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Determination and evaluation of trace elements in the blood of radiography workers using graphite furnace atomic absorption spectrometry

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ABSTRACT

This study aimed to evaluate the potentially toxic effects of trace elements in the blood of Iraqi medical radiography workers by analyzing them through the graphite furnace-atomic absorption spectrometry method (GF-AAS). The study involved 50 male blood radiography workers from the medical imaging field at Al-Shatrah General Hospital in Thi-Qar City, Iraq. All workers were aged between 35-50 years and had worked for less than 10 years. The study compared these workers with 50 healthy men. The study found a significant increase in the levels of Cu, Pb, Cd, and Ca among radiography workers compared to the healthy control group, while Zn and Se levels decreased significantly. Moreover, Specificity and confidence interval (95%) were estimated via the receiver operating characteristic curve (ROC). The study provided conclusive evidence of disturbances in the levels of trace elements in the blood of radiographer workers, which makes them more susceptible to many diseases because of their radiation exposure. which portends the use of more preventive measures and commitment to the principles of radiation protective protocols to reduce the effects of radiation exposure and an increase in the occupational dose. The linear range of Cd, Cu, Zn, Se and Pb in human serum were obtained 0.2-6.0 μg dL^{-1}, 6.0-200 μg dL^{-1}, 8.0-200 μg dL^{-1}, 10-250 μg dL^{-1}, 4-120 μg dL^{-1} by GF-AAS after dilution samples with DW up to 20 (n=10, RSD< 5%).

1. Introduction

The utilization of medical imaging procedures like X-rays, CT scans, and MRI, which have quickly advanced over the last three decades, has become standard practice in healthcare facilities. The main advantage of such technologies is the structural information they provide about the human organism, which helps with the identification of illnesses, internal body tissue investigation, evaluation, and medical treatment. As a result, these technologies are crucial to both the safety and quality of healthcare for patients [1]. Radiographers' radiation exposure risk is a danger associated with all medical imaging modalities, but it varies greatly depending on the characteristics of the person who is the radiographer. Radiographers may be more susceptible to cancer or have higher quantities of trace elements in their blood, which could lead to damage to their DNA [2, 3]. There are many ways that DNA can change, but one of them involves reacting ROS that break DNA strands and crosslink DNA proteins. According to recent studies, radiation may indirectly alter DNA through the accumulation of trace elements and minerals [4, 5]. Throughout the human body, trace elements are present in minute amounts and support the functions of the body. Trace elements are categorized into essential and non-essential elements

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based on the demands of the organism [6]. Essential elements, which make up 0.02% of body weight, are crucial in physiological functions like metabolic processes, blood cell production, reproduction, and immunity. The occurrence of trace elements in quantities that are either too low or too excessive can lead to metabolic problems and disruptions of cellular growth, including the occurrence of cancer and mutagenesis [7]. Because they can disrupt cellular processes and change the expression patterns of numerous genes, trace elements are hazardous because they increase the likelihood of developing disease [8]. Trace elements can interact with DNA in a variety of ways. Large numbers of strongly bonded metal ions can be found in nucleic acids. The incorporation of genetic mistakes may increase due to changes in DNA polymerase activity caused by the binding of particular metals. Trace elements interfere with the function of reparative proteins, altering the outcome of the repair of double-strand breaks (DSBs), and also changing the distribution of altered DNA, potentially increasing the risk of developing any type of cancer. ROS generation from exposures to trace elements can result in damage to DNA [9]. Human radiation exposure has an impact on trace element levels, as long-term exposure leads to changes in the physiological functions of the organism's body [10]. The normal range of elements such as Cd (>0.3 µg dL^{-1}), Cu(130-160 µg dL^{-1}), Zn (120-170 µg dL^{-1}), Se (15-25 µg dL^{-1}), Pb(5-25 µg dL^{-1}), and Ca (85 to 100 mg L^{-1}) were reported in human serum samples [11]. Spectroscopy techniques have been developed in many areas of analytical chemistry, such as the environmental, medical, and industrial fields [12]. The purpose of this investigation was to evaluate the potentially toxic effects of radiation on the levels of trace elements in the blood of Iraqi medical radiography workers by estimating them through the atomic absorption spectrometry method

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2. Materials and methods

2.1. Instruments

A centrifuge (Biofuge B, Heraeus–Christ, Germany) was used to separate blood samples, and an oil bath (F3– HAKE, Germany) to heat samples. Copper (Cu), lead (Pb), calcium (Ca), cadmium (Cd), zinc (Zn), and selenium (Se) were determined by Graphite furnace atomic absorption Spectrophotometer (280Z AA/ Agilent, USA).

