

Influence of Electromagnetic Fields on Lead Toxicity: A Study of Conformational Changes in Human Blood Proteins

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Received 2015 February 18; Revised 2015 April 04; Accepted 2015 April 13.

Abstract

Background: Electromagnetic fields (EMF) are associated with oxidative stress, which is in turn associated with reactive oxygen species (ROS), anemia, and hypoxia.

Objectives: This study focused on the synergistic effects of lead ions and EMF on oxidative modifications in hemoglobin (Hb) and plasma proteins.

Patients and Methods: In this experimental study, the blood samples were obtained from age- and sex-matched healthy subjects at Arak University of Medical Sciences, Arak, Iran. The collected bloods were prepared as 55 samples and then divided into different groups for incubating with 0 to 100 μ M of lead ions in 2 mT and 50 Hz of EMF for 120 minutes. The carbonyl group was determined to be an oxidative biomarker in plasma proteins. The ferric reducing ability of plasma (FRAP) was considered to be an antioxidant power of human plasma. The conformational changes in hemoglobin, met-Hb, and hemichrome were considered to be oxidative markers in red blood cells. To predict the factors affecting the oxyHb, the artificial neural network (MLP: 11,2,2,1) in SPSS software was applied.

Results: The test subjects showed increased concentrations of metHb (1.8 ± 0.19 vs. 1.36 ± 0.25) and hemichrome (6.01 ± 0.57) in relation to the control subjects. The decreased absorbance at 340 nm (0.88 ± 0.09 vs. 1.07 ± 0.08) demonstrated the reduced interaction between the globin chain and the heme ring. The decreased absorbance at 420 nm (Soret band) (2.96 ± 0.13) and the increased absorbance at 630 nm (0.07 ± 0.002 vs. 0.064 ± 0.005) indicated the conversion of oxyHb to metHb, which confirmed the oxidative damage to the erythrocytes. The linear regression analysis showed significant positive correlations between lead concentration and the percentage of plasma carbonyl content ($R^2 = 0.96$), the relation of plasma carbonyl content to Hb absorbance at 630 nm ($R^2 = 0.97$), and the relation of plasma carbonyl content to metHb concentration ($R^2 = 0.95$) after 120 minutes incubation with lead ions in 20 millitesla and 50 hertz EMF. The artificial neural network analysis showed the significant importance of hemichrome, PCO, metHb, and lead concentration to the oxyHb content of erythrocytes.

Conclusions: Lead contamination in the presence of an EMF exacerbates the oxidative damage to plasma proteins as well as the conformational changes in Hb. An artificial neural network can be used as a predictive tool for the oxidative danger posed to workers in industrial fields, battery manufacturing companies, and power plants.

Keywords: Artificial Neural Network, Electromagnetic Field, Hemoglobin, Lead, Oxidative Stress

1. Background

The world health organization (WHO) has identified lead (Pb) as a toxic element that can have a major impact on public health, which necessitates action by member states to protect the health of young people, industrial workers, and women. Lead exposure is estimated to account for 0.6% of the global burden of disease, with the highest burden being seen in developing countries. Lead exposure during childhood is estimated to contribute to about 600,000 new cases of children with intellectual disabilities every year (1). Further, lead contamination results in respiratory symptoms and lower pulmonary function testing (PFT) val-

ues among workers exposed to lead in car battery factories (2). Additionally, chronic lead exposure may result in hypertension (3).

The WHO has prepared guidelines on the management and prevention of lead exposure, which are intended to provide policy makers and health professionals with guidance on the measures that they can implement to maintain the health of young and adult people and avoid lead contamination (4).

Studies have shown that abnormal hemoglobin concentrations during pregnancy are correlated with both low Apgar scores and low birth weight in neonates (5).

Lead toxicity is related to behavioral, biochemical, and

physiological dysfunctions in laboratory animals and humans. Lead contamination affects the intelligence quotient (6), heme biosynthesis (7), nervous system (8), erythrocytes (9), hematopoietic system (10, 11), kidney (12), cardiovascular system (13), reproductive system (14), bone (15), and general quality of life (16).

In recent years, industrial developments have resulted in increased exposure to electromagnetic fields (EMF), which has become a matter of considerable public concern. In addition to epidemiological research, *in vitro* and *in vivo* studies have been of great importance in this regard. The biological changes that may occur during exposure to EMF have been studied in relation to both static and frequency magnetic field sources. Many studies have reported that static magnetic fields induce apoptosis in a time-dependent manner (17) as well as increasing the malonyldialdehyde (MDA) concentration and catalase activity in rat kidney (18). Additionally, static magnetic fields can decrease glutathione peroxidase (GPx) activity in rat kidney and increase superoxide dismutase (SOD) activity in rat liver (19).

The international agency for research on cancer (IARC) has reported that EMF must be considered as a potential human carcinogen (20). Many studies have reported that extremely low frequency electromagnetic fields (ELF-EMF) can reduce eosinophils, mean platelet volume, and hemoglobin concentration with respect to the period of exposure (21). Extremely low frequency electromagnetic fields of a 50 Hz frequency and 0.5 mT intensity can increase the blood glucose level, change the proopiomelanocortin mRNA level, and enhance depression-like behaviors (22). Cho et al. have shown that the number of neuronal nitric oxide synthase (nNOS) immunoreactivity (IR) neurons was significantly increased in the cerebral cortex, striatum, and hippocampus, leading to physiological interference with intracellular signaling activation (23). Many studies have shown that EMF induce oxidative stress. For instance, Ciejka et al. found that EMF of 7 mT and 40 Hz produce reactive oxygen species (ROS) in the brain tissue of experimental animals, which correlated with exposure time. Their results showed that exposure to EMF can generate free radicals and increase both lipid peroxidation and free sulfhydryl groups (24). Further, there is some evidence that EMF in the vicinity of chemical and physical agents can aggravate their toxic properties. Amara reported the synergistic effects of static magnetic fields and cadmium on the activity of antioxidant enzymes, which led to oxidative damage in rat brain tissue (25). Suzuki showed that static magnetic fields enhance the mutagenic effects of chemical agents (26). Suzuki also reported that the mutagenic effects of carboquone, colcemid, mitomycin C, vincristine, sodium fluoride, and 1-ethyl-1-nitrosourea can increase af-

ter exposure to 4.7 Tesla static magnetic fields (26). The international commission on non-ionizing radiation protection (ICNIRP) has established the adverse health effects of high-level, short-term exposure to EMF (27).

