Organochlorine pesticides, oxidative stress biomarkers, and leukemia: a case-control study

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ABSTRACT

Exposure to pesticides has been linked to an elevated risk of leukemia. The present research aimed to evaluate the relationship between organochlorine (OC) pesticides and biomarkers of oxidative stress in patients with leukemia. This work was conducted on 109 patients with leukemia and 109 healthy controls. The serum concentrations of seven derivatives of OCs including alpha-hexachlorocyclohexane (HCH), beta-HCH, gamma-HCH, 2,4-dichlorodiphenyltrichloroethane (DDT), 4,4-DDT, 2,4-dichlorodiphenyldichloroethylene (DDE), and 4,4-DDE along with acetylcholinesterase (AChE), glutathione peroxidase (GPx), superoxide dismutase (SOD), paraoxonase-1 (PON1), and catalase (CAT) activities as well as total antioxidant capacity (TAC), nitric oxide (NO), protein carbonyl (PC), and malondialdehyde (MDA) levels were measured in all the subjects. Levels of OCs were remarkably higher in patients with leukemia compared with the controls ($p<0.05$). In addition, levels of SOD, AChE, GPx, PON1, and TAC were remarkably lower in patients with leukemia compared with controls ($p<0.05$). In contrast, MDA, NO, and PC concentrations were higher in patients with leukemia than in the controls ($p<0.05$). Moreover, the serum level of 4,4-DDE was negatively associated with GPx activity ($p=0.038$). Our findings suggest that OCs may play a role in the development of leukemia by disrupting the oxidant/antioxidant balance.

Significance of this study

What is already known about this subject?

- One hypothesis is that leukemia results from exposure to carcinogens, including pesticides.
- Organochlorine pesticides (OCPs) are one of the oldest pesticides; however, the possible mechanism of OCPs on the development of leukemia is not fully understood.

What are the new findings?

- This study evaluated the levels of OCPs including alpha-hexachlorocyclohexane (HCH), beta-HCH, gamma-HCH, 2,4-dichlorodipheny ltrichloroethane (DDT), 4,4-DDT, 2,4-dichloro diphenyldichloroethylene (DDE), and 4,4-DDE in patients with acute lymphoblastic leukemia and acute myeloid leukemia.
- We also evaluated association of OCPs with the oxidative stress markers, acetylcholinesterase, and paraoxonase-1 activities in patients with leukemia in the region of southeastern Iran (Kerman), which is an agricultural center.
- Our results showed that OCPs may reduce the body's antioxidant capacity and eventually induce oxidative stress by disrupting the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.

How might these results change the focus of research or clinical practice?

- We showed that disturbance in the oxidant-antioxidant balance in the body is a possible effect of OCPs and may play a role in the development of leukemia.
- Therefore, people engaged in the farmer activity or who live in the agricultural fields should be considered from the oxidant-antioxidant balance.
- These findings increase our understanding of both leukemia pathophysiology and the possible mechanisms of OCPs in leukemia development.

INTRODUCTION

Leukemia is the most common cancer in children with rapid progression, and early diagnosis is essential.[1] One hypothesis is that leukemia results from mutations caused by exposure to carcinogens, including pesticides, during pregnancy or infancy.[2]

Pesticides are a critical group of environmental pollutants with the highest use in agriculture, which are widely employed to protect against diseases and pests.[3] Their use improves the quality of agricultural products while also introducing pesticides into the human diet.[4] This issue is currently one of the major human concerns. Contamination with these chemicals in the short term mainly affects the nervous system, but there is a growing concern about their toxic effects on non-target tissues, which is one of the long-term and chronic effects of these substances.[5] Most people are constantly exposed to low concentrations of organophosphates, and long-term studies have shown that the risks of side effects outweigh the risk

of cancer.[6] The findings on the effects of pesticides on animals show that these compounds have detrimental impacts on various organs including the immune system, reproductive system, liver, and kidney, as well as various biochemical parameters and blood, in human and animal studies.[6 7] These studies have also indicated that these pesticides increase oxidative stress in testicular, kidney, liver, brain, and oligodendrocyte precursor cells.[8]

Organochlorines (OCs) are one of the most essential pesticides, which are carcinogenic due to their long-term persistence in the environment and food.[9] The physical and chemical properties of OCs and their metabolites allow these compounds to enter organisms easily.[10] The high fat solubility and low water solubility of these compounds lead to their accumulation in adipose tissue.[11] The amount of accumulation in the body varies according to the concentration of pesticides and the type and duration of contact in environmental conditions.[11] This accumulation suggests that toxic effects can occur in organisms and areas farther away from the contaminated area.[11] The rate of biological and chemical decomposition of these compounds is very low, and they are easily absorbed by soil and sediments, which can be a source of pollution for humans in the long run.[10]

OCs have irreversible effects on human health through various mechanisms such as abnormal synthesis of sex hormones, genetic changes, endocrine system imbalance, changes in the activity of antioxidant enzymes, and increased oxidative stress.[12] One of these effects is an increased risk of cancer in individuals exposed to these pesticides.[13]

Pesticides bind to the serine residues at the active site of the enzyme acetylcholinesterase (AChE), leading to the inactivation of the enzyme and its dysfunction, which may contribute to the development of leukemia.[14 15]

A growing body of evidence has shown increased levels of oxidative stress in various malignancies while antioxidant enzymes act to prevent the progression of these malignancies.[16]

Paraoxonase-1 (PON1) is an enzyme that plays a role in the antioxidative process by hydrolyzing organophosphates.[17] Studies have shown that the paraoxonase activity is reduced in various cancers due to the high levels of oxidative stress.[18]