2.2. Chemicals

Each chemical has been sourced to the highest possible level of purity and from discreet origins, including hydrochloric acid (HCl 37%, CAS Number:7647-01-0, Merck, Germany), nitric acid (HNO_3 69%, CAS Number:7697-37-2, Scharlau-Espane), perchloric acid ($HClO_4$ 70%, CAS Number: 7601-90-3, HIMEDIA, India), and sulfuric acid (H_2SO_4 98%, CAS Number: 7664-93-9, HIMEDIA, India). Also, Standard solutions of copper, lead, cadmium, calcium, zinc, and selenium (1000 ± 51 mg L^{-1} in 2 % HNO_3) were prepared from Merck (Germany).

2.3. Subjects

The current study included 50 male radiography workers working in the field of medical imaging (MRI, CT-scan, and X-rays departments) at Al-Shatrah General Hospital in Thi-Qar City Iraq, with an age range of 35-50 years and 10 years of periods of working. The subjects compared with 50 males who did not work in the field of medical imaging, with the same age range of 35-50 years, and good health served as a control group. Participants suffering from chronic diseases such as heart disease, hypertension, thyroid disorders, and diabetes were excluded. Also, The Body Mass Index was determined via the Equation 1 [13]:

$$BMI\ (kg\ m^{-2}) = Weight\ (kg) / [Height(m)]^2$$

(Eq.1)

2.4. Blood Samples Collection

Blood samples were obtained from radiography workers and health control groups at AL-Shatrah General Hospital. Around 5.0 mL of blood was haggard from a vein and allowable to clot for about 15 min at ٢٥ °C and then centrifuged at ٣٠٠٠ rpm for ١٠ min. The obtained serum was stored in a deep freeze at -١٨°C and was ready for use in the measurement of trace elements.

Everyone who participated in the study gave their consent, and it was made public. The 1964 Declaration of Helsinki and its later amendments or comparable ethical norms were followed in conducting this study, which was approved by the Al-Ayen University Ethical Committee) (Date: 5 Jan 2024, AUEC, Number ECN:2024- 892)

2.5. Determination of Trace Elements

2.5.1. Preparation of Standard Solutions

Standard solutions of copper, lead, cadmium, calcium, zinc, and selenium (1000 ± 51 mg L^{-1} in 2 % HNO) were obtained from Merck company. required working solutions were ready by diluting a specific stock solution with aquatic to obtain a standard calibration curve.

2.5.2.Samples Preparation and procedure

Serum samples were digested by incorporating 2.0 mL of concentrated HNO_3 and 1.0 mL of concentrated $HClO_4$ to 0.5 mL of serum in a Pyrex tube heated in an oil bath for 1 hour at 165 °C. So that the digested samples were clear, the tubes were cooled at ۲۵ °C, and the quantity was increased to ۱۰ mL by 0.3 M HCl. Graphite furnace-atomic absorption spectrometry (GF-AAS) was utilized to analyze of copper, lead, cadmium, calcium, zinc, and selenium in standards and sample solutions under the ideal conditions shown in Table 1. Also, the elements in serum samples of medical radiography workers was determined by the GF-AAS which was shown in Table 2. The proposed method was validated by certified reference material which was analyzed by ICP-MS in serum samples (Table 3).