2. Objectives

The main aim of this study was to demonstrate whether lead ions in the presence of EMF induce oxidative stress and to determine whether such synergistic effects affect the biological functions of erythrocytes and plasma. This work also aimed to substantiate the conformational modifications in hemoglobin, the oxidative damage in proteins, and the antioxidant power of human plasma.

3. Patients and Methods

3.1. Preparation of Whole Blood and Isolation of Blood Cell Fractions

Based on our previous studies on oxidative modifications in plasma proteins (28) and structural changes in Hb (29), the confidence level specified is 95 percent, $E = 0.3$, the Z-value is 1.96, and $q = p = 0.5$. The sample size for each group is

$$\begin{aligned} \frac{Z_{\frac{\alpha}{2}}^2 pq}{E^2} &= \frac{(1.96)^2 \times (0.5)}{(0.3)^2} \\ &= 10.67 \\ &\approx 11 \end{aligned} \quad (1)$$

Therefore, 55 samples were divided into five groups for the control and the test.

In this experimental study, the blood samples were obtained from healthy donors from the faculty of medicine in Arak, Iran, between 1st May 2013 and 1st October 2013. The volunteers who participated in this study did so according to the written permission of the human ethics committee of the faculty of medicine in Arak and in accordance with the ethical guidelines of the 1975 declaration of Helsinki.

The structural analysis of Hb, the ferric reducing ability of plasma (FRAP) assay, and the carbonyl estimations were performed at the molecular and medicine research center, while the artificial neural network (ANN) analysis was performed at the department of biochemistry and Genetics.

3.2. Equipment and Chemicals

The reagents were purchased from Merck Company and they were all of analytical reagent grade. Further, the spectrophotometer UV/Vis model 6505 was obtained from JENWAY, the balance from Sartorius, the centrifuge 5804 R

from Eppendorf, the pH meter from METTLER, the pipettes from ISOLAB, the glass wares from LASSCO, and the micropipettes from SOCOREX.

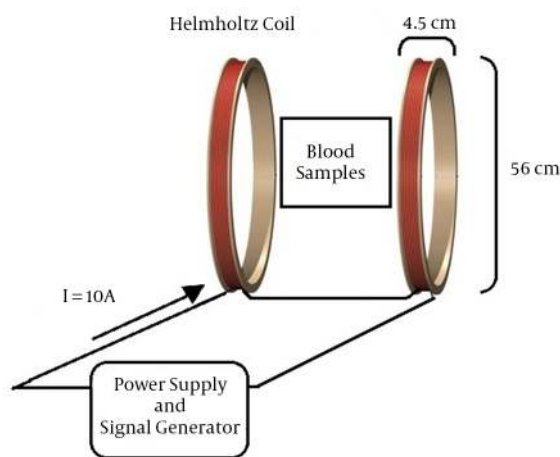
3.3. Induction of Oxidative Stress by Electromagnetic Field and Lead Ions

Lead chloride (PbCl_2) was employed as a source of lead ions. The plasma and erythrocytes samples were incubated in an isotonic phosphate buffer (100 mmol/L, pH 7.4) containing 0-100 micromole lead ions for 30-120 hours at 37°C under aerobic conditions.

Two millitesla and 50 hertz EMF were induced by a pair of parallel coils in a Helmholtz configuration (Figure 1). The external diameter and width of each coil were 56 cm and 4.5 cm, respectively. Ten ampere of current was generated by the signal generator and the power supply caused an EMF to form in the space between the coils. The uniformity of the EMF between the coils was checked with a gaussmeter (Model GM2, AlphaLab Inc., Utah, USA).

After incubation, the oxidative effects were stopped by isolating the erythrocytes with centrifugation and then washing them with an isotonic phosphate buffer three times. The hemoglobin assay was performed according to the Drabkin method. The hemoglobin concentration was adjusted to 40 micromolar with a phosphate buffer and the optical density of each sample was measured at 630, 577, 560, 542, and 340 nanometers.

Figure 1. Magnetic Field Generation With a System Consisting of a Pair of Parallel Coils in a Helmholtz Configuration



A, ampere; Cm, centimeter; I, electric current.

3.4. Determination of Hemoglobin Derivative Concentrations

The concentrations of oxyHb, metHb, and hemichrome were estimated as micromolars accord-

ing to the following equations (30):

$$\text{Hemichrome} = -133A_{577} - 114A_{630} + 233A_{560}$$

$$\text{MetHb} = 28A_{577} + 307A_{630} - 55A_{560}$$

$$\text{OxyHb} = 119A_{577} - 39A_{630} - 89A_{560}$$

3.5. Carbonyl Assay in Plasma Proteins

The carbonyl assays were performed according to the method introduced by Evans (31). Briefly, the proteins were suspended in an acidic solution of DNPH (2, 4-dinitrophenylhydrazine, 10 mM, in hydrochloric acid, 2 mM) and incubated at room temperature. The derivatized proteins were precipitated with trichloroacetic acid (20% w/v) and then dissolved in guanidinium hydrochloride (pH 2.3, 6 mol/L). The carbonyl content of the plasma proteins was calculated from the optical density at 360 nm using a molar absorption coefficient of $22,000 \text{ M}^{-1} \text{ cm}^{-1}$ and considering the following equations: $C = \text{Absorption}/\epsilon$, Carbonyl concentration (nmol/mg of protein) = carbonyl groups (nmol/mL)/protein concentration (mg/mL), C: concentration of dinitrophenylhydrazine/mL, ϵ : 2.2×10^{-2} nanomole/mL.

