of other risk factors rather than the genetic factors in the development of SLE; suggesting the influence of environmental triggers on disease expression [8, 41, 42].

This finding was in line with a study that reported that autoimmune diseases in family members have not been associated with SLE [43]. On the other hand, this finding is in contrast with other previous studies that stated that family history of an auto immune disease in first degree relatives (parents or siblings) was associated with increased SLE risk [44, 45].

Estimation of SLE risk in relation to hormonal factors

The findings of the present study showed the prevalence of menstrual disorders and hormonal disturbances among SLE patients as demonstrated in (table 3). Early menopause was present in (31%) of SLE patients compared to (0%) of controls, irregular menstrual cycle was prevalent in (40%) of SLE patients compared to (8%) of controls, (37.9%) of cases used hormonal therapy compared to (11.1%) of controls, and (31%) of cases had problems in uterus versus (7.4%) of controls.

There was a statistically significant increase in SLE risk with early menopause, menstrual irregularities, use of hormonal therapy, and presence of problems in uterus (OR =31.26, 95%CI = 1.7-575.54; OR =7.67, 95%CI =1.3-45.29; OR =4.889, 95%CI = 1.19-20.13; OR= 5.625, 95%CI= 1.09-29.03 respectively).

The results of the current study was in concordance with a population-based Japanese case control study that revealed that menstrual irregularity was associated with an increased SLE risk (OR =3.79, 95% CI =1.43-10.01) [46]. In the same line comes a cross sectional study of 61 SLE patients, in which 49.2 % of the patients had menstrual irregularities, of which 60% had sustained amenorrhoea (premature menopause) compared to the control group (16.7%) [47]. Moreover, a cross sectional study (N=87) showed menstrual alterations in 37.9% of SLE patients and amenorrhea in 11.5% of patients which was higher than the general population [48]. Additionally, an increased SLE risk was reported in the NHS (Nurses' Health Study) with the use of estrogen replacement therapy [49]. Moreover, a population-based nested case control study found that the current use of COCs (Combined Oral Contraceptives) was associated with an increased SLE risk (RR =1.54, 95% CI =1.15–2.07) [50].

Other studies contradicted the findings of the current study and stated that the use of HRT (Hormone Replacement Therapy) in postmenopausal SLE women did not appear to increase the rate of lupus flares and appeared to be well tolerated and safe in postmenopausal SLE patients [51-53].

Table 3: SLE risk estimation in relation to family history of any auto immune disease and hormonal factors (Rheumatology clinic, Alexandria University Hospital, 2020).

Item		Cases (n=29)		Controls (n=27)		Test of Significance (P-value)	Odds Ratio OR (95%CI)
		N	(%)	N	(%)		
Family history of any auto immune disease						$\chi^2 = 8.513$ (0.004)*	
No (Ref)		28	96.6%	18	66.7%		1
Yes		1	3.4%	9	33.3%		0.07 (0.01-0.61)
Menopause	Early	9	31%	0	0%	0.001**	31.26 (1.7- 575.54)
	Yes	5	17.2%	2	7.4%		4.17 (0.72- 24.23)
	No (Ref)	15	51.7%	25	92.6%		1
Menstrual cycle	Regular (Ref)	9	60%	23	92%	0.036 [†] *	1
	Irregular	6	40%	2	8%		7.67 (1.3- 45.29)
Taking hormonal therapy	Yes	11	37.9%	3	11.1%	$\chi^2 = 5.364$ (P =0.021)*	4.889 (1.19- 20.13)
	No (Ref)	18	62.1%	24	88.9%		1
Problems in uterus	Yes	9	31%	2	7.4%	$\chi^2 = 4.945$ (P =0.026)*	5.625 (1.09- 29.03)
	No (Ref)	20	69%	25	92.6%		1

Ref is reference, χ^2 = Chi-square, + Monte Carlo, [†] Fisher, * P <0.05.

Estimation of SLE risk in relation to blood levels of lead, cadmium and zinc

As shown in (table 4), the range of values of blood lead levels (Pb) in the cases group was very extensive from minimum 0.0237 mg/ L to maximum 0.6951 mg/ L and it is higher than the range of values in the controls group. The median concentration of blood lead in the cases group (0.115±0.165) was significantly higher than in the controls group (0.067±0.077). There were significantly higher blood lead levels in the cases group compared to the controls group (U =210, P =0.003).

