GSC Advanced Research and Reviews
eISSN: 2582-4597 CODEN (USA): GARRC2
Cross Ref DOI: 10.30574/gscarr
Journal homepage: https://gsconlinepress.com/journals/gscarr/

(Review Article)

The use of atomic absorption technology as a diagnostic and monitoring tool for some diseases: A review between 2010 and 2022

Shaymaa Awad kadhim [1,*], Noor Hassan Abdulrudha [2], Ali Jaafar Azeez [1] and Yaqdhan Yousif abbas alhadmawi [3]

[1] *Department of Physics, Faculty of Science, University of Kufa, Najaf, Iraq.*
[2] *Faculty of Density, University of Kufa, Najaf, Iraq.*
[3] *Ministry of Education/Najaf Education Directorate, Najaf, Iraq.*

GSC Advanced Research and Reviews, 2024, 18(02), 001–008

Publication history: Received on 16 December 2023; revised on 28 January 2024; accepted on 31 January 2024

Article DOI: https://doi.org/10.30574/gscarr.2024.18.2.0021

Abstract

Atomic absorption technology is considered one of the important technologies in many different scientific fields because it is of a specification and diagnosis nature. Therefore, we decided to make a review about this technology over the years from 2010 to 2022 and the studies that used this technology in the fields of chemistry, physics, and medicine, and it's very important relationship in diagnosing the levels of element concentrations. Necessary, trace, toxic, etc.

We have found that it is very effective in this aspect and provides scientific research with many relevant positives. Therefore, we advise researchers to use it and to delve into broader fields by using it in more comprehensive and advanced research and to work on developing the technology in terms of work and diagnosis.

Keywords: Atomic Absorption Spectroscopy; Trace; Toxic; Technique

1. Introduction

The fact that about seventy-five percent of the chemical elements on Earth are metals underscores the importance of methods like AAS in various scientific and industrial applications. While metals play crucial roles in many processes and materials, they can also be pollutants or toxins at elevated concentrations. Therefore, assessing the metal content in different samples is vital for environmental monitoring, toxicology studies, and ensuring quality control in various industries.(1).

AAS is a technique widely used in analytical chemistry to determine the concentration of specific elements in a sample. Here's a breakdown of the key concepts you've mentioned: light absorption, element specific wavelength , selective absorption, concertation proportional absorption for example, If you have a sample containing both nickel and copper, and you expose it to light with a wavelength specific to copper, only the copper atoms or ions will absorb the light. The degree of absorption will be proportional to the concentration of copper in the sample(2)

The key concept here is quantization of energy levels. Electrons can only exist at specific energy levels within an atom, and the energy difference between these levels is discrete. When an electron absorbs a photon of the right energy, it gains exactly the energy difference between its current energy level and the higher energy level, allowing it to move to the excited state(3).

* Corresponding author: Shaymaa Awad kadhim; ORCID: 0000-0002-3991-5318; Email: Shaymaa.alshebly@uokufa.edu.iq

Exactly, you've provided a succinct overview of the main components of an atomic absorption spectrometer (AAS). Let's delve a bit deeper into each component : light source, atomization system, monochromator ,detection system(4).

The sample, whether in liquid or solid form, is atomized to convert it into free atoms. This is typically achieved by introducing the sample into a flame or a graphite furnace. In flame atomization, the sample is vaporized and atomized in a flame, creating a gaseous mixture of free atoms. In graphite furnace atomization, the sample is introduced into a high-temperature graphite tube, where it is vaporized and atomized(5).

Placing a monochromator between the sample and the detector serves to isolate the specific wavelength of light that corresponds to the electronic transition of interest for the element being analyzed. The monochromator ensures that only the targeted wavelength reaches the detector, effectively filtering out unwanted wavelengths and reducing background interference. The monochromator ensures that only the targeted wavelength reaches the detector, effectively filtering out unwanted wavelengths and reducing background interference(6).

AAS is indeed most commonly used for the analysis of metal atoms. This is because the electronic transitions and characteristic wavelengths of metals are well-suited for the technique. AAS is particularly effective for trace metal analysis, allowing for the detection and quantification of minute concentrations of metal ions in a sample(7).

The absorption and emission lines for metals are typically narrow, meaning they occur at very specific wavelengths. This narrowness is advantageous for analytical techniques like AAS because it allows for precise identification and measurement of the absorbed or emitted light (8).