3.6. Protein Assay

The protein concentration was estimated by measuring the absorbance of the plasma samples at 280 nm. Standard curves were plotted according to 1 to 10 mg/mL of bovine serum albumin in an isotonic phosphate buffer (100 mmol/L, pH 7.4).

3.7. FRAP Assay

This assay was performed according to the method described by Benzie and Strain (32), albeit with some modifications. This method measures the antioxidant power of biologic samples that reduce ferric ions to the ferrous form and produce a complex with 2, 4, 6-tripiridyl-s-triazine (TPTZ), which absorbs light at 593 nm. The stock solutions included a 300 mM acetate buffer (pH 3.6), a 10 mM TPTZ solution in 40 mM HCl, and a 20 mM FeCl_3 solution. The antioxidant power of the plasma was calculated by plotting a standard curve of absorbance against 250-1000 micromole of ferrous solution.

3.8. Statistical Analysis

The control and test samples were analyzed in duplicate and the results were reported as mean \pm SD. Statistical significance at the 0.05 level within and between the groups was determined by the student's t-test and ANOVA methods of multiple comparison. The homogeneity of variances were calculated with the Leven test, while the post hoc multiple comparisons were calculated according to the Scheffe or Dunnett's T3 method.

3.9. Artificial Neural Networks

In medical research, ANN have been widely used to solve critical and complicated problems. In this study, the utilized ANN was a multi-layered perceptron (11,2,2,1). The input layer comprised 11 neurons (processing elements), while the output layer comprised one neuron (oxyHb). Some 70% of the variables were considered for establishing the optimal number of processing elements in the hidden layers. Our results showed that two hidden layers with two neurons in each layer produced the least error during training (Figure 4). Training was implemented using batch learning.

3.10. Statistical Software

The statistical significance, homogeneity of variances, post hoc multiple comparisons, linear regression, and artificial neural network were all calculating using SPSS software version 20.

3.11. Providing the Data Source

The data sources were presented using Microsoft office excel 2007 software.

3.12. Foundation Project

This work was supported by the molecular and medicine research center and biochemistry and genetics department of the faculty of medicine in Arak according to grant number 1164 and by the human ethics committee in medical research according to grant number 90-122-8 on 7th March 2012.

4. Results

4.1. Electromagnetic Field- and Lead-Mediated Oxidation of Hemoglobin

The time and dose schedule used in this study is based on our preliminary experiments carried out in human erythrocytes treated with MCO systems in vitro (33). Spectrophotometric studies can reveal heme interaction with oxygen or distal histidine as well as conformational changes in globin (30). Hemoglobin shows a characteristic absorption spectrum in the range of 200 to 700 nm. OxyHb is indicated by an increase in absorbance at 577 and 542 nm, metHb gives a peak at 630 nm, hemichrome gives a groove at 560 nm, heme-heme interaction (Soret band) occurs at 420 nm, and globin-heme interaction occurs at 340 nm (30).

Our results showed an increase in the optical density of Hb at 630 nm from 0.07 ± 0.002 to 0.064 ± 0.005 (Figure 2F, $P < 0.05$), which indicates the conversion of oxyHb

into metHb in the EMF- and lead-treated erythrocytes (Figure 2B, $P < 0.05$). This conversion showed the oxidative effects of EMF and lead on the Hb structure, resulting in an increased concentration of metHb from 1.8 ± 0.19 to 1.36 ± 0.25 μM (Figure 2B, $P < 0.05$), which in turn increased the hemichrome concentration from 4.85 ± 0.5 to 6.01 ± 0.57 (Figure 2C, $P < 0.05$).

Figure 2D shows that EMF and lead treatment induces a decrease in the absorbance values at 340 nm from 0.88 ± 0.09 to 1.07 ± 0.08 μM ($P < 0.05$), which indicated the reduced interaction between the globin chain and the heme ring. The increase in Hb absorbance at 630 nm from 0.064 ± 0.005 to 0.07 ± 0.002 (Figure 2F) corresponds with the conversion of oxyHb into metHb after 120 minutes incubation with lead ions in an EMF. Figure 2E shows a decrease in absorbance at 420 nm from 3.28 ± 0.26 to 2.96 ± 0.13 , which corresponds with the Soret band and shows a diminished heme-heme interaction in the EMF- and lead-treated erythrocytes.

4.2. Effect of Electromagnetic Field and Lead on the Carbonyl Content of Plasma

The measurement of the carbonyl content of the plasma proteins in EMF- and lead-treated subjects revealed a significant increase in the proportion of carbonyl content from 0.24 to 23.81 (Figure 3A, $R^2 = 0.9633$, $P < 0.05$). Additionally, there was a positive correlation between lead concentration and both the relation of plasma carbonyl content to Hb absorbance at 630 nm (Figure 3B, $R^2 = 0.967$, $P < 0.05$) and the relation of plasma carbonyl content to metHb (Figure 3C, $R^2 = 0.948$, $P < 0.05$), which indicates the positive correlation between oxidative damage in plasma and erythrocytes.