After categorization of the level of blood lead in the sample, the SLE risk associated with blood lead levels ≥ 0.09 mg/L was higher and statistically significant (OR =7.71, 95%CI =1.85-32.21) in comparison with subjects having blood lead

level < 0.05 mg/L. A statistically significant trend was computed (Chi-square trend for Odds =7.77, P value =0.005) showing that the more the increase in blood lead level, the more the chance of SLE occurrence.

It was observed that the median blood lead levels in the cases group as well as the controls group in this study were higher than the CDC permissible range of less than 5 µg/dl of lead in children and adults (0.05 mg/L) and this is indicative of the extent of environmental lead pollution [54].

The median levels of blood cadmium (Cd) in cases group (0.059±0.102 mg/L) were significantly higher than in the controls group (0.017±0.042 mg/L) as U =216, P =0.004. It was noticed that the median blood cadmium levels for both cases and controls in this study were higher than the WHO permissible range of 0.03-0.12 µg/dl of Cd [55].

As presented in (table 4), the level of blood cadmium in the sample was categorized into groups, females having blood cadmium level from 0.03 to less than 0.07 and ≥ 0.07 mg/L blood had 6.68 and 4.45 times more risk to develop SLE in comparison with females having blood cadmium level < 0.03 mg/L blood and the risks for these upper two categories were statistically significant (95%CI =1.58-28.29; 1.18-16.8 respectively). The observed increased trend was found to be statistically significant (X^2 trend for Odds =4.98, P =0.026).

Regarding blood zinc levels (Zn), as shown in table (4), The median concentration of zinc in the controls group (2.275± 0.707 mg/L blood) was lower than the median concentration of zinc in the cases group (2.660 ± 0.970 mg/L blood), but the median blood zinc values in the cases and controls groups did not differ significantly (U =290.5, P > 0.05).

It was observed that the median blood zinc levels were not consistent with reference ranges of zinc in blood (70 - 120 µg/dl) [56], requiring further consideration of zinc levels to avoid overdose and toxicity.

Data from experimental studies suggested that heavy metals may enhance systemic autoimmunity or accelerate disease progression in experimental models of lupus and that co-exposure to certain heavy metals may increase the risk associated with other exposures [5].

In contrast to the current study is a recent similar case control study that reported that SLE diagnosis was associated with lower serum Zn (P = 0.003), and Pb (P =0.020) [57]. Decreased serum Zn levels in SLE patients compared to healthy controls were also reported in other studies [23, 58]. However, some studies did not observe a significant difference in serum Zn concentrations between SLE patients and healthy controls which is similar to the finding of the present study [59, 60].

Table 4: SLE risk estimation in relation to blood levels of lead, cadmium, and zinc(Rheumatology clinic, Alexandria University Hospital, Alexandria 2020).

Blood level		Cases (n=29)		Controls (n=27)		Test of Significance (P-value)	OR (95%CI)
		N	(%)	N	(%)		
Lead (mg/L)	<0.05 (Ref)	4	13.8%	12	44.4%	$\chi^2=8.847$ (P =0.012)*	1
	0.05-						
	0.09+	7	24.1%	8	29.6%	Chi-square trend for Odds =7.77 (P =0.005)*	2.63 (0.57-12)
		18	62.1%	7	25.9%		7.71 (1.85-32.21)
Minimum value		0.0237		0.0175			
Maximum value		0.6951		0.4447			
Range (maximum - minimum)		0.6714		0.4272			
Median±IQR		0.115±0.165		0.067±0.077		U=210,	
Mean rank		34.76		21.78		(P =0.003)*	
Cadmium (mg/L)	<0.03 (Ref)	7	24.1%	17	63%	$\chi^2=8.844$ (P =0.012)*	1
	0.03-	11	37.9%	4	14.8%		6.68 (1.58-28.29)
	0.07+	11	37.9%	6	22.2%	Chi-square trend for Odds =4.98 (P =0.026)*	4.45 (1.18-16.8)
Minimum value		0.0068		0.0032			
Maximum value		0.3233		0.1470			
Range (maximum – minimum)		0.3165		0.1438			
Median±IQR		0.059±0.102		0.017±0.042		U=216,	
Mean rank		34.55		22		(P =0.004)*	
Zinc (mg/L)							
Minimum value		0.5412		1.1170			
Maximum value		4.7166		3.3590			
Range (maximum–minimum)		4.1754		2.2420			
Median ± IQR		2.660 ± 0.970		2.275± 0.707		U=290.5, (0.098)	