Malondialdehyde (MDA) is a carcinogenic compound derived from lipid peroxidation and is known as a biomarker of oxidative stress.[19] Thus, increasing MDA levels indicate that oxidative stress has increased in individuals. Moreover, there is evidence of an association between elevated MDA levels and the risk of hematological malignancies.[20]

Total antioxidant capacity (TAC) is another primary parameter for assessing oxidative stress.[21] It is a non-enzymatic estimation of the antioxidative system with a cumulative impact on other cancer prevention agents.[22] The non-enzymatic antioxidants are ascorbic acid, albumin, glutathione, uric acid, and bilirubin.[23] The total amount of antioxidants can be assessed to determine the effect of nutritional elements on the development of leukemia.[24] Instead of examining nutritional antioxidants, TAC provides a broader chart of dietary patterns, which suggests that TAC might be involved in the pathophysiology of leukemia.[25]

Nitric oxide (NO) is produced by various cells, including monocytes, and plays an important role in non-specific defense.[26] In addition to its various physiological and pathophysiological roles, NO also has anticancer properties.[26] Studies have shown that pesticides can alter the levels of NO, which is secreted by changing the balance of intracellular ions (especially calcium), via acting on the constituent NO.[27] Due to the fact that patients with leukemia tend to have some form of imbalance and defect in their immune system, including the production of NO, the evaluation of NO levels and its relationship with the level of OCs in these patients can be of considerable importance.[28]

Pesticides may reduce the body's antioxidant capacity and eventually induce oxidative stress by disrupting the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).[29] Antioxidant enzymes appear to be essential factors in carcinogenicity due to their ability to suppress oxygen-free radicals. As a result, the enzymatic antioxidant system has a fundamental commitment to protecting fats and proteins from oxidative damage.[30] Basal changes in these enzymatic activities seem to occur after prolonged contact with pesticides.[31]

Southeastern Iran (Kerman) is an agricultural center, producing crops such as pistachios, vegetables, citrus, and other agricultural products, and farmers in this region have been using pesticides to eliminate agricultural pests. Therefore, the farmer is affected by these compounds, and the produced crops can expose the consumers to these pesticides.

Thus, we aimed to evaluate the levels of alpha-hexachlorocyclohexane (HCH), beta-HCH, gamma-HCH, 2,4-dichlorodiphenyltrichloroethane (DDT), 4,4-DDT, 2,4-dichlorodiphenyldichloroethylene (DDE), and 4,4-DDE and their effect on the activity of AChE, PON1, SOD, CAT, and GPx enzymes as well as oxidative stress biomarkers, that is, MDA, TAC, NO, and protein carbonyl (PC), in patients with leukemia to better understand the possible mechanism of the effect of OCs on the development of leukemia.

MATERIALS AND METHODS

Subjects and sampling

A total of 109 patients with leukemia (acute lymphoblastic leukemia (ALL)=72, acute myeloid leukemia (AML)=37) referred to the blood and cancer ward of Bahonar and Afzalipour Hospitals in Kerman (from 2019 to 2021) were selected. In addition, 109 healthy individuals were chosen as the control group. The participants were matched regarding age, sex, and body mass index (BMI). Inclusion criteria included age over 10 years, leukemia confirmation by a hematologist using Wright-Giemsa staining and Sudan black, subgroup confirmation by flow cytometry, and being a resident of Kerman. Exclusion criteria were antioxidant supplementation within 6 months prior to diagnosis, any malignancy other than leukemia, and prior chemotherapy. About 5 mL of blood was taken from healthy individuals and patients before chemotherapy and transferred to two tubes, one containing EDTA to measure the activity of erythrocyte AChE and the other without EDTA to isolate serum in order to measure OC derivatives and biochemistry factors. An informed consent form was obtained from all participants before blood sampling.

Measurement of PON1 activity

PON1 activity was measured using a commercial kit (Relassay, Turkey). The amount of paraoxon (diethyl p-nitrophenylphosphate) hydrolysis was evaluated by observing an elevation in the absorption at 412 nm and 37°C.[32]

Measurement of erythrocyte AChE activity

Compressed blood samples were used at room temperature to test erythrocyte AChE activity. First, the hemoglobin (Hb) concentration was determined using Drabkin's solution. Then 50 μL of the blood sample was added to 3 mL of distilled water. The tubes were vortexed for 30 s. AChE activity was evaluated using thiocholine substrate via the modified Ellman method.[33] The concentration of AChE was divided into grams of Hb to obtain the AChE activity in grams of Hb.

Measurement of TAC

TAC in serum samples was determined based on the Benzie method. This assay determines the ferric reducing antioxidant power. The reduction of the ferric ion (Fe^{3+}), which is colorless, to the ferrous ion (Fe^{2+}) produces a blue color and its absorbance can be measured at 590 nm.[34]

Measurement of PC

PC concentrations were assessed using the Levine method. For this purpose, trichloroacetic acid was added for protein precipitation. The pellets were washed with ethanol-ethyl acetic acid to expel the free reagent. At that point, the precipitated proteins were broken down in 0.8 mL of the guanidine solution, and then the carbonyl content was computed from the maximum absorbance (360 nm).[35]

Measurement of NO

The levels of NO were assessed using the Griess method. First, plasma was deproteinized using $ZnSO_4$ in the presence of 0.3 M NaOH. Afterward, vanadium chloride was added to the deproteinated plasma in order to convert nitrate to nitrite. Next, the Griess reagent was added and the solution was incubated for 30 min at 37°C. Eventually, the absorbance was determined at 570 nm.[36]

MDA measurement

Serum MDA levels were evaluated using the Janero approach. According to this method, serum levels of MDA were assessed as thiobarbituric acid (TBA) reagents. The absorbance of the color produced by the MDA-TBA complex was determined at 532 nm. Total TBA reactive substances were used to measure MDA. MDA levels are expressed in nmol/mL.[37]

Evaluation of SOD and GPx activities

Enzyme activities of GPx and SOD were evaluated using the Randox Assay Kit (London, UK) according to the manufacturer's instructions. The results are reported in U/L and U/mL, respectively.