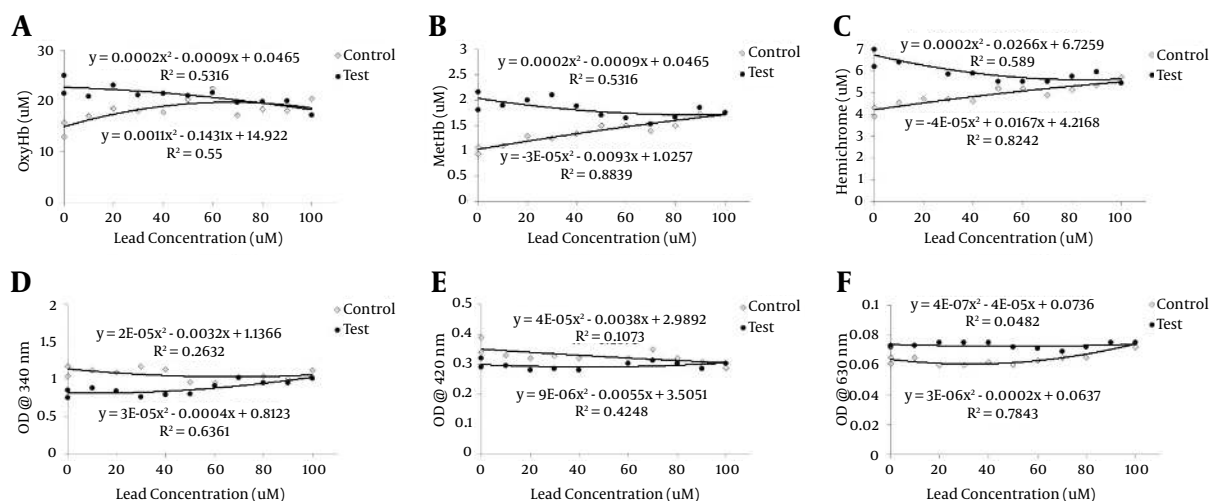
4.3. Effect of Electromagnetic Field and Lead on the Antioxidant Power of Plasma

The results showed no differences in the FRAP values after incubation in an EMF with different concentrations of lead (Figure 3D).

4.4. Artificial Neural Network Analysis

In this study, a feed-forward architecture with two hidden layers (MLP 11, 2, 2, 1) was designed. The description diagram for this ANN is presented in Figure 4. The diagram displays information about the input, hidden, and output layers. After optimizing the designed ANN and training within 2000 epochs, the network's predictive power was tested. The relative errors for the training and testing steps were 0.07 and 0.151, respectively (Table 1). Further, the sum of the squares errors for the training and testing steps were 0.209 and 0.131, respectively (Table 1).

Figure 2. Correlations Between the Lead Concentration and the oxyHb, metHb, hemichrome, and Hb Absorbance at Different Wavelengths



All of the samples were incubated for 120 minutes in 20 millitesla and 50 hertz EMF. Each sample was analyzed in duplicate. The correlation between the variables was calculated with the Pearson's test. MetHb, methemoglobin; OD, Hb absorbance at indicated wavelengths; OxyHb, oxyhemoglobin; uM: micromole.

The artificial neural network analysis showed a positive and linear correlation between the predicted and observed concentrations of oxyHb (Figure 5A). The residual by predicted chart (Figure 5B) shows no visible patterns between the predicted and residual values. Additionally, the significant importance of hemichrome, PCO, metHb, and lead concentration to the oxyHb content of erythrocytes can be seen (Figure 6).

squares errors in the training and testing groups, the relative errors in the training and testing groups, the stopping rule used to stop training, and the training time. OxyHb: oxyhemoglobin.

5. Discussion

In this work, a reproducible and reliable set of experiments was presented for evaluating the oxidative damage to human plasma and erythrocytes that were exposed to EMF and different concentrations of lead ions. For this purpose, the response of the erythrocytes to lead- and EMF-mediated oxidative stress, the oxidation state of heme-iron, the conformational changes in Hb, and the structural modifications in plasma proteins have been investigated.

The decreased absorption at 340 nm indicates the conversion of oxyHb into metHb in this oxidative condition (Figure 2D). Such a reduction in the Hb concentration and elevation in the metHb concentration may disrupt the erythrocyte structure and cause anemia, although toxic methemoglobinemia can be successfully treated with ascorbic acid (34).

If the peptide chains of globin are denatured, metHb can convert into hemichrome in which several ligands occupy the sixth coordination position of the ferric ion in heme (Figure 2C). Hemichromes can then precipitate and form Heinz bodies. This phenomenon is explained by the production of superoxide anions during the oxidation of ferrous ions.