The limit of detection (LOD) for trace elements was intended by Equation 2. The limit of quantification (LOQ) for trace elements was calculated by Equation 3. Where is the standard deviation of blank solution for 10 reads and S is the slope of the standard curve.

$$LOD=3.3\ SD/S \qquad (Eq.2)$$

$$LOQ= 10SD/S \qquad (Eq.3)$$

2.6. Statistical Examining

The data were assessed using the statistical program SPSS, version 23.0. A Student's t-test was used to determine the significance of any differences that were found to exist between each group. The mean ± standard deviation (SD) of the data was displayed. The operating characteristics (ROC) curve was used to calculate specificity, specificity, and the 95% confidence interval. All p-values were two-tailed and $p<0.05$ was deemed significant for statistical analysis.

Table 1. Ideal circumstances for the experiment were employed to determine Cu, Pb, Cd, Ca, Zn, and Se by GF-AAS with Zeeman background correction.

Parameters	Cu	Pb	Ca	Cd	Zn	Se
Lamp Current (mA)	5	10	10	4	5	5
Wavelength (nm)	234.8	283.3	422.4	228.8	217	196
Slit Width (nm)	1	1	1	1	1	1
Ac FR($dm^3\ min^{-1}$)	0.8 -1	0.8-1	0.8-1	0.8-1	0.8-1	0.8-1
Read Time (s)	3	3	3	3	3	3

Ac FR: Acetylene flow rate

Table 2. Statistical results for determination elements in serum workers by GF-AAS (µg dL^{-1})

GF-AAS	Cd	Cu	Zn	Se	Pb
LR	0.01-0.3	0.3-10.0	0.4-12.0	0.5-12.5	0.2-6.0
LOD	0.003	0.084	0.12	0.14	0.066
LOQ	0.011	0.302	0.40	0.50	0.201
*LR Method	0.2-6.0	6.0-200	8.0-200	10.0-250.0	4.0-120
LOD	0.06	2.02	2.60	3.30	1.33
LOQ	0.20	6.03	8.00	10.0	4.00
Mean RSD%	2.20	2.01	1.33	1.34	3.42

*Procedure: Dilution Factor=20 (0.5 mL of Serum+10 mL HCL/DW)
LR: Linear Range, Concentration of µg dL^{-1}= 10× µg L^{-1}

Table 3. Validation procedure by certified reference material by ICP-MS in serum samples (µg dL^{-1})

Elements	Certified Value (ICP-MS)	Found (GF-AAS)	Recovery (%)
Cu	83.28±1.52	81.28±3.39	97.6
Pb	20.96±0.77	19.84±0.98	94.7
Cd	0.902±0.02	0.817±0.04	95.1
Ca	9.13±0.35	8.98±1.44	98.4
Zn	79.26±2.22	76.44±3.75	96.4
Se	148.93±31.16	138.77±30.96	۹۳.1

3. Results and Discussion

The descriptive data used in the current study indicates as in Table 4, that there were no significant variances in the mean of age and BMI between the study groups (37.93±4.44 years, and 33.87±1.55, respectively compared to 38.01±4.68 years, and 34.22±2.76, respectively). and this is due to the selection of the ages and BMI of the control group close to the ages and BMI of the radiography workers being studied so that there was no discrepancy between the ages and BMI of the studied subjects. Therefore, non-significant variances ($p>0.05$) were in the age and BMI of the studied groups [14].

The results of this investigation displayed a high significant increase ($p<0.01$) in the levels of serum copper (Cu) in the blood of radiography workers (81.28±16.39 µg dL^{-1}) compared to the control group (60.1±15.21 µg dL^{-1}) as in Figure 1. It also showed the acquired AUC data that serum copper (Cu) might be a more precise predictive biomarkers in radiography workers (AUC = 0.819) as demonstrated in Figure 2.