Evaluation of CAT activity

CAT activity in the serum was assessed by the Sinha method using hydrogen peroxide (H_2O_2) as a substrate. Briefly, 1 mL of H_2O_2 was added to the assay mixture containing 2 mL of phosphate buffer, distilled water, and the necessary enzyme in order to initiate the reaction. Then the solution was incubated at 37°C for 2 min. In the next step, the reaction was terminated via adding 2.5 mL of the DDA reagent (potassium dichromate solution 5% with glacial acetic acid) (1:3 v/v). The absorbance of the reaction mixture was assessed at 570 nm.[38]

Measurement of OCs

The OC residues were extracted using sulfuric acid and n-hexane according to the Zombado method.[39] For this purpose, the internal standard (4,4-dichlorobenzophenone) was added to 250 μL of serum. Samples were extracted two times with 2 mL of n-hexane. Afterward, 400 μL of concentrated sulfuric acid was added to the extract, which led to the separation of the organic part. Dehydration of the obtained organic part was performed using 200 mg of anhydrous sodium sulfate (Na_2SO_4). The removed organic phase was fully concentrated at ambient temperature via centrifugation. The concentrated samples were identified and measured using a gas chromatograph (GC) (Agilent 7890 A, USA) with a flame ionization detector. The retention time of each OC measured by GC is shown in online supplemental figure 1. In this study, seven OC derivatives, that is, alpha-HCH, beta-HCH, gamma-HCH, 2,4-DDT, 4,4-DDT, 2,4-DDE, and 4,4-DDE, were measured in the participants' sera.

Statistical analysis

Statistical analyses were performed by SPSS V.22.0. The results are expressed as mean±SD. The differences between the groups were assessed using the independent sample t-test. The Spearman's test was employed to determine the correlations between variables. The effect of OCs on oxidative stress and leukemia development was investigated using a regression test. The significance level was $p<0.05$.

RESULTS

Characteristics of subjects

Table 1 lists the demographic and clinical characteristics of the subjects. In total, 60.55% of the participants were boys and 39.45% were girls. There was no significant difference between the case and control groups regarding age, sex, and BMI ($p>0.05$). White blood cells, triglyceride (TG), cholesterol, and low-density lipoprotein levels were higher in patients with ALL and AML than in the control ($p<0.001$). However, Hb, platelets, and high-density lipoprotein (HDL) levels in patients with ALL and AML showed a significant decrease compared with healthy individuals ($p<0.05$).

OC pesticides

Table 2 shows that the levels of seven OC pesticides in patients with ALL and AML were significantly higher than in healthy individuals ($p<0.001$). In addition, the results of linear regression (table 3) showed that the levels of 2,4-DDE (adjusted OR (AOR)=−0.49), 2,4-DDT (AOR=−0.78), 4,4-DDE (AOR=−0.94), 4,4-DDT (AOR=−0.83), alpha-HCH (AOR=−0.65), beta-HCH (AOR=−0.98), and gamma-HCH (AOR=−0.90) had a significant negative relationship with PON1 activity ($p<0.05$). The levels of 2,4-DDE (AOR=−0.02), alpha-HCH (AOR=−0.02), and beta-HCH

Table 1 Demographic and clinical characteristics of patients with leukemia and healthy individuals

Variables		ALL (n=72)	Control (n=72)	P value	AML (n=37)	Control (n=37)	P value
Sex	Boy	46 (63.9%)	45 (62.5%)	0.863	21 (56.8%)	20 (54.1%)	0.815
	Girl	26 (36.1%)	27 (37.5%)		16 (43.2%)	17 (45.9%)	
Age (year)		14.33±3.36	14.06±3.29	0.783	29.72±3.89	28.32±3.60	0.112
BMI (kg/m^2)		23.50±3.54	22.55±3.66	0.114	25.63±3.21	25.49±4.39	0.882
Stage	M0	None	None		2 (5.4%)	None	
	M1	None	None		2 (5.4%)	None	
	M2	None	None		5 (13.5%)	None	
	M3	None	None		6 (16.2%)	None	
	M4	None	None		16 (43.2%)	None	
	M5	None	None		6 (16.2%)	None	
	L1	34 (47.2%)	None		None	None	
	L2	27 (37.5%)	None		None	None	
	L3	11 (15.3%)	None		None	None	
WBC (×10^9/L)		43.87±18.73	6.82±1.52	**<0.001***	49.75±16.77	6.74±1.64	**<0.001***
Platelets (×10^9/L)		63.06±44.61	360.5±54.52	**<0.001***	57.56±39.08	357.4±54.37	**<0.001***
Hb (g/L)		9.23±1.08	14.61±1.12	**<0.001***	9.21±1.02	14.46±1.06	**<0.001***
CHOL (mg/dL)		224.45±56.23	171.40±38.28	**<0.001***	230.05±55.86	179.13±40.72	**0.001***
TG (mg/dL)		219.63±54.88	156.73±37.98	**<0.001***	220.24±54.89	155.48±36.92	**0.001***
HDL (mg/dL)		42.13±13.67	49.35±13.02	**0.001***	44.40±12.37	48.96±12.72	0.123
LDL (mg/dL)		127.05±43.63	95.31±38.99	**<0.001***	127.89±38.97	103.60±40.83	**0.011***

Comparisons were made by using the χ^2 test and independent sample t-test.
*P<0.05.
ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BMI, body mass index; CHOL, cholesterol; Hb, hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; WBC, white blood cell.