Table 1. Model Summary of the Artificial Neural Network^a

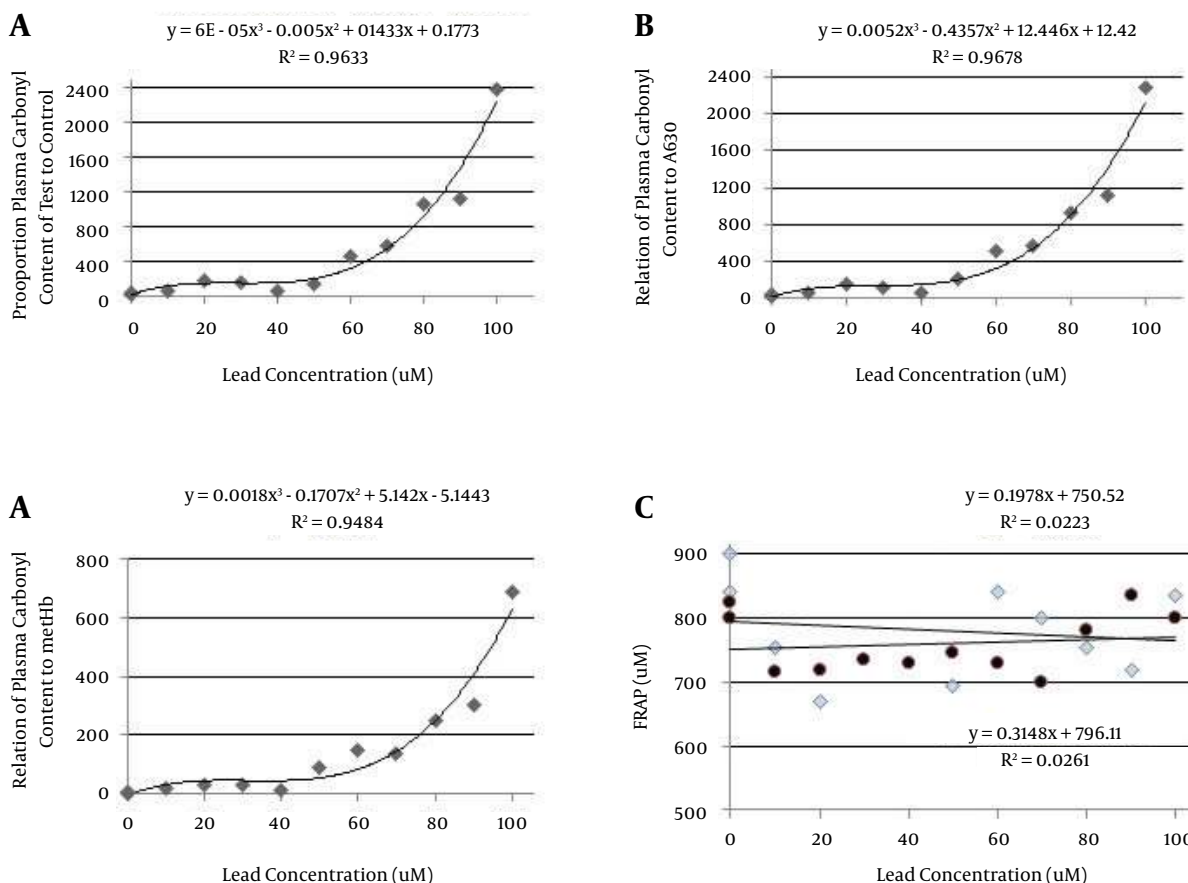
Model Summary	Value
Training	
Sum of squares error	0.209
Relative error	0.070
Stopping rule used	One consecutive step(s) with no decrease in error ^b
Training time	0:00:00.00
Testing	
Sum of squares error	0.131
Relative error	0.151

^aThis model displays a summary of the neural network results by partition and overall, including the sum of squares errors in the training and testing groups, the relative errors in the training and testing groups, the stopping rule used to stop training, and the training time. OxyHb: oxyhemoglobin.

^bDependent variable: OxyHb.

This model displays a summary of the neural network results by partition and overall, including the sum of

Figure 3. Correlations Between the Lead Concentration and Oxidative Modifications in the Plasma and Erythrocytes



Each data represents the correlation between the lead concentration and the percentage of plasma carbonyl content; A, the relation of plasma carbonyl content to Hb absorbance at 630 nm; B, the relation of plasma carbonyl content to metHb concentration; C, and the antioxidant power of plasma; D, after 120 minutes incubation in 20 millitesla and 50 hertz EMF. Each sample was analyzed in duplicate. The correlation between the variables was calculated with Pearson's test. FRAP, ferric reducing ability of plasma; MetHb, methemoglobin; OD, Hb absorbance at indicated wavelengths; OxyHb, oxyhemoglobin; uM, micromole.

Superoxide dismutase converts superoxide into hydrogen peroxide that produces hydroxyl radicals in the presence of metal ions such as lead. On the other hand, methemoglobin plays a central role in oxidative damage and cytoskeleton defects. Such oxidative effects on the cell membrane lead to hemolysis (35) and the release of Hb and metHb into blood and urine. Further, the pseudo-peroxidase activity of metHb produces a large amount of ROS (36), which results in protein carbonylation and membrane damage in erythrocytes (37).