Table 4. The general descriptive data for every individual taking part in this research

The traits	Radiography workers group	Control group	p-value
Number (N)	50	50	------
Age± SD (year)	37.93±4.44	38.01±4.68	0.183
BMI± SD (kg m^{-2})	33.87±1.55	34.22±2.76	0.114

BMI: body mass index; SD: Standard devation; P>0.05: non-significant variance; P<0.05 significant variance; P<0.01: high significant variance

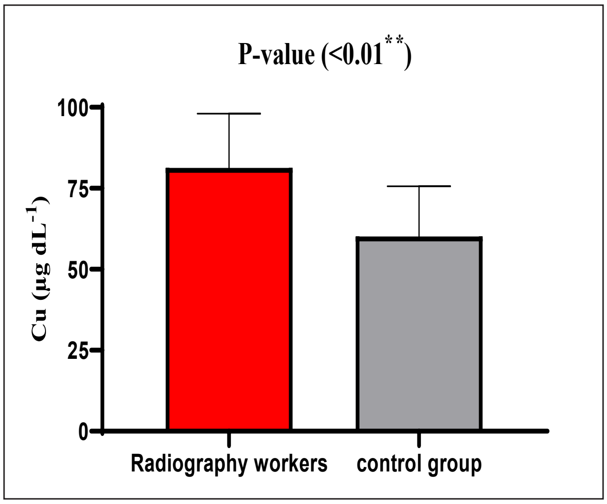

Fig.1. Serum levels of Copper (Cu) in the radiography workers group and the control group

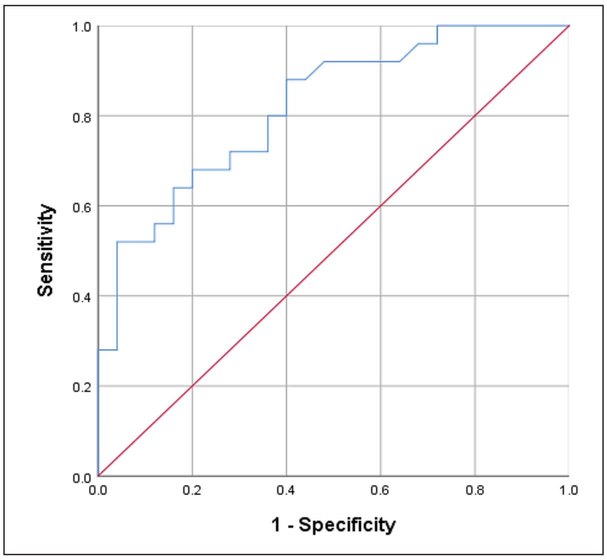

Fig.2. The ROC curve for levels of serum Copper (Cu) in the radiography workers group and the control group

Regarding of serum lead (Pb), the results of this investigation displayed a high significant increase (P< 0.01) in the blood of radiography workers (19.84±6.8 µg dL^{-1}) compared to the control group (10.14±3.44 µg dL^{-1}) as in Figure 3. It also showed the acquired AUC data that serum copper (Cu) might be a more precise predictive biomarkers in radiography workers (AUC = 0. 891) as demonstrated in Figure 4.

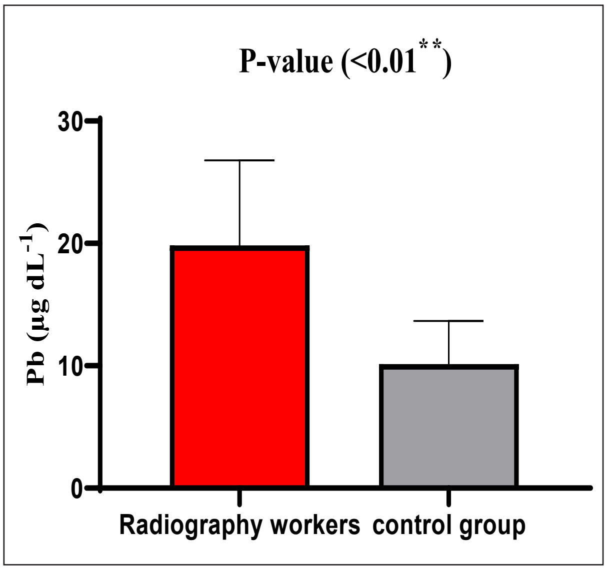

Fig.3. Serum levels of Lead (Pb) in the radiography workers group and the control group