(AOR=−0.09) were negatively correlated with AChE activity (p<0.05). Moreover, 2,4-DDE (AOR=−5.72), 2,4-DDT (AOR=−10.2), 4,4-DDE (AOR=−10.6), 4,4-DDT (AOR=−7.5), alpha-HCH (AOR=−7.13), beta-HCH (AOR=−12.7), and gamma-HCH (AOR=−9.67) had a significant inverse relationship with TAC (p=0.001). Only 2,4-DDE (AOR=0.01) was positively correlated with MDA (p<0.05). All OC pesticides except for 4,4-DDT (AOR=0.02) had a significant direct relationship with PC (p<0.05). 2,4-DDE (AOR=0.07), 4,4-DDE (AOR=0.08),

Table 2 Organochlorines (OCs) levels in patients with leukemia compared with healthy individuals

OCs (ng/mL)	LOD (ng/mL)	ALL (n=72) Mean±SD	ALL (n=72) Median (min-max)	Control (n=72) Mean±SD	Control (n=72) Median (min-max)	P value	AML (n=37) Mean±SD	AML (n=37) Median (min-max)	Control (n=37) Mean±SD	Control (n=37) Median (min-max)	P value
Alpha-HCH	0.11	1.55±0.93	1.45 (0.1–3.13)	0.24±0.21	0.10 (0.1–0.92)	**<0.001***	1.56±0.95	1.52 (0.1–3.00)	0.26±0.24	0.14 (0.1–0.92)	**<0.001***
Beta-HCH	0.14	0.65±0.39	0.52 (0.1–2.04)	0.20±0.19	0.10 (0.1–0.94)	**<0.001***	0.62±0.38	0.46 (0.3–2.04)	0.24±0.23	0.10 (0.1–0.94)	**<0.001***
Gamma-HCH	0.19	0.72±0.61	0.51 (0.1–2.51)	0.15±0.10	0.10 (0.1–0.61)	**<0.001***	0.68±0.56	0.51 (0.1–2.26)	0.15±0.11	0.10 (0.1–0.61)	**<0.001***
2,4-DDE	0.029	2.15±1.17	2.89 (0.1–3.61)	0.28±0.25	0.10 (0.1–0.89)	**<0.001***	1.87±1.21	2.27 (0.1–3.38)	0.30±0.26	0.10 (0.1–0.89)	**<0.001***
4,4-DDE	0.038	0.70±0.66	0.48 (0.1–2.92)	0.12±0.06	0.10 (0.1–0.41)	**<0.001***	0.71±0.71	0.44 (0.1–2.92)	0.13±0.08	0.10 (0.01–0.41)	**<0.001***
2,4-DDT	0.043	0.58±0.57	0.46 (0.1–2.00)	0.21±0.18	0.10 (0.09–0.82)	**<0.001***	0.55±0.59	0.44 (0.1–2.00)	0.18±0.16	0.10 (0.1–0.82)	**<0.001***
4,4-DDT	0.08	0.63±0.67	0.12 (0.1–1.80)	0.13±0.12	0.10 (0.1–0.95)	**<0.001***	0.60±0.66	0.10 (0.1–1.80)	0.13±0.14	0.10 (0.1–0.95)	**<0.001***

OCs levels were significantly higher in patients with leukemia than in healthy individuals (p<0.001). The results are stated as mean±SD, LOD, median, minimum, and maximum.
*P<0.05.
ALL, acute lymphoblastic leukemia; alpha-HCH, alpha-hexachlorocyclohexane; AML, acute myeloid leukemia; beta-HCH, beta-hexachlorocyclohexane; 2,4-DDE, 2,4-dichlorodiphenyldichloroethylene; 4,4-DDE, 4,4-dichlorodiphenyldichloroethylene; 2,4-DDT, 2,4-dichlorodiphenyltrichloroethane; 4,4-DDT, 4,4-dichlorodiphenyltrichloroethane; gamma-HCH, gamma-hexachlorocyclohexane; LOD, limit of detection.