Mean rank	31.98	24.76
-----------	-------	-------

Ref is reference, X²=chi-square, IQR Inter Quartile Range, U=non- parametric Mann Whitney test, *=P <0.05.

Pearson’s correlation coefficient was calculated to demonstrate the relationship between lead, cadmium, and zinc blood levels with each other and yielded a result of r =0.548, P =0.002, portraying a positive, moderate, linear, and significant correlation between lead and cadmium blood levels for the cases group (figure 1), whereas a non significant correlation was observed in the controls group (r =0.123, P=0.542).

A case control study reported a positive correlation between lead and cadmium blood levels for the exposed group (p =0.39, P =0.023) which was consistent with the finding of the current study, whereas blood zinc levels correlated negatively with both lead (p = - 0.41, P =0.015) and cadmium blood levels (p = - 0.44, P =0.009). In addition, no correlations between the studied metals were found in the control group and the study suggested that zinc insufficiency is more likely to occur in cases of combined exposure to cadmium and lead, because of competition between similar ions for receptors involved in absorption, transport, storage or function, and this would explain the negative correlation in the controls group between zinc blood levels with either lead and cadmium levels [61].

Analysis of risk factors affecting SLE risk by stepwise logistic regression

A multivariate stepwise logistic regression model was built in order to determine which of these predictors really contribute to predicting SLE risk, and exclude those who don’t.

Negelkerke R Square suggests that the model explains 78.4% of the variance in the outcome. The accuracy of the model was 87.5%, depicting that the model can correctly classify 87.5% of the cases. The sensitivity and specificity of the model were calculated, and they were 86.2%, and 88.9% respectively.

As illustrated in (table 5), the final model revealed that only five factors showed significant association with SLE. The risk was highest for subjects living near agricultural areas with an OR of 58.556 (95% CI =1.897-1807.759), followed by subjects exposed to passive smoking with an OR of 24.116 (95%CI =1.763-329.799), then subjects having blood lead levels ≥ 0.075 mg/L who were 18.981 times (95%CI =1.228-293.364) more likely to have SLE than those having blood lead levels < 0.075 mg/L, followed by subjects exposed to the sunlight who were at increased risk of SLE by 9.549 (95%CI =1.299-70.224). Whereas walking or doing exercise were significantly protective against SLE (p = 0.006); the table demonstrated a negative B coefficient (-5.246) which indicates that a decrease in the walking and exercise is associated with a greater likelihood of SLE risk.

Table 5: Results of the stepwise logistic regression analysis for risk factors affecting SLE risk (Rheumatology outpatient clinic, Alexandria University Hospital, Alexandria, 2020).

Variable	Regression coefficient B	P.value	Adjusted OR	95% CI	
				Lower	Upper
Living near agricultural areas (Ref1)	4.070	0.020*	58.556	1.897	1807.759
Walking or exercise (Ref2)	-5.246	0.006*	0.005	0.000	0.220
Sun exposure (Ref3)	2.256	0.027*	9.549	1.299	70.224
Passive smoking (Ref4)	3.183	0.017*	24.116	1.763	329.799
Lead (Ref5)	2.943	0.035*	18.981	1.228	293.364
Constant	-3.477	0.010*	0.031		

Ref1: Not living near agricultural areas, Ref2: Not walking or doing exercise, Ref3: No sun exposure, Ref4: No passive smoking, Ref5: < 0.075 mg/L

Model: Variables initially included: Living near agricultural areas, walking and exercise, Level of education, early menopause, Sun exposure, hormones, Passive smoking, and Lead, cadmium, and Zinc. $\text{pseudoR}^2_{\text{model}}=78.4\%$, which mean that the model could explain about 78% of variance in SLE outcome.