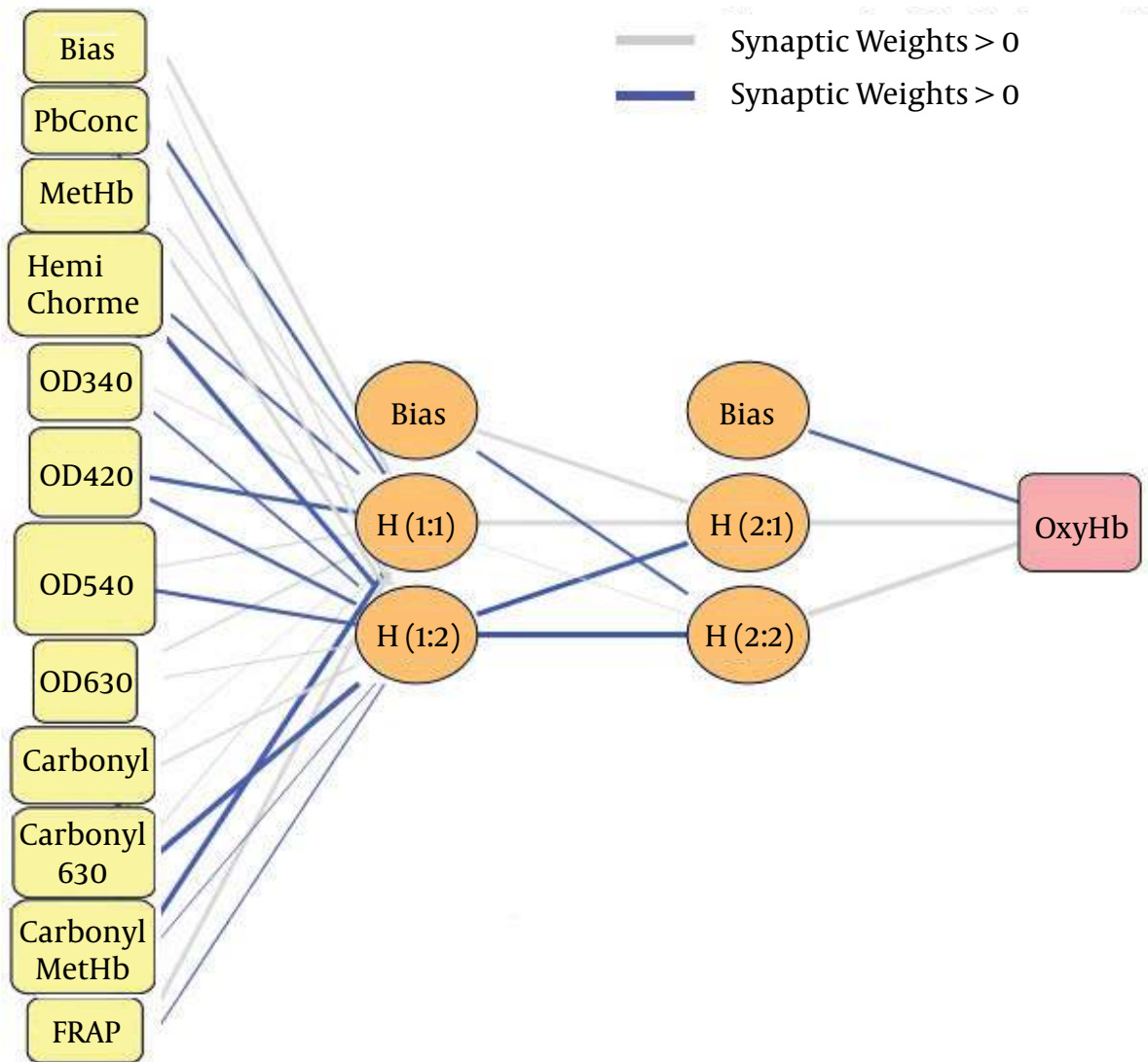
As the reduced form of plasma proteins and heme iron is necessary for their biologic activity, the oxidative damage can explain the shorter erythrocyte lifespan and pathological changes associated with anemia (38). The increased concentration of oxygen radicals seen in lead- and EMF-

treated erythrocytes has an effect on the heme iron oxidation observed in this study.

One of the most important biomarkers in protein oxidation is the carbonyl group, which induces conformational modifications, impaired function, and proteolysis (39). In this work, we assessed the oxidative effects of lead and EMF on plasma proteins as the carbonyl group. Interestingly, our study showed a significant elevation in the carbonyl content of the proteins, which positively correlated with the EMF and lead concentration (Figure 3). This result demonstrates that EMF and lead have synergistic effects that produce reactive oxygen species and promote chemical and conformational modifications in erythrocyte and plasma proteins.

We anticipated that in human erythrocytes the Hb con-

Figure 4. Feed-Forward Architecture for the Artificial Neural Network as a Multilayer Perceptron (11,2,2,1).



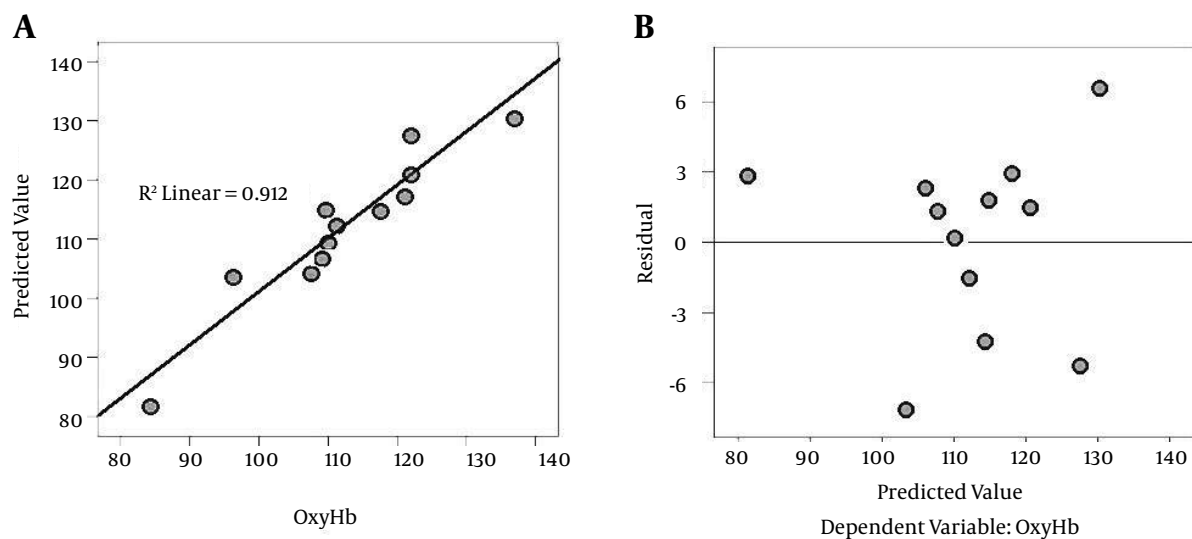
This description diagram displays information about the designed artificial neural network, including the dependent variables, oxyHb as the output unit, two hidden layers with two units, and hyperbolic tangent and identity as the activation functions in the hidden layer and the output layer, respectively. Carbonyl/630: relation of plasma carbonyl content to Hb absorbance at 630 nm, Carbonyl/MetHb, relation of plasma carbonyl content to methemoglobin concentration; FRAP, ferric reducing ability of plasma; H, hidden layer, MetHb: methemoglobin; OD, Hb absorbance at indicated wavelengths; OxyHb, oxyhemoglobin.

formation would be modified both primarily by the EMF-induced oxidation and secondarily by the impairments caused by the direct reaction of lead ions with heme groups. In previous works, we exposed erythrocytes to a copper-mediated MCO system and reported that cupric ions could direct erythrocytes toward an oxidative challenge (40).