Metals often have well-defined electronic transitions between energy levels. When these transitions occur, they result in the absorption or emission of light at very specific wavelengths (7).

In summary, the focus of AAS on metal atoms is due to the advantageous spectral characteristics of metals, making the technique highly effective for the precise and selective analysis of metals. The use of graphite furnaces extends the applicability of AAS to solid samples, providing a versatile analytical tool in the field of elemental analysis(9).

Figure 1 Atomic absorption spectroscopy(4)

In terms of physical, chemical, and functional processes, the normal state of humans is a condition of precise physiological balance, which is maintained by a complex of yet-unknown mechanisms; thus, sickness is an indicator of breakdown and derangement of control mechanisms, as well as disruption of other organ system(10). Thalassemia is a category of hereditary illnesses characterized by a decrease in globin chain synthesis. Beta-thalassemia is the most well-known kind of thalassemia. It is characterized by reduced synthesis of normal adult hemoglobin (Hb A), the most common form of hemoglobin from birth to death. The globin component of Hb A is made up of four protein portions known as polypeptide chains. Two of these chains are called alpha chains, while the other two are called beta chains. Beta globin synthesis is decreased or nonexistent in beta-thalassemia patients(11). Growth failure in thalassemic children has been related to growth hormone insufficiency, hypothyroidism, delayed sexual maturation, hypogonadism, diabetes mellitus, zinc deficiency, low hemoglobin levels, bone abnormalities, and desferrioxamine toxicity(12).

According to WHO and TIF, the global prevalence of thalassemia is estimated to be around 1.4 per 100000 people(13). To treat their anemia, people with thalassemia frequently need frequent blood transfusions. As you indicated, an excess of iron buildup, or iron overload, can result in a number of health issues. Indeed, iron excess has been linked to known issues in patients receiving repeated blood transfusions, including diabetes mellitus, growth retardation, parathyroid and thyroid insufficiency, and heart failure(12).

Table 1 The frequency distribution, male to female ratio, and prevalence rates of various forms of inherited anemias in patients registered at Iraq's 16 thalassemia facilities in 2015(13).

Type of Hereditary Anemias	number	percentage	Ratio of male to female	Prevalence (per 100000)
thalassemia	11165	66.2	1.1	37.14
β -TM	8,246	73.9	1.1	27.43
β -TI	2,485	23.3	1	8.27
α -Thalassemia	391	3.4	1	1.3
Hb E thalassemia	29	0.3	1.6	0.1
$^{\delta}$-thalassemia	14	0.1	1	0.05
sickle cell disorders	5,025	29.8	1.1	16.72
Other hereditary anemias	672	4	1.1	2.24

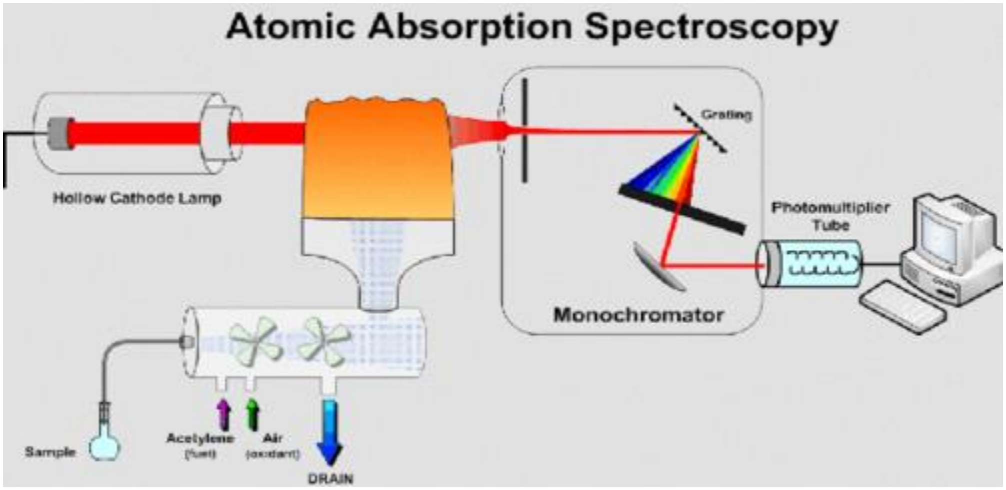

Figure 2 Atomic absorption spectroscopy(15)

Atomic absorption spectroscopy (AAS), emission spectrography, fluorometry, polarography, colorimetry, neutron activation studies, and x-ray fluorescence AAS is a recommended option for trace element analysis due to its specificity, sensitivity, accuracy, ease of use, and affordability. Furthermore, you bring out the developments in AAS technology, particularly the availability of burner heads that can aspirate highly-solid matrix materials. This advancement has simplified the procedure, doing away with the requirement for laborious and perhaps imprecise sample preparation techniques like precipitation and extraction(14).