Limitations Of The Study

- The potential of case control studies for recall bias and misclassification error; as most exposure information were based on self-reported history so some inaccuracies can be expected. The design of the study also limited the ability to ascribe causal relationships to the associations detected and to control for all potential confounders.
- Absence of genetic information for participants, so the potential effects of genetic heterogeneity on the association between risk factors and SLE risk could not be determined.
- Human population are rarely exposed to a single agent over time, and there may be a significant delay between exposure and the onset of the disease.

Conclusion & Recommendations

To date, our knowledge about the etiology of SLE is still unclear and limited; genetic and environmental interactions were suggested.

From the present case control study, it is concluded that the risk portion attributed to unsafe and unhealthy environment was found to be quite significant, showing how the environment can play an important role in SLE occurrence.

Exposure to potential environmental risk factors specifically heavy metals should not be under-estimated. Hence, there is an urgent need for interventions to reduce environmental risk factors exposure in order to achieve substantial public health gains.

Increasing the awareness of patients about the environmental pollutants and the ways to protect their health is necessary. As well as the awareness of health care providers about environmental risk factors, so they can advise the patients about the ways of reduction of exposure to environmental hazards. Educational awareness programs to patients and their family should be carried out through media and non-governmental organizations (NGOs) to raise their knowledge about environmental hazards and how to minimize the sources of their exposure and their consequent negative impacts.

Additional experimental and epidemiological studies are required to determine the causative role of several environmental exposures, to confirm data from case control studies. Additionally, the combinatory effects of genetic susceptibility with non-genetic factors need to be addressed.

List Of Abbreviations

Cd	Cadmium
CDC	Centers for Disease Control and prevention
CI	Confidence Interval
COCs	Combined Oral Contraceptives
CPD	number of Cigarettes smoked Per Day
dsDNA	Double stranded DNA
ETS	Environmental Tobacco Smoke
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
ng/ml	nanogram/milliliter
NGOs	Non-Governmental Organizations
NHS	Nurses' Health Study
NZB/NZW	Hybrid New Zealand Black/ White mice
P	Probability
Pb	Lead
r	Pearson correlation coefficient (parametric)
R ²	Coefficient of determination
Sig.	Significance
SLE	Systemic Lupus Erythematosus
SLICC	Systemic Lupus International Collaborating Clinics
SPSS	Statistical Package for Social Sciences
UK	United Kingdom
UV	Ultraviolet
WHO	World Health Organization
Zn	Zinc

Declarations

- Availability of data and material

The datasets used and/or analyzed during the current study are not publicly due to privacy and ethical concerns but are available from the corresponding author on reasonable request.

- **Competing interests**

The authors declare that they have no competing interests.

- **Funding**

Not applicable.

- **Authors' contributions**

All authors contributed to the conception and design of the work.

RR is assigned as the corresponding author. She accomplished all the practical part of the research, in addition to interpreting and analyzing the results and discussing it with the previous results, then writing the manuscript. The corresponding author ensures that all listed authors have approved the manuscript before submission.

BM helped at all stages of the research, followed up the laboratory part of the study, assisted in preparing the data collection tool and helped in data analysis and interpretation of the results.

AA guided the selection of cases that were suitable for the research according to the inclusion and exclusion criteria, supervised the sampling process, and revised the results.

AM helped in reviewing the results and revising the work.

All authors read and approved the final submitted manuscript and agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