Table 3 Predicting the effects of OCs on oxidative stress parameters

	Alpha-HCH			Beta-HCH			Gamma-HCH			2,4-DDE			4,4-DDE			2,4-DDT			4,4-DDT		
	AOR	95% CI	P value	AOR	95% CI	P value	AOR	95% CI	P value	AOR	95% CI	P value	AOR	95% CI	P value	AOR	95% CI	P value	AOR	95% CI	P value
PON1	−0.65	−0.99 to −0.30	**0.001***	−0.98	−1.89 to −0.08	**0.03***	−0.90	−1.52 to −0.27	**0.005***	−0.49	−0.74 to −0.24	**0.001***	−0.94	−1.56 to −0.32	**0.003***	−0.78	−1.46 to −0.1	**0.02***	−0.83	−1.43 to −0.24	**0.006***
AChE	−0.02	−0.05 to −0.002	**0.03***	−0.09	−0.15 to −0.03	**0.003***	−0.01	−0.06 to 0.02	0.45	−0.02	−0.04 to -0.11	**0.002***	−0.03	−0.07 to 0.01	0.12	−0.01	−0.06 to 0.03	0.46	−0.03	−0.08 to 0.01	0.08
TAC	−7.13	−9.67 to −4.59	**0.001***	−12.7	−19.6 to −5.88	**0.001***	−9.67	−14.4 to −4.94	**0.001***	−5.72	−7.51 to −3.93	**0.001***	−10.6	−15.2 to −6.02	**0.001***	−10.2	−15.3 to −5.19	**0.001***	−7.5	−12.2 to −2.9	**0.001***
MDA	0.006	−0.001 to 0.01	0.09	0.01	−0.004 to 0.03	0.14	0.01	−0.002 to 0.02	0.08	0.01	0.001 to 0.01	**0.01***	0.01	0 to 0.02	0.05	0.01	−0 to 0.02	0.17	0.008	−0.01 to 0.02	0.18
PC	0.02	0.01 to 0.04	**0.001***	0.08	0.05 to 0.12	**0.001***	0.04	0.01 to 0.06	**0.001***	0.02	0.01 to 0.03	**0.001***	0.04	0.01 to 0.06	**0.001***	0.03	0 to (0.05)	**0.02***	0.02	−0.01 to 0.05	0.07
NO	0.07	0.03 to 0.11	**0.001***	0.17	0.06 to 0.27	**0.001***	0.11	0.04 to 0.19	**0.001***	0.07	0.04 to 0.10	**0.001***	0.08	0.01 to 0.16	**0.01***	−0.01	−0.08 to 0.07	0.85	0.11	0.03 to 0.17	**0.002***
SOD	−0.93	−1.24 to −0.61	**0.001***	−2.26	−3.08 to −1.44	**0.001***	−1.37	−1.96 to −0.79	**0.001***	−0.60	−0.83 to −0.37	**0.001***	−1.44	−2.01 to −0.87	**0.001***	−1.26	−1.9 to −0.62	**0.001***	−0.91	−1.48 to −0.32	**0.002***
GPx	−0.21	−0.41 to −0.02	**0.02***	−0.44	−0.93 to 0.05	0.08	−0.31	−0.65 to 0.03	0.07	−0.21	−0.35 to −0.07	**0.002***	−0.69	−1.01 to −0.36	**0.001***	−0.43	−0.79 to −0.06	**0.02***	−0.36	−0.68 to −0.03	**0.03***
CAT	0.09	−0.32 to 0.51	0.66	0.5	−0.57 to 1.57	0.35	0.51	−0.23 to 1.25	0.17	0.11	−0.18 to 0.41	0.45	1.01	0.28 to 1.72	**0.007***	0.49	−0.3 to 1.29	0.22	0.30	−0.41 to 1.01	0.41

*P<0.05.
AChE, acetylcholinesterase; alpha-HCH, alpha-hexachlorocyclohexane; AOR, adjusted OR; beta-HCH, beta-hexachlorocyclohexane; BMI, body mass index; CAT, catalase; 2,4-DDE, 2,4-dichlorodiphenyldichloroethylene; 4,4-DDE, 4,4-dichlorodiphenyldichloroethylene; 2,4-DDT, 2,4-dichlorodiphenyltrichloroethane; 4,4-DDT, 4,4-dichlorodiphenyltrichloroethane; gamma-HCH, gamma-hexachlorocyclohexane; GPx, glutathione peroxidase; MDA, malondialdehyde; NO, nitric oxide; OC, organochlorine; PC, protein carbonyl; PON1, paraoxonase-1; SOD, superoxide dismutase; TAC, total antioxidant capacity.

4,4-DDT (AOR=0.11), alpha-HCH (AOR=0.07), beta-HCH (AOR=0.17), and gamma-HCH (AOR=0.11) had a significant positive relationship with NO levels (p<0.05). SOD had a significant negative relationship with 2,4-DDE (AOR=−0.6), 2,4-DDT (AOR=−1.26), 4,4-DDE (AOR=−1.44), 4,4-DDT (AOR=−0.91,), alpha-HCH (AOR=−0.93), beta-HCH (AOR=−2.26), and gamma-HCH (AOR=−1.37) (p<0.001). Moreover, 2,4-DDE (AOR=−0.21), 2,4-DDT (AOR=−0.43), 4,4-DDE (AOR=−0.69), 4,4-DDT (AOR=−0.36), and alpha-HCH (AOR=−0.21) were inversely related to GPx (p<0.05). Of the seven OC pesticides, only 4,4-DDE (AOR=1.01) had a direct and significant relationship with CAT (p=0.007). In general, it can be said that exposure to OCs increases the level of oxidative stress by reducing the body's antioxidant factors and enhancing the level of oxidant parameters (table 3).

In table 4, logistic regression analysis showed that exposure to high concentrations of OC pesticides was associated with a higher risk of leukemia. The quartiles of some OCs such as alpha-HCH (Q4: AOR=4.82), beta-HCH (Q3: AOR=4.11 and Q4: AOR=3.23), gamma-HCH (Q3: AOR=3.14 and Q4: AOR=3.15), 2,4-DDE (Q2: AOR=2.80 and Q4: AOR=4.55), 4,4-DDE (Q3: AOR=2.32 and Q4: AOR=4.42), 2,4-DDT (Q3: AOR=3.29 and Q4: AOR=3.41), and 4,4-DDT (Q4: AOR=5.01) showed significant AORs in patients with leukemia (p<0.05).

Oxidative stress

Table 5 indicates the differences in AChE activity and several oxidative stress factors between patients with leukemia and healthy individuals. AChE, SOD, GPx, and PON1 activities as well as TAC levels in patients with ALL and AML showed a significant decrease compared with healthy controls (p<0.05). Moreover, MDA, PC, and NO levels in patients with ALL and AML were significantly higher than in healthy subjects (p<0.05). However, the CAT level in patients with ALL and AML was not significantly different from that in healthy subjects (p=0.919).