The main concept is to regard the flame into which the spray is delivered as if it were a trough of absorbing solution in spectrophotometry, and to measure the absorbance of the flame. The absorbance of the flame for light with a resonant wave length is a direct measure of the concentration of absorbing atoms in the flame, and hence of the concentration of atoms in the dissolved substance(15).

This study's objective is to highlight some of the most significant research that evaluated atomic absorption spectroscopy and its potential to distinguish between normal and thalassemia patients to varied degrees and that were published between 2010 and 2022.

There have been many studies in the field of measuring the concentrations of trace elements and toxic elements in blood serum, hair, nails, and other biological parts of the human body, using atomic technology(10)(16)(17)(18).

2. Applications of atomic absorption Spectroscopy to Analyze Biological Samples of thalassemia patients

atomic absorption spectroscopy (AAS) as an analytical technique for elemental determination due to AAS is capable of detecting a broad variety of elements in various matrices, such as water, biological fluids, air particles, and pharmaceuticals. Because of its adaptability, it may be used in a variety of scientific fields. AAS is frequently seen of as being inexpensive, particularly when contrasted with some other methods. It is therefore a sensible option for regular analysis and research initiatives(19). Purpose of Acid digestion is to break down complex matrices and solubilize the analytes of interest, making them available for subsequent spectroscopic analysis. The process involves the use of strong acids to dissolve the sample and convert the elements into a form suitable for measurement(20). The determination of heavy metal traces in biological samples often involves complex procedures to extract and quantify the metals of interest. The use of dithizone and controlled pH settings is one such method, commonly employed for the determination of heavy metals like lead, cadmium, and zinc(21).

essential trace elements, which include both essential and probably essential elements, play crucial roles in various biological processes in humans. While the essentiality of certain elements like iron, zinc, and copper has been well-established, there is ongoing research regarding the essentiality and potential health effects of elements like vanadium, boron, and nickel(22). some elements considered toxic in high doses may still have essential roles at lower concentrations, while others, even in trace amounts, can have beneficial pharmacological properties. Here are examples of lithium and fluorine(23). As our understanding of trace element biology continues to grow, it is likely that more specific and pathologically relevant indicators of deficiency or excess will be identified, facilitating earlier and more accurate detection of trace element-related disorders.(18). In addition, Individual variations in metabolism, genetics, and lifestyle can influence how the body absorbs, utilizes, and excretes trace elements. This variability adds to the complexity of detecting and diagnosing trace element-related disorders(17).

In 2010 the scientists Abolfazl Mahyar et al. studied atomic absorption spectrophotometer was used to evaluate zinc and copper levels in serum. This study found that hypozincemia is widespread in thalassemic patients, although there is no copper deficit. More research is needed in this area(24).

In 2010, Bijan Keikhae et al. conducted research on this. The mean serum zinc levels in the TM with DFO, TM with DFO + DEF combination treatment, and control group were 68.9721.12g/dl, 78.1028.50g/dl, and 80.1626.54g/dl, respectively, to compare blood zinc levels in Thalassemia Major (TM) patients with the general population. The patients' and the control group's association was not statistically significant. This study shows that low serum zinc levels are a health concern in South West Iran, affecting both the general population and individuals with thalassemia.(25).