References

1. Ferenkeh-Koroma A. Systemic lupus erythematosus: nurse and patient education. *Nursing Standard*. 2012; 26(39): 49–57. doi: 10.7748/ns2012.05.26.39.49.c9134
2. Streifler JY, Molad Y. Connective tissue disorders: systemic lupus erythematosus, Sjogren's syndrome, and scleroderma. *Handbook of Clinical Neurology*. 2014; 119: 463–73. doi: 10.1016/B978-0-7020-4086-3.00030-8
3. Cattalini M, Soliani M, Caparello MC, Cimaz R. Sex Differences in Pediatric Rheumatology. *Clinical Reviews in Allergy & Immunology*. 2019; 56(3): 293–307. doi: 10.1007/s12016-017-8642-3
4. Brodin P, Jojic V, Gao T, Bhattacharya S, Angel CJ, Furman D, et al. Variation in the human immune system is largely driven by non-heritable influences. *Cell*. 2015; 160(1-2): 37–47. doi: 10.1016/j.cell.2014.12.020
5. Barbhaiya M, Costenbader KH. Environmental exposures and the development of systemic lupus erythematosus. *Current Opinion in Rheumatology*. 2016; 28(5): 497–505. doi: 10.1097/BOR.0000000000000318
6. Gergianak I, Fanouriakis A, Repa A, Tzanakakis M, Adamichou C, Pompieri A, et al. Epidemiology and burden of systemic lupus erythematosus in a Southern European population: data from the community-based lupus registry of Crete, Greece. *Annals of the Rheumatic Diseases* 2017;76: 1992–2000. doi: 10.1136/annrheumdis-2017-211206

7. Deng Y, Tsao BP. Genetic susceptibility to systemic lupus erythematosus in the genomic era. *Nature Reviews Rheumatology*. 2010; 6(12): 683–92. doi: 10.1038/nrrheum.2010.176
8. Leffers HCB, Lange T, Collins C, Ulff-Møller CJ, Jacobsen S. The study of interactions between genome and exposome in the development of systemic lupus erythematosus. *Autoimmunity Reviews*. 2019; 18(4): 382–92. doi: 10.1016/j.autrev.2018.11.005
9. Gulati G, Brunner HI. Environmental triggers in systemic lupus erythematosus. *Seminars in Arthritis and Rheumatism*. 2018; 47(5): 710–17. doi: 10.1016/j.semarthrit.2017.10.001
10. Edwards TM, Myers JP. Environmental exposures and gene regulation in disease etiology. *Ciência & Saúde Coletiva*. 2008; 13(1): 269–81. doi: 10.1590/s1413-81232008000100030
11. ATSDR [internet]. ATSDR's Substance Priority List. 2019. Retrieved from <https://www.atsdr.cdc.gov/spl/index.html>
12. Lemos VA, de Carvalho AL. Determination of cadmium and lead in human biological samples by spectrometric techniques: a review. *Environmental Monitoring and Assessment*. 2010; 171(1-4): 255–65. doi: 10.1007/s10661-009-1276-z
13. Jan AT, Azam M, Siddiqui K, Ali A, Choi I, Haq QM. Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. *International journal of molecular sciences*. 2015; 16(12): 29592–630. <https://doi.org/10.3390/ijms161226183>
14. Mannino DM, Homa DM, Matte T, Hernandez-Avila M. Active and passive smoking and blood lead levels in U.S. adults: data from the Third National Health and Nutrition Examination Survey. *Nicotine Tob Res*. 2005; 7(4):557-64. doi: 10.1080/14622200500185264. PMID: 16085527.
15. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. *Experientia Supplementum*. 2012; 101, 133–64. doi: 10.1007/978-3-7643-8340-4_6
16. Virani S, Rentschler KM, Nishijo M, Ruangyuttikarn W, Swaddiwudhipong W, Basu N, Rozek LS. DNA methylation is differentially associated with environmental cadmium exposure based on sex and smoking status. *Chemosphere*. 2016; 145: 284–90. doi: 10.1016/j.chemosphere.2015.10.123
17. Joshi BC, Dwivedi C, Powell A, Holscher M. Immune complex nephritis in rats induced by long-term oral exposure to cadmium. *Journal of Comparative Pathology*. 1981; 91(1): 11–5. doi: 10.1016/0021-9975(81)90040-2
18. Hudson CA, Cao L, Kasten-Jolly J, Kirkwood JN, Lawrence DA. Susceptibility of lupus-prone NZM mouse strains to lead exacerbation of systemic lupus erythematosus symptoms. *Journal of Toxicology and Environmental Health (Part A)*. 2003; 66(10): 895–918. doi: 10.1080/15287390306456
19. Leffel EK, Wolf C, Poklis A, White KL. Drinking water exposure to cadmium, an environmental contaminant, results in the exacerbation of autoimmune disease in the murine model. *Toxicology*. 2003; 188(2-3): 233–50. doi: 10.1016/s0300-483x(03)00092-1
20. Cooper GS, Wither J, Bernatsky S, Claudio JO, Clarke A, Rioux JD, et al. Occupational and environmental exposures and risk of systemic lupus erythematosus: silica, sunlight, solvents. *Journal of Rheumatology (Oxford)*. 2010; 49(11): 2172–80. doi: 10.1093/rheumatology/keq214
21. Ibs KH, Rink L. Zinc-altered immune function. *Journal of Nutrition*. 2003; 133(5 Suppl 1): 1452S-6S. doi: 10.1093/jn/133.5.1452S
22. Maywald M, Wessels I, Rink L. Zinc Signals and Immunity. *International Journal of Molecular Sciences*. 2017; 18(10): 2222. doi:10.3390/ijms18102222
23. Sahebari M, Abrishami-Moghaddam M, Moezzi A, Ghayour-Mobarhan M, Mirfeizi Z, Esmaily H, Ferns G. Association between serum trace element concentrations and the disease activity of systemic lupus erythematosus. *Lupus*. 2014; 23(8): 793–801. doi: 10.1177/0961203314530792