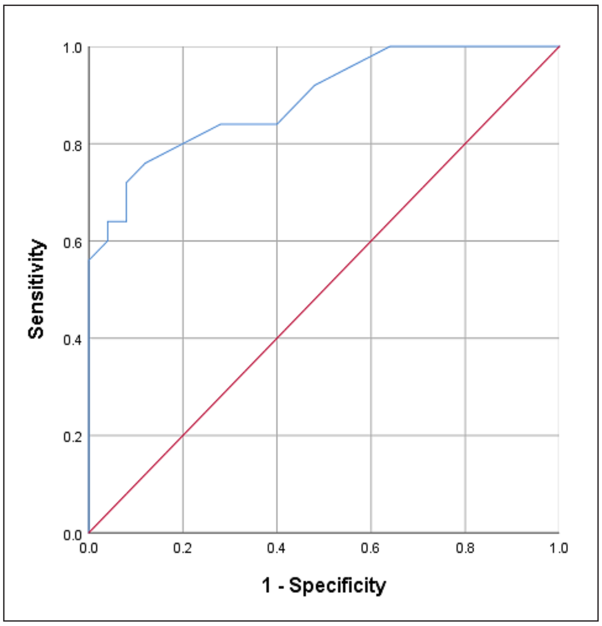

Fig.4. The ROC curve for levels of serum Lead (Pb) in the radiography workers group and the control group Regarding serum cadmium (Cd), the results of this investigation displayed a highly significant increase (P< 0.01) in the blood of radiography workers (0.81±0.18 µg dL^{-1}) compared to the control group (0.42±0.07 µg dL^{-1}) as in Figure 5. It also showed the acquired AUC data that serum copper (Cu) might be a more precise predictive biomarker in radiography workers (AUC= 0.990) as demonstrated in Figure 6.

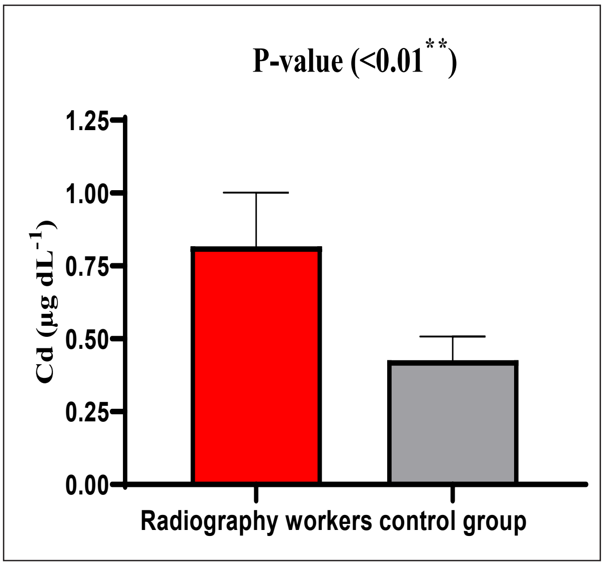

Fig.5. Serum levels of Cadmium (Cd) in the radiography workers group and the control group

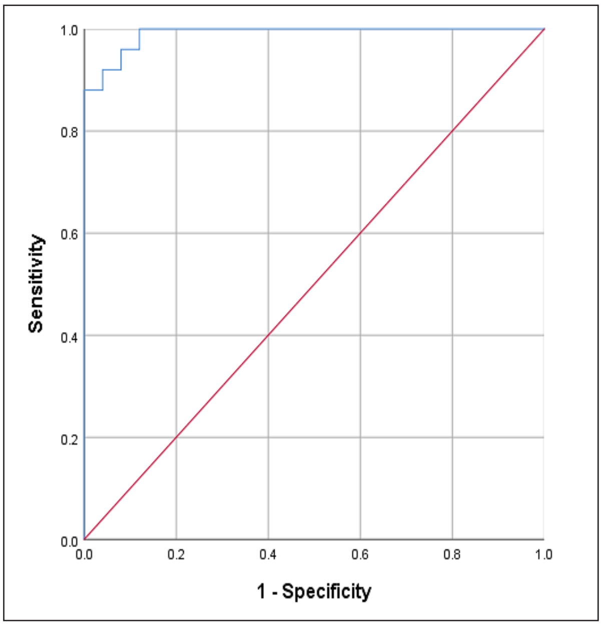

Fig.6. The ROC curve for levels of serum Cadmium (Cd) in the radiography workers group and the control group

Regarding of serum Calcium (Ca), the results of this investigation displayed a high significant increase ($p < 0.01$) in the blood of radiography workers (8.98±1.95 µg dL^{-1}) compared to the control group (6.18±1.66 µg dL^{-1}) as in Figure 7. It also showed the acquired AUC data that serum copper (Cu) might be a more precise predictive biomarkers in radiography workers (AUC = 0. 862) as demonstrated in Figure 8.