2011; Y. OZTAS et al. Thirteen SCA patients in stable condition, ten carriers, and ten controls had their blood samples taken. The amounts of plasma protein carbonyl, total sulfhydryl, triglyceride, phospholipids, total cholesterol, and bilirubin were assessed using spectrophotometric methods. The amounts of copper, zinc, and iron in the plasma were measured using atomic absorption spectrophotometry. The hemolysate lipid extract's triglyceride, cholesterol, and ion levels were also assessed. The protein carbonyl levels in the patients were greater and the total sulfhydryl level was lower than in the controls Compared to controls, patients with SCA exhibited lower plasma cholesterol and higher triglyceride levels. Compared to carriers and controls, patients reported higher levels of hemolysate copper and plasma iron. In one investigation, the patients' plasma iron and hemolysate zinc levels were shown to be correlated with their plasma protein carbonyl levels. In the plasma and erythrocytes of individuals with steady-state sickle cell anemia, the researchers found alterations in proteins, lipids, and ions. A few of these modifications are linked to the oxidative stress present in the illness as well as to one another (26).

Mohamad Ali Mashhadi was looked into in 2012. In this study, 370 individuals with thalassemia major who were older than five were enrolled. Before the test, all patients were told to fast for twelve hours. In southeast Iran, observations were made using an atomic-absorption spectrophotometer. The study included 141 female participants and 192 male participants. Of the total, 49% were above the age of 15, 90 (27%) were between the ages of 5 and 10, and 80 (24%) were between the ages of 10 and 15. Dessferroxamine, deferasirox, and combinations of dessferroxamine and deferiprone were used as iron chelation treatments in 61.5% (204 patients), 24.2% (82 cases), and 14.3% (47 cases), respectively. Among the 370 patients, 153 (45.9%) had normal copper levels, 107 (32.1%) had copper overload, and 73 (21.9%) had copper deficit. He comes to the conclusion that, out of 333 individuals with thalassemia major, a significant proportion had copper deficiency, with about 50% of patients having normal copper levels. This study highlighted the need of micronutrient evaluation for treatment planning in thalassemic patients worldwide(27).

In 2014, Zahra Heidari and colleagues published their findings. In this study, 369 people with significant thalassemia for more than 5 years were included. In the south east of Iran, selenium levels were tested in all eligible individuals after 12 hours of fasting using graphite enstrum furnace atomic absorption spectrometry. 333 eligible individuals were assessed out of 369 cases. The average age was 15.637.4 years. One hundred ninety-two instances were male, while the remaining 141 cases were female. About 27% (90) of the cases were between the ages of 5 and 10, 24% (80) were between the ages of 10-15, and 49% were above the age of 15. Dessferrioxamine was the iron chelator in 62.2% of cases, Deferiprone in 15.5%, and a combination of the two in 22.3%. There were 85 instances (25.52%) with selenium shortage, 35.43% (118 cases) of normal levels, and 39% (130 cases) of excess selenium. The research of 333 significant thalassemia patients revealed that selenium levels ranged from deficient to greater than normal. It was not like other stories from throughout the world(28).

The aim of the 2015 study by Sadia SULTAN et al. was to determine the prevalence of zinc insufficiency in beta thalassemia major patients receiving desferrioxamine for iron chelation. Over the course of six months, a cross-sectional, prospective, descriptive research was conducted. 63 patients with beta thalassemia major who were between the ages of five and fifteen were included, and they had been on desferrioxamine for a minimum of a year. Atomic absorption spectrophotometry was used to assess the serum zinc levels, and the patients' mean age ranged from 5.15 to 10.84±3.47 years. There were 28 (44.4%) girls and 35 (55.6%) males among them. Zinc deficiency, defined as zinc levels less than 50 µg/dl, affected 22.2% of the population. Males who had been unwell for more than 10 years had greater proportions of deficit(29).

In a prospective study carried out by Ibrahim H. Bucak et al. in 2017, 35 patients with β-thalassemia were recruited, and they were regularly monitored at a tertiary university hospital. Studies discovered a strong relationship between VSCC and iron levels in β-thalassemia patients. In this study, the patients' skin tones darkened in correlation with increases in ferritin ($p < 0.001$) and iron (p ¼ 0.001). Furthermore, age ($p = 0.002$) and the number of transfusions received yearly ($p = 0.022$) were shown to be associated with darker skin tones. Our review of the literature revealed no previous studies using a VSCC to evaluate skin color and iron values in people with β-thalassemia(30).