Online supplemental table 6 shows the relationship of seven OC pesticides with the activities of AChE, PON1, GPx, CAT, SOD, and serum levels of MDA, PC, NO, and TAC (online supplemental table 6). In correlation analysis, it was demonstrated that gamma-HCH (r=0.250) and 4,4-DDE (r=0.234) were positively associated with CAT activity. However, 4,4-DDE levels were inversely associated with GPx (r=−0.245) and SOD (r=−0.135) activities as well as TAC levels (r=−0.114). NO levels were shown to have a significant negative association with 2,4-DDT (r=−0.255). In addition, NO was positively associated with PC levels (r=0.273) and negatively associated with GPx (r=−0.252) and SOD (r=−0.268) activities. AChE activity levels were shown to have a significant positive association with PON1 (r=0.223) and SOD (r=0.256) activities and TAC levels (r=0.312). Moreover, AChE had a negative association with PC (r=−0.196) and NO (r=−0.199) levels. PON1 activity was positively correlated with SOD (r=0.301) and GPx (r=0.199) activities and TAC levels (r=0.401), while it was negatively associated with PC (r=−0.308) and NO (r=−0.246) levels. TAC

Table 4 Logistic regression analyses

	Crude				Adjusted			
	B	OR	95% CI	P value	B	OR	95% CI	P value
Alpha-HCH	0.27	1.31	1.44 to 1.19	<0.001	0.29	1.34	1.20 to 1.49	<0.001
Q1 (≤1)	Ref	Ref	Ref	–	Ref	Ref	Ref	–
Q2 (1–2)	0.77	2.17	0.81 to 5.79	0.11	0.75	2.12	0.76 to 5.88	0.14
Q3 (2–3)	0.61	1.84	0.75 to 4.49	0.17	0.48	1.63	0.64 to 4.11	0.31
Q4 (>3)	1.49	4.42	1.75 to 11.15	**0.002***	1.57	4.82	1.83 to 12.68	**0.001***
Beta-HCH	0.43	1.53	1.31 to 1.79	<0.001	0.43	1.54	1.30 to 1.82	<0.001
Q1 (≤1.74)	Ref	Ref	Ref	–	Ref	Ref	Ref	–
Q2 (1.74–4.34)	0.72	2.06	0.84 to 5.07	0.11	0.74	2.11	0.83 to 5.37	0.11
Q3 (4.34–7.11)	1.42	4.13	1.62 to 10.54	**0.003***	1.41	4.11	1.55 to 10.87	**0.004***
Q4 (>7.11)	1.16	3.19	1.21 to 8.37	**0.01***	1.17	3.23	1.17 to 8.92	**0.02***
Gamma-HCH	0.44	1.56	1.31 to 1.84	<0.001	0.46	1.58	1.31 to 1.91	<0.001
Q1 (≤1.38)	Ref	Ref	Ref	–	Ref	Ref	Ref	–
Q2 (1.38–2.92)	0.57	1.77	0.63 to 4.94	0.27	0.36	1.44	0.48 to 4.22	0.51
Q3 (2.92–6.71)	1.07	2.91	1.21 to 7.05	**0.01***	1.14	3.14	1.23 to 8.03	**0.01***
Q4 (>6.71)	1.32	3.73	1.52 to 9.17	**0.004***	1.15	3.15	1.23 to 8.07	**0.01***
2,4-DDE	0.18	1.21	1.13 to 1.28	<0.001	0.19	1.21	1.13 to 1.30	<0.001
Q1 (≤1.57)	Ref	Ref	Ref	–	Ref	Ref	Ref	–
Q2 (1.57–8.38)	0.97	2.66	1.01 to 6.97	**0.04***	1.03	2.80	1.02 to 7.67	**0.04***
Q3 (8.38–27.33)	0.93	2.52	1.03 to 6.16	**0.04***	0.99	2.71	1.06 to 6.89	**0.03***
Q4 (>27.33)	1.54	4.67	1.78 to 12.15	**0.002***	1.52	4.55	1.70 to 12.19	**0.003***
4,4-DDE	0.64	1.91	1.48 to 2.45	<0.001	0.64	1.89	1.42 to 2.52	<0.001
Q1 (≤1.42)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	–
Q2 (1.42–1.91)	0.34	1.40	0.58 to 3.40	0.45	0.23	1.27	0.51 to 3.16	0.61
Q3 (1.91–5.65)	1.12	3.06	1.18 to 7.93	**0.02***	0.84	2.32	0.85 to 6.29	0.09
Q4 (>5.65)	1.55	4.72	1.77 to 12.60	**0.002***	1.48	4.42	1.62 to 12.13	**0.004***
2,4-DDT	0.15	1.16	1.07 to 1.27	0.001	0.15	1.16	1.05 to 1.28	0.002
Q1 (≤1.37)	Ref	Ref	Ref	–	Ref	Ref	Ref	–
Q2 (1.37–2.30)	−0.05	0.95	0.36 to 2.51	0.91	−0.21	0.81	0.29 to 2.23	0.68
Q3 (2.30–7.21)	1.07	1.03	1.02 to 8.41	**0.04***	1.19	3.29	1.10 to 9.85	**0.03***
Q4 (>7.21)	1.23	1.46	1.46 to 8.05	**0.005***	1.22	3.41	1.37 to 8.47	**0.008***
4,4-DDT	0.21	1.24	1.11 to 1.38	<0.001	0.20	1.22	1.09 to 1.38	0.001
Q1 (≤1.27)	Ref	Ref	Ref	–	Ref	Ref	Ref	–
Q2 (1.27–1.53)	−0.40	0.67	0.25 to 1.80	0.42	−0.42	0.65	0.23 to 1.78	0.41
Q3 (1.53–4.45)	0.93	2.55	0.98 to 6.60	0.05	0.73	2.08	0.77 to 5.61	0.14
Q4 (>4.45)	1.64	5.17	1.98 to 13.44	**0.001***	1.61	5.01	1.85 to 13.47	**0.001***