24. Beach RS, Gershwin ME, Hurley LS. Nutritional factors and autoimmunity. II. Prolongation of survival in zinc-deprived /W mice. *The Journal of Immunology*. 1982; 128(1): 308-13. Available from: <https://www.jimmunol.org/content/128/1/308>
25. Leiba A, Amital H, Gershwin ME, Shoenfeld Y. Diet and lupus. *Lupus*. 2001; 10(3): 246–48. doi: 10.1191/096120301674681790
26. Constantin MM, Nita IE, Olteanu R, Constantin T, Bucur S, Matei C, Raducan A. Significance and impact of dietary factors on systemic lupus erythematosus pathogenesis. *Experimental and Therapeutic Medicine*. 2019; 17(2): 1085–90. doi: 10.3892/etm.2018.6986
27. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and Validation of Systemic Lupus International Collaborating Clinics Classification Criteria for Systemic Lupus Erythematosus. *Arthritis and Rheumatism*. 2012; 64(8): 2677–86. doi: 10.1002/art.34473
28. Toth CN, Baranyai E, Csipo I, Tarr T, Zeher M, Posta J, Fabian I. Elemental Analysis of Whole and Protein Separated Blood Serum of Patients with Systemic Lupus Erythematosus and Sjogren's Syndrome. *Biological Trace Element Research*. 2017; 179(1): 14–22. doi: 10.1007/s12011-017-0945-y
29. Ruxton GD, Neuhauser M, Freckleton R. Review of alternative approaches to calculation of a confidence interval for the odds ratio of a 2 × 2 contingency table. *Methods in Ecology and Evolution*. 2013; 4(1): 9–13. doi: 10.1111/j.2041-210x.2012.00250.x
30. Valenzuela C. [2 Solutions for Estimating Odds Ratios With Zeros]. *Revista medica de Chile*. 1993; 121(12): 1441-4. Available from: <https://pubmed.ncbi.nlm.nih.gov/8085071/>
31. Schober P, Boer C, Schwarte LA. Correlation Coefficients: Appropriate Use and Interpretation. *Anesthesia & Analgesia*. 2018; 126(5): 1763–8. doi: 10.1213/ANE.0000000000002864
32. Ward MM. Education level and mortality in systemic lupus erythematosus (SLE): evidence of underascertainment of deaths due to SLE in ethnic minorities with low education levels. *Arthritis & Rheumatology*. 2004; 51(4): 616–24. doi: 10.1002/art.20526
33. Margiotta DPE, Basta F, Dolcini G, Batani V, Lo Vullo M, Vernuccio A, et al. Physical activity and sedentary behavior in patients with Systemic Lupus Erythematosus. *PLoS One*. 2018; 13(3): e0193728. doi: 10.1371/journal.pone.0193728
34. Bengtsson AA, Rylander L, Hagmar L, Nived O, Sturfelt G. Risk factors for developing systemic lupus erythematosus: a case-control study in southern Sweden. *Journal of Rheumatology (Oxford)*. 2002; 41(5): 563–71. doi: 10.1093/rheumatology/41.5.563
35. Parks C, Long S, Beane-Freeman L, Jonathan H, Dale S. Systemic Lupus Erythematosus and Sjögren's Syndrome in the Agricultural Health Study: Lower Risk Associated with Childhood Farm Residence and Raising Livestock [abstract]. *Arthritis & Rheumatology*. 2019; 71 (suppl 10). Available from: <https://acrabstracts.org/abstract/systemic-lupus-erythematosus-and-sjogrens-syndrome-in-the-agricultural-health-study-lower-risk-associated-with-childhood-farm-residence-and-raising-livestock/>
36. Zamansky GB. Sunlight-induced pathogenesis in systemic lupus erythematosus. *Journal of Investigative Dermatology*. 1985; 85(3): 179–80. doi: 10.1111/1523-1747.ep1227664.
37. Mak A, Tay SH. Environmental factors, toxicants and systemic lupus erythematosus. *International Journal of Molecular Sciences*. 2014; 15(9): 16043–56. doi: 10.3390/ijms150916043
38. Montes RA, Mocarzel LO, Lanzieri PG, Lopes LM, Carvalho A, Almeida JR. Smoking and Its Association With Morbidity in Systemic Lupus Erythematosus Evaluated by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index: Preliminary Data and Systematic Review. *Arthritis & Rheumatology*. 2016; 68(2): 441–8. doi: 10.1002/art.39427