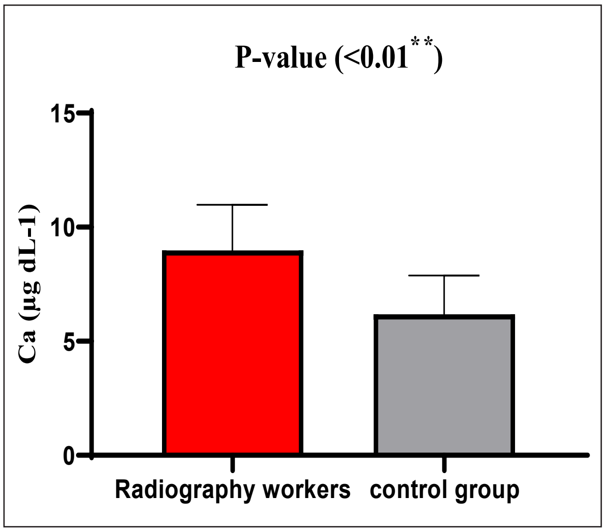

Fig.7. Serum levels of Calcium (Ca) in the radiography workers group and the control group

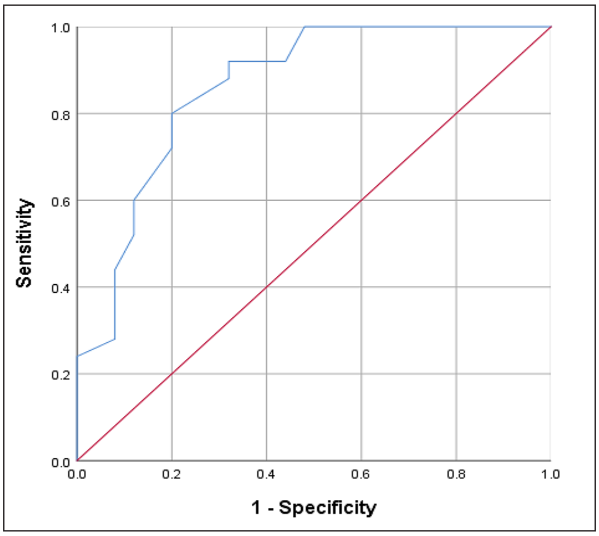

Fig.8. The ROC curve for levels of serum Calcium (Ca) in the radiography workers group and the control group

Regarding of serum Zinc (Zn), the results of this investigation displayed a high significant increase ($p<0.01$) in the blood of radiography workers (76.44±10.22 µg dL^{-1}) compared to the control group (96.31±12.88 µg dL^{-1}) as in Figure 9. It also showed the acquired AUC data that serum copper (Cu) could not be a predictive biomarker in radiography workers (AUC = 0. 118) as demonstrated in Figure 10.

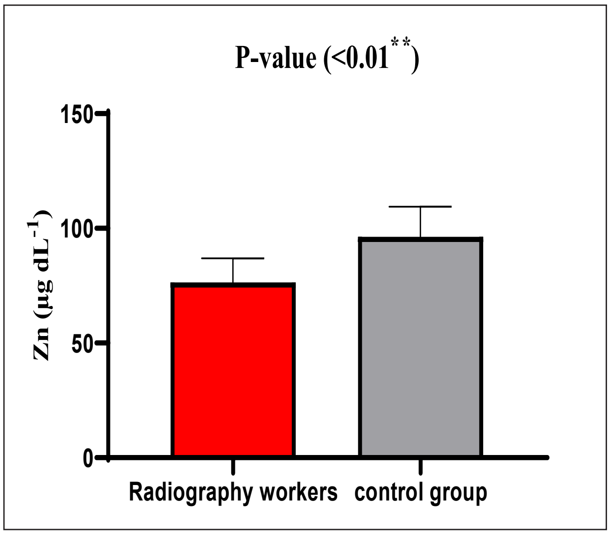

Fig.9. Serum levels of Zinc (Zn) in the radiography workers group and the control group