Multiple logistic regression. Models were adjusted for total lipid, sex, age, BMI, and disease stage.
*P<0.05.
alpha-HCH, alpha-hexachlorocyclohexane; beta-HCH, beta-hexachlorocyclohexane; BMI, body mass index; 2,4-DDE, 2,4-dichlorodiphenyldichloroethylene; 4,4-DDE, 4,4-dichlorodiphenyldichloroethylene; 2,4-DDT, 2,4-dichlorodiphenyltrichloroethane; 4,4-DDT, 4,4-dichlorodiphenyltrichloroethane; gamma-HCH, gamma-hexachlorocyclohexane; Ref, reference.

had a significant positive relationship with SOD (r=0.301) and GPx (r=0.277) but was negatively correlated with NO (r=−0.293) and PC (r=−0.483). MDA showed a positive relationship with PC (r=0.175) while it had a negative relationship with TAC level (r=−0.268) and GPx activity (r=−0.164). PC was negatively related to GPx (r=−0.182) and SOD (r=−0.319) activities.

DISCUSSION

The results showed that the levels of all seven OC pesticides in patients with ALL and AML were significantly higher than those in healthy individuals. Past research has revealed a significant relationship between different concentrations of OCs and the incidence of malignancies.[40] In line with our results, Ward *et al* showed that exposure to pesticides was linked to a raised risk of leukemia.[41] However, contrary to our findings, Scheele *et al* reported no link between these compounds and leukemia.[42] This inconsistency can be due to differences in the types of pesticides, individuals' behaviors, environment, utilization rate, agricultural strategy, and other factors. Moreover, Ward *et al* showed that even though some pesticides play a crucial part in the development of leukemia, others are not directly related to its incidence.[41]

In addition, the cumulation of OCs in adipose masses and the increment of TG and BMI in individuals may lead

Table 5 Levels of biomarkers of oxidative stress in patients with leukemia compared with healthy individuals

	ALL (n=72)	Control (n=72)		AML (n=37)	Control (n=37)	
Variables	**Mean±SD**	**Mean±SD**	**P value**	**Mean±SD**	**Mean±SD**	**P value**
SOD (U/mL)	40.47±15.42	69.20±19.94	**<0.001***	37.40±14.45	68.95±20.75	**<0.001***
GPx (U/L)	42.05±13.19	50.19±10.83	**<0.001***	45.78±14.12	49.58±10.38	**0.039***
CAT (KU/L)	167.18±30.26	166.72±24.26	0.919	165.59±28.89	169.17±22.29	0.552
TAC (nmol/Fe^{2+})	439.21±104.27	676.29±166.25	**<0.001***	447.21±111.99	695.7±166.93	**<0.001***
MDA (nmol/mL)	1.37±0.33	1.11±0.51	**<0.001***	1.32±0.33	1.11±0.51	**0.041***
NO (nmol/mL)	20.04±2.76	17.13±1.72	**<0.001***	19.78±2.82	17.36±1.50	**<0.001***
PC (nmol/mg protein)	3.35±0.94	2.28±0.59	**<0.001***	3.36±1.01	2.29±0.52	**<0.001***
AChE (U/g Hb)	5.68±1.80	6.89±1.22	**<0.001***	5.99±1.92	6.91±0.98	**0.011***
PON1 (U/L)	58.01±20.94	82.93±18.79	**<0.001***	56.89±20.74	84.67±21.26	**<0.001***

MDA, PC, and NO levels increased significantly, and TAC, SOD, GPx, AChE, and PON1 decreased significantly in patient with leukemia than in healthy individuals ($p<0.05$). However, no significant change in CAT level was observed between the groups ($p>0.05$).

*P<0.05.

AChE, acetylcholinesterase; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CAT, catalase; GPx, glutathione peroxidase; MDA, malondialdehyde; NO, nitric oxide; PC, protein carbonyl; PON1, paraoxonase-1; SOD, superoxide dismutase; TAC, total antioxidant capacity.

to the persistence of these compounds in the body for a long time, causing elevated oxidative stress levels during this period.[43] According to prior results, the increase of BMI and TG contributes to the accumulation of pesticides in body tissues.[44] Therefore, due to remarkable differences in TG concentrations in patients with leukemia compared with healthy individuals, increased TG levels may lead to higher OC concentrations in the body.

Moreover, our results showed that increased levels of OC pesticides, including alpha-HCH, beta-HCH, gamma-HCH, 2,4-DDE, and 4,4-DDT, were associated with decreased AChE activity in patients with leukemia. This decrease in the level of AChE activity indicates that in addition to OC pesticides, patients might have also been exposed to organophosphate pesticides.[45] Reduced AChE activity leads to the cumulation of acetylcholine, and the constant stimulation of its receptors in the cells disrupts the regulation of immune function and may eventually enhance the development of malignancies.[46] In addition, given that AChE plays a role in the process of apoptosis[47] and due to the increase of OC levels in patients with leukemia and the decrease of AChE activity, cells may deviate from their apoptotic pathway, and the risk of cancer might be elevated by disrupting the balance of cell death. Moreover, increased oxidative stress due to a reduced AChE activity may lead to the development of malignancies.[15]

Generally, PONs perform various activities within the body, namely inactivating pro-oxidant and pro-inflammatory agents and metabolizing specific medications and xenobiotics, as well as playing a role in cell proliferation.[48] Our study showed that PON1 activity was lower in patients with leukemia than in healthy individuals and was inversely related to OC levels. This result was in agreement with that of the report by de Aguiar Gonçalves *et al*.[49] In contrast, Çebi *et al* did not observe a significant reduction in the PON activity in patients with leukemia.[50] A possible reason for this inconsistency could be exposure to chemical substances such as pesticides, differences in diet, the number of samples, duration of exposure, subjects' behavior, race, environment, agricultural strategy, and other similar factors. The results of this study revealed that the level of HDL (PON1 enzyme carrier) in patients with leukemia was significantly reduced compared with healthy individuals. In addition, a significant increase was observed in oxidative stress biomarkers in patients with leukemia. These alterations may be the reasons for decreased PON1 activity.