39. Minkin SJ, Slan SN, Gilkeson GS, Kamen DL. Smoking and secondhand smoke among patients with systemic lupus erythematosus and controls: associations with disease and disease damage. *Arthritis Research & Therapy*. 2014; 16(Suppl 1): A40. doi: 10.1186/ar4656
40. Simard JF, Costenbader KH, Liang MH, Karlson EW, Mittleman MA. Early-life exposure to cigarette smoke and adult-onset SLE. *Lupus*. 2009; 18(5): 431–5. doi: 10.1177/0961203308098186
41. Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P, et al. A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis & Rheumatology*. 1992; 35(3): 311–8. doi: 10.1002/art.1780350310
42. Kamen DL. Environmental influences on systemic lupus erythematosus expression. *Rheumatic Disease Clinics of North America*. 2014; 40(3): 401-12, vii. doi: 10.1016/j.rdc.2014.05.003
43. Grimaldi-Bensouda L, Le Guern V, Kone-Paut I, Aubrun E, Fain O, Ruel M, et al. The risk of systemic lupus erythematosus associated with vaccines: an international case-control study. *Arthritis & Rheumatology*. 2014; 66(6): 1559–67. doi: 10.1002/art.38429
44. Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS. Hormonal and reproductive risk factors for development of systemic lupus erythematosus: results of a population-based, case-control study. *Arthritis & Rheumatology*. 2002; 46(7): 1830–9. doi: 10.1002/art.10365
45. Kuo CF, Grainge MJ, Valdes AM, See LC, Luo SF, Yu KH, et al. Familial Aggregation of Systemic Lupus Erythematosus and Coaggregation of Autoimmune Diseases in Affected Families. *JAMA Internal Medicine*. 2015; 175(9): 1518–26. doi: 10.1001/jamainternmed.2015.3528
46. Minami Y, Sasaki T, Komatsu S, Nishikori M, Fukao A, Yoshinaga K, Hisamichi S. Female systemic lupus erythematosus in Miyagi Prefecture, Japan: a case-control study of dietary and reproductive factors. *The Tohoku Journal of Experimental Medicine*. 1993; 169(3): 245–52. doi: 10.1620/tjem.169.245
47. Fatnoon NN, Azarisman SM, Zainal D. Prevalence and risk factors for menstrual disorders among systemic lupus erythematosus patients. *Singapore medical journal*. 2008; 49(5): 413-8. Available from: <https://pubmed.ncbi.nlm.nih.gov/18465054/>
48. Nonato DR, Barbosa VS, Rodrigues DL, Amaral PC, Assis MR, Antonio da Silva N. Menstrual Disturbances in Systemic Lupus Erythematosus Patients Using Immunossuppressants. *Revista Brasileira de Reumatologia*. 2010; 50(5) : 501–15.
49. Sanchez-Guerrero J, Liang MH, Karlson EW, Hunter DJ, Colditz GA. Postmenopausal estrogen therapy and the risk for developing systemic lupus erythematosus. *Annals of Internal Medicine*. 1995; 122(6): 430–3. doi: 10.7326/0003-4819-122-6-199503150-00005
50. Bernier MO, Mikaeloff Y, Hudson M, Suissa S. Combined oral contraceptive use and the risk of systemic lupus erythematosus. *Arthritis and Rheumatology*. 2009; 61(4): 476–81. doi: 10.1002/art.24398
51. Buyon JP, Petri MA, Kim MY, Kalunian KC, Grossman J, Hahn BH, et al. The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. *Annals of Internal Medicine*. 2005; 142(12 Pt 1): 953–62. doi: 10.7326/0003-4819-142-12_part_1-200506210-00004
52. Kreidstein S, Urowitz MB, Gladman DD, Gough J. Hormone Replacement Therapy in Systemic Lupus Erythematosus. *Journal of Rheumatology*. 1997; 24(11): 2149–52.
53. Mok C C, Lau CS, Ho CT, Lee KW, Mok MY, Wong RW. Safety of hormonal replacement therapy in postmenopausal patients with systemic lupus erythematosus. *Scandinavian Journal of Rheumatology*. 1998; 27(5): 342–6. doi: 10.1080/03009749850154357