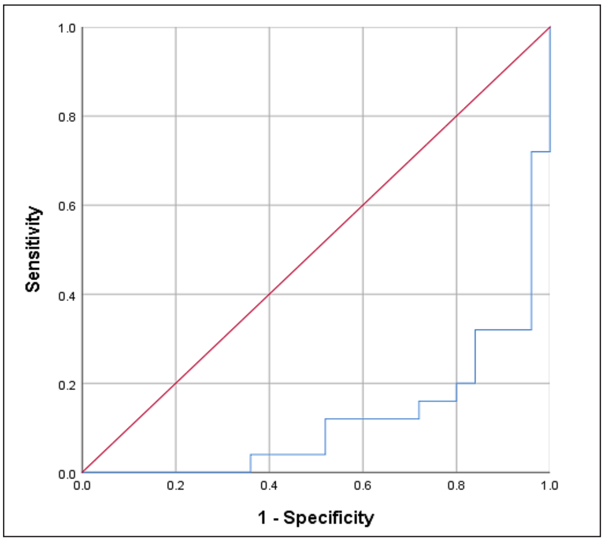

Fig.10. The ROC curve for levels of serum Zinc (Zn) in the radiography workers group and the control group

Finally, The results of this study showed a significant decrease ($p< 0.01$) in the levels of serum Selenium (Se) in the blood of radiography workers (131.77±30.96 ng dL^{-1}) compared to the control group (154.37±۳3.85 ng dL^{-1}) as in Figure 11. It also showed the acquired AUC data that serum Selenium (Se) could not be a predictive biomarker in radiography workers (AUC= 0.322) as demonstrated in Figure 12.

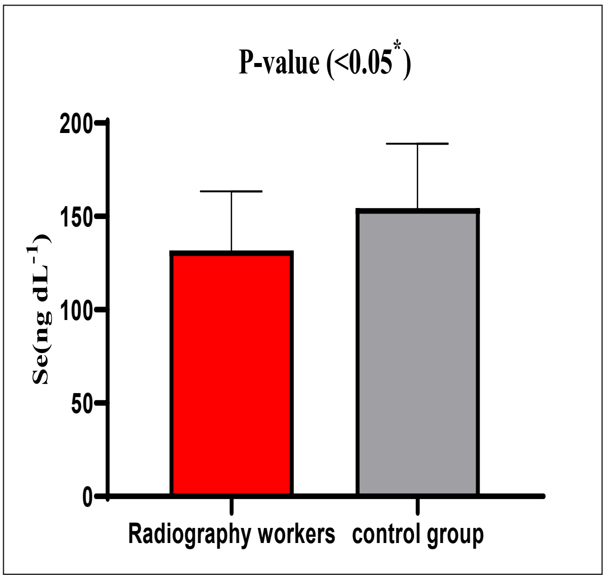

Fig.11. Serum levels of Selenium (Se) in the radiography workers group and the control group

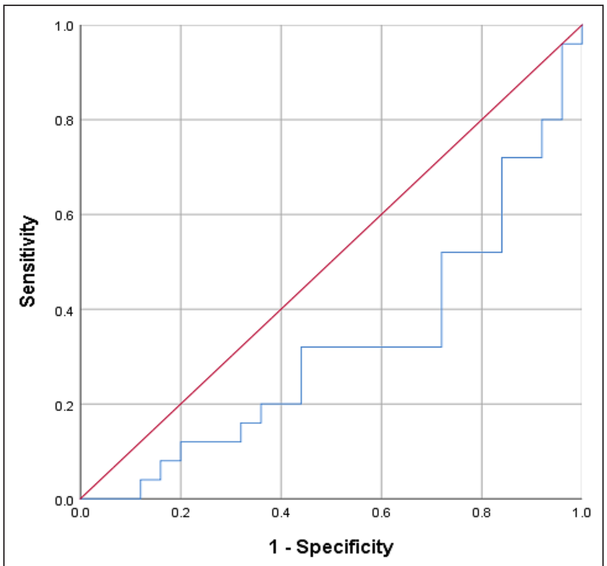

Fig.12. The ROC curve for levels of serum Selenium (Se) in the radiography workers group and the control group