The participation of lead in the oxidation-induced impairment of the erythrocyte membrane through the MCO

system has been confirmed. Many studies have shown that oxidative stress contributes to eryptosis (41) and a decrease in deformability (42).

The conformational integrity of the plasma proteins is necessary for biochemical and biological activities. Oxidized proteins become highly sensitive to proteolysis (43). Reactive oxygen species can directly attack the protein backbone, leading to protein degradation and fragmentation (44).

Figure 5. Predicted by Observed and Residual by Predicted Charts for the Artificial Neural Network

There is a positive correlation between the predicted and observed values; A, but no visible pattern between the residual and predicted values for each scale-dependent variable; B, in the artificial neural network. OxyHb: oxyhemoglobin.

According to Dean's study, the modified movement of proteins in electrophoresis was observed during oxidative stress (45). Based on this study, the erythrocyte membrane is an important target for ROS attack. However, the authors did not make a distinction between the membrane regions that are more accessible to ROS. A final consideration needs to be mentioned, namely that aerobic MCO conditions not only accelerate the oxidative damage to erythrocyte and plasma proteins but also activate proteolytic mechanisms and hemolysis.

Our results have important implications in relation to EMF exposure and lead contamination in workers in industrial sectors, battery manufactures, and power plants. The importance of erythrocyte as a useful model for evaluating oxidative conditions has become evident from the various abnormal features illustrated in the cytoskeleton and Hb structure.

Innovations and breakthroughs: The main purpose of our study was to introduce accurate and sensitive techniques for estimating the oxidative effects of electromagnetic fields and lead on the structure of blood proteins. In the present study, chemical and conformational analyses confirmed the structural modifications in plasma and erythrocyte proteins.

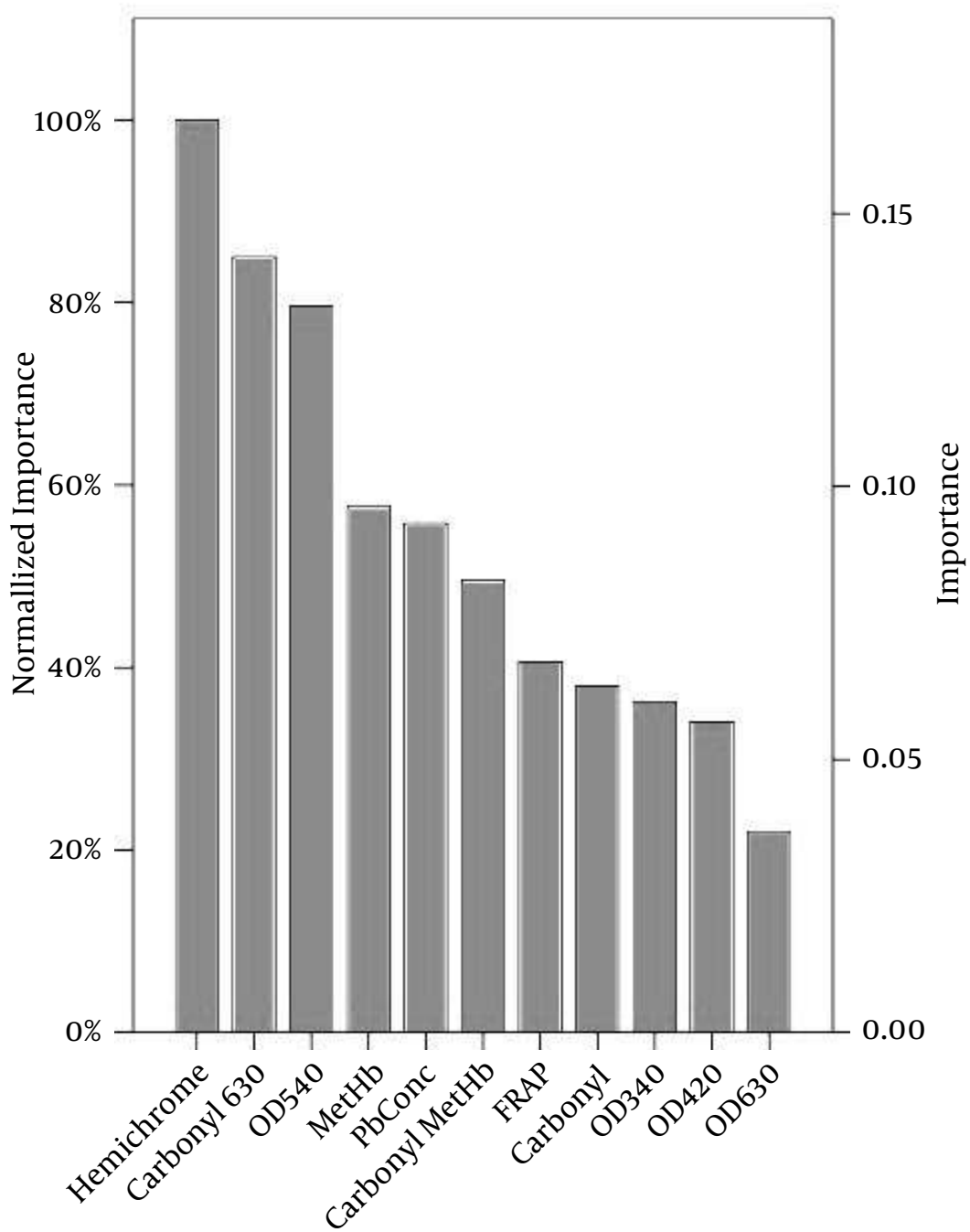
Significantly, an increase in the carbonyl content of the plasma was demonstrated in the presence of EMF and lead ions. Additionally, there was a positive correlation between the carbonyl content of plasma and the oxidative

modifications to Hb.