MDA is created by the peroxidation of lipids in unsaturated fatty acids and its levels are elevated in various types of cancer.[51] In our study, MDA levels showed a significant increase in patients with leukemia compared with healthy individuals, which may be due to oxidative stress caused by exposure to OC pesticides in these patients. since our results showed that the levels of MDA have a direct and significant relationship with the level of OCs, especially 2,4-DDE. On the other hand, this increase may be due to the reduction in the capacity of antioxidants and the disturbance of the oxidant-antioxidant balance.[52] Moreover, MDA can enhance the risk of cancer by altering the structure of DNA.[53] Therefore, exposure to OCs may be associated with high production of MDA and reactive oxygen species (ROS), which eventually raises the incidence of cancer. Furthermore, several previous studies have demonstrated that peroxidation of lipids increases during the progression of malignancies, which is indicated by the high levels of MDA.[54]

NO is a multifunctional molecule involved in a wide range of physiological and pathological processes.[55] Various studies have shown that NO synthase activity and NO synthesis are high in tumor tissues and the plasma of patients with cancer.[56] In this study, NO levels in patients with leukemia were significantly higher than those in healthy individuals and had a significant positive relationship with OCs levels. This result was in agreement with the findings of the report by Bakan *et al*.[52] The role of NO in tumor biology is still poorly understood. Studies have suggested that NO might play a dual role in cancer progression, depending on its concentration, and can act as a protumor or antitumor factor.[57] Therefore, elevated NO levels in patients with leukemia can lead to a variety of chemical reactions in the biological system, including the induction of lipid peroxidation, inhibition of mitochondrial

electron transfer, disruption of the antioxidant system, and ultimately exacerbation of the disease.[58]

PC is created as a result of irrevocable non-enzymatic oxidation or carbonylation of proteins.[59] It causes protein damage and dysfunction and is considered an oxidative stress factor in patients.[59] In the present study, PC concentration in patients with leukemia was significantly higher than that in healthy individuals. Similar studies have shown that PC levels are remarkably increased in different cancers, including leukemia and Hodgkin's lymphoma, as well as in vitro human cells.[20] The suggested reason for high PC concentrations is that myeloid cells are a significant source of free radicals, which cause lipid peroxidation and irrevocable protein oxidation in cells.[60] Our results indicated that OC levels are positively correlated with NO and PC levels. Therefore, exposure to OCs may lead to an increase in free radicals, lipid peroxidation, NO, and PC levels and ultimately an increase in the incidence of leukemia.

The simultaneous effect of all the antioxidants in the body against free radicals is much stronger than the activity of an antioxidant alone.[61] Recently, TAC levels have been assessed in several neoplastic conditions.[62] In the present research, TAC levels in patients with leukemia were significantly lower than those in healthy participants and had a significant negative relationship with OC levels. Contrary to our findings, Buico *et al* reported increased TAC levels in patients with leukemia.[63] These discrepancies may be due to dietary factors including the consumption of fruits and vegetables that contain varying amounts of antioxidants along with OC pesticides.

The human body has robust endogenous antioxidant systems to prevent the potential damage of free radicals.[64] An imbalance between the generation of ROS and the compensation mechanisms of antioxidative systems, both physiological and biochemical, leads to high ROS levels, which increases the risk of leukemia.[65] The findings of different investigations on antioxidant enzymes in various types of human cancers are inconsistent. Our findings revealed a significant reduction in GPx and SOD activities in patients with leukemia compared with healthy individuals, which was consistent with the results reported by Bakan *et al*.[52] However, the present findings are different from those of several previous reports. In some patients with leukemia, increased GPx activity and glutathione concentration have been reported.[66] Disruption of the antioxidant system may lead to the accumulation of ROS. On the other hand, it is possible that cancer progression disrupts the metabolism of antioxidants and ultimately impairs the function of the antioxidant system. Our results showed that GPx and SOD activities are negatively correlated with OCs. Therefore, exposure to OCs may be associated with decreased SOD and GPx activity, leading to the accumulation of free radicals in red blood cells and other cell types. These processes may be necessary for the toxicity of OC pesticides and ultimately responsible for cell damage and the increased incidence of leukemia.

CONCLUSIONS

We observed an increase in the levels of OCs, MDA, NO, and PC, and a decrease in the activity levels of GPx, SOD, AChE, and PON1, as well as the level of TAC, in patients with leukemia compared with healthy individuals. Thus, it is likely that one of the possible mechanisms of OCs in the development of leukemia is disrupting the function of antioxidant enzymes and increasing the levels of oxidative stress. Therefore, OCs may be considered crucial factors in the development of leukemia in southeastern Iran (Kerman). The importance of the current research was that it was one of the very few studies to measure the precise amount of seven OC derivatives and assess the enzymatic and non-enzymatic antioxidant systems in the participants to determine the correlation of exposure to the mentioned pesticides and leukemia. Moreover, we showed that disturbance in the oxidant-antioxidant balance in the body is a possible effect of OCs and may play a role in the development of leukemia.

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