54. ATSDR. Lead Toxicity. What Are U.S. Standards for Lead Levels? [2019-b].Retrieved from:
<https://www.atsdr.cdc.gov/csem/csem.asp?csem=34&po=8>
55. Alli LA. Blood level of cadmium and lead in occupationally exposed persons in Gwagwalada, Abuja, Nigeria. *Interdisciplinary Toxicology*. 2015; 8(3): 146–50. doi: 10.1515/intox-2015-0022
56. Mashhadi MA, Bakhshipour A, Zakeri Z, Ansari- Moghadam A. Reference Range for Zinc Level in Young Healthy Population in Southeast of Iran. *Health Scope*. 2016; 6(1). doi: 10.17795/jhealthscope-18181
57. Pedro EM, da Rosa Franchi Santos LF, Scavuzzi BM, Iriyoda TMV, Peixe TS, Lozovoy M AB, et al. Trace Elements Associated with Systemic Lupus Erythematosus and Insulin Resistance. *Biological Trace Element Research*. 2019; 191(1): 34–44. doi: 10.1007/s12011-018-1592-7
58. Yilmaz A, Sari RA, Gundogdu M, Kose N, Dag E. Trace elements and some extracellular antioxidant proteins levels in serum of patients with systemic lupus erythematosus. *Clinical Rheumatology*. 2005; 24(4): 331–5. doi: 10.1007/s10067-004-1028-y
59. Almroth G, Westberg NG, Sandstrom BM. Normal zinc and selenium levels in patients with systemic lupus erythematosus. *Journal of Rheumatology*. 1985; 12(3): 633-4. Available from: <background-color:#FF3300;vertical-align:super;><https://www.ncbi.nlm.nih.gov/pubmed/4045869></background-color:#FF3300;vertical-align:super;>
60. Nossent J, Lester S, Rischmueller M, Zalewski P. No Zinc Deficiency But a Putative Immunosuppressive Role for Labile Zn in Patients with Systemic Autoimmune Disease. *Current Rheumatology Reviews*. 2017; 13(1): 59–64. doi: 10.2174/1573397111666151026223501
61. Gidikova PL. Blood Lead, Cadmium and Zinc Correlations in Elderly Rural Residents. *Folia Medica (Plovdiv)*. 2019; 61(1) : 113–9. doi: 10.2478/folmed-2018-0051

Figures

Figure 1

Correlation between blood lead and cadmium levels for cases and controls (Rheumatology outpatient clinic, Alexandria University Hospital, Alexandria, 2020).