Medical radiography workers run a greater risk of poisoning since they are frequently exposed to electromagnetic radiation. Iraqi medical radiography workers exposure to radiation is evaluated for possible hazardous effects using several parameters including measurements of trace element levels. Trace elements are necessary for many different biological activities. On the other hand, these elements can cause metabolic problems and disturb cellular growth, leading to the emergence of genetic abnormalities and cancer, if their levels are too high or low [15, 16]. Many risk factors, including smoking and hepatic, renal, cardiovascular, and DM illnesses, can raise the body's levels of elements. A diagnostic for prolonged exposure, an increase in blood trace element values revealed the body's stress [17, 18]. The best way to determine the exposure to hazardous elements (like Lead) that cling tightly to intracellular proteins is to analyze serum [19, 20]. It has been shown that radiation exposure raises Cd and Pb levels, which in turn overproduces Reactive oxygen species (ROS) and damages DNA in male sperm cells [21]. Moreover, working-related Cd exposure impairs lung function or may result in COPD (chronic obstructive pulmonary disease) [22]. Additionally, studies show that high plasma Pb concentrations harmed the reproductive system of men, leading to sperm quality declines, morphological changes, and broken-down DNA [23, 24]. Furthermore, it has been proposed that elevated blood levels of copper and iron may elevate the likelihood of developing Alzheimer's illness [25, 26].

Radiation exposure may cause problems with lipid membranes, chromosomal integrity, enzyme activities, and cell-to-cell communication. These problems can then affect tissue respiration, proliferation, and hemopoiesis [27, 28]. Previous studies have also proven that exposure to radiation for a long period leads to the appearance of problems in the hair follicles due to damage to the keratin protein, which leads to the appearance of structural symptoms in the hair [29, 30]. Other layers of cells are then affected by the low molecular mass for degraded protein fragments that migrate outside the skin cells, leading to skin-related problems when exposed to low amounts of radiation [31, 32]. The results of this study offer compelling evidence that for a long time, radiation exposure puts radiologists at elevated risk for specific disorders, each of which has a unique effect on trace element quantities. To fully understand the relationship between long-time radiation exposure and the amounts of trace elements in human beings, more research is necessary [33]. Professional radiographers should implement safety precautions and preventive measures, such as wearing protective clothing and shielding, using beam-limiting devices, and using appropriate beam filtering, based on this study's results. In addition, basic guidelines regarding distance, duration, and shields must be followed to reduce radiation exposure in a work environment [34, 35]. Also, some methodology was used for the determination of trace elements by ET-AAS or AAS [36-39]. Additionally, radiographers who have been exposed to prolonged radiation need to have frequent blood element testing performed to avoid or diagnose illnesses [40].

4. Conclusion

Spectroscopy techniques have been developed in many areas of analytical chemistry, such as the environmental, medical, and industrial fields. This study aimed to evaluate the potentially toxic effects of radiation on the levels of trace elements in the blood of Iraqi medical radiographer workers by estimating them through the atomic absorption spectroscopy method (RSD< 5%), which has proven its efficiency in the measurement and estimation process. LOD of Cd, Cu, Zn, Se, and Pb were obtained at 0.06, 2.02, 2.60, 3.30, and 1.33, respectively (n=10). The study provided conclusive evidence of disturbances in the levels of trace elements in the blood radiographer workers in the medical radiation field, which makes them more susceptible to many diseases because of their radiation exposure. which portends the use of more preventive measures and commitment to the principles of radiation protective protocols to reduce the effects of radiation exposure and an increase in the occupational dose.

5. Acknowledgements

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