Regarding the synergistic effect of lead contamination and EMF exposure, the results of this research are applicable to industrial health and biochemical analysis as well as to the design of methods to test for further effects of EMF and lead contamination and other environmental pollutants.

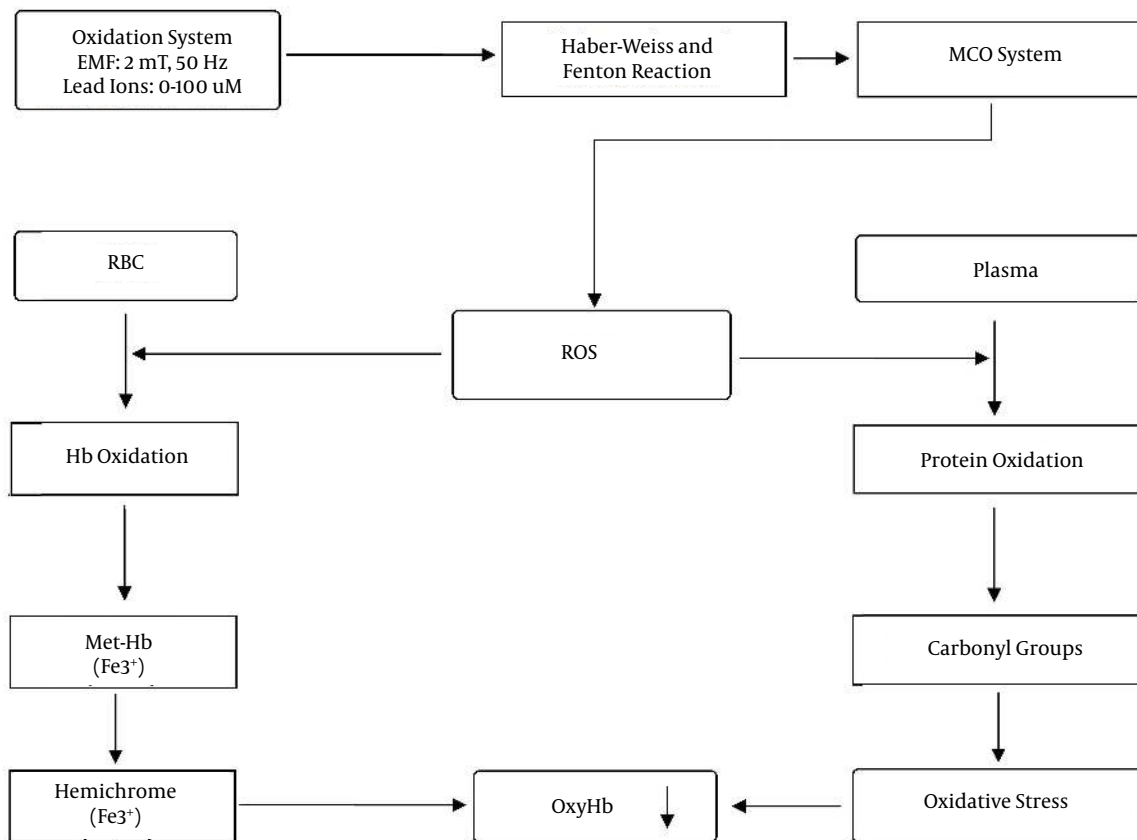
Further, the ANN analysis showed the significant importance of hemichrome, PCO, metHb, and lead ions to the oxyHb concentration.

Figure 6. Independent Variable Importance Analysis for the Artificial Neural Network



This illustration displays a sensitivity analysis, which computes the importance of each predictor in determining the artificial neural network. The analysis is based on the combined training and testing samples. Carbonyl/630, ratio of plasma carbonyl content to Hb absorbance at 630 nm; Carbonyl/MetHb, ratio of plasma carbonyl content to methemoglobin concentration; FRAP, ferric reducing ability of plasma; MetHb, methemoglobin; OD340, Hb absorbance at 340 nm; OD, Hb absorbance at indicated wavelengths.

Figure 7. Diagrammatic Representation of the Relations Between the Electromagnetic Field, Lead Ions, and Oxidative Damage in the Plasma and Erythrocytes



EMF, electromagnetic fields; Hb, Hemoglobin; Hz, hertz; MCO, metal-catalyzed oxidation; Met-Hb, met-hemoglobin; OxyHb, oxyhemoglobin; RBC, red blood cells; ROS, reactive oxygen species.

Acknowledgments

This research was supported by the molecular and medicine research center and clinical biochemistry and genetics department of Arak University of Medical Sciences.

Footnotes

Authors' Contribution: Study concept and design, Hadi Ansarihadipour and Mohamadreza Bayatiani; acquisition of data, Hadi Ansarihadipour; 3, analysis and interpretation of data, Hadi Ansarihadipour; 4, drafting of the manuscript, Hadi Ansarihadipour; 5, critical revision of the manuscript for important intellectual content, Hadi Ansarihadipour and Mohamadreza Bayatiani; 6, statistical analysis, Hadi Ansarihadipour.

Financial Disclosure: Hadi Ansarihadipour and Mohamadreza Bayatiani both reported receiving research

grants from Arak University of Medical Sciences.

Funding/Support: This research was supported by the molecular and medicine research center and clinical biochemistry and genetics department of Arak University of Medical Sciences.

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