In a case-control cross-sectional investigation, Zekavat et al. (2018) examined 43 TM patients and 43 age- and sex-matched healthy controls from Jahrom University of Medical Sciences (southern Iran). Individuals who had an enrollment at the Thalassemia Clinic. Zinc and copper levels in the serum were examined for each participant. Zinc and copper dietary intake was also assessed. While patients' mean copper levels were considerably greater than those of the control group, participants' median zinc levels were significantly lower than those of the latter. But the sick group's mean dietary consumption of zinc and copper was significantly lower than that of the control group. There was no significant difference in the mean blood levels of copper and zinc across the patient group based on the kind of iron chelation treatment. Furthermore, based on ferritin level, age, and length of therapy, the patient's group's zinc and copper levels were not statistically significant. In individuals with TM, essential trace element levels may fluctuate and deficiencies may arise essential trace element levels can vary in TM patients, and deficits can develop.(31).

Yasir Sharif and colleagues in 2019 In this research, 408 participants were included; 204 patients and the same number of controls were drawn from the Sundas Foundation Thalassemia and Blood Transfusion Center in Lahore, Pakistan. We used an atomic spectrometer to measure the amounts of copper and. β-thalassemia patients had an average copper level of 39.55 ± 8.766 µg/dL, compared to controls' 88.33 ± 8.633 µg/dL. typical level of selenium.86.80±8.601 µg/L was recorded in the controls; in the β-thalassemia. The values in patients were 39.18 ± 7.207 µg/L. Both β-thalassemia patients and controls showed a substantial positive association ($r = 0.89$, $p = 0.000$) between copper and selenium(32).

Ghone RA et al. (2020) conducted a research in the Department of Biochemistry, ACPM Medical College, Dhule, with 104 patients in total. comprising 52 previously diagnosed children with β-thalassemia (17 males and 35 females) and 52 age- and sex-matched healthy normal youngsters (28 males and 24 females). Every patient with thalassemia receives a daily oral dosage of deferiprone, an iron chelator. The youngsters were between the ages of 4 and 10 and reliant on blood transfusions. Using atomic absorption spectrophotometry, blood zinc and copper levels were measured. The results indicate that, in patients with beta-thalassemia major, serum zinc levels were much lower and copper levels were significantly higher than in healthy normal subjects before to supplementation(33).

Sudha K. et al. in 2022 The study's participants were split into two groups: group I consisted of 40 beta thalassemia T patients, while group II contained 40 age- and sex-matched normal persons. Hemoglobin variant analysis was performed using cation exchange HPLC. The spectrophotometric technique was used to measure the plasma iron, copper, ceruloplasmin, and SOD. Atomic absorption spectrometry was used to evaluate the zinc content, and ECLIA was used to measure the ferritin content. When beta thalassemia was compared to healthy controls, there was a noticeable drop in plasma trace elements. There was a statistically significant decline in copper ($p<0.04$) and zinc ($p<0.001$). BTT patients had significantly decreased levels of ferritin and SOD ($p<0.001$). On the other hand, ceruloplasmin levels appeared to be declining. In beta thalassemia patients, iron and ferritin had a strong positive association ($r=0.84$ $p=0.01$), while copper and ceruloplasmin exhibited a positive correlation ($r=0.92$ $p=0.001$). It's interesting to see that HbF had negative correlations with each of the three trace elements(34).

3. Discussion

In the current ten years, atomic absorption spectrometry has emerged as the most prevalent technique in the analytical sector. Atomic absorption spectrometry provides an effective and sensitive method for measuring all elements trace elements as Zinc , Toxic elements as lead and heavy metals in small biological samples. This approach has several advantages, but it also has some drawbacks, including the possibility of errors arising from sample digestion processes and spectroscopic issues during heavy metal analysis. These studies can shed insight on the role that trace elements play in oxidative stress in thalassemia patients and emphasize the need of routinely testing metalloenzymes. It was demonstrated that ferritin and iron were positively connected. Positive correlations were found between copper and zinc and all metalloproteins examined. Patients with thalassemia were shown to be deficient in zinc, copper, and other trace metals in every study.

In addition to implementing the detailed information for practical and effective management of trace element status in clinical diagnosis and healthcare situations, the previous review was updated to emphasize in detail the significance of knowing this technique and relation with trace elements thus far in human physiology and nutrition. Additionally, it used the WHO categorization system to classify trace elements as it had in the past. In this classification, the trace elements have been divided into three groups from the point of their nutritional significance in humans, as follows: essential elements; elements which are probably essential; and potentially toxic elements, some of which may nevertheless have some essential functions at even low levels. Homoeostasis for different trace elements is maintained by different mechanisms regulating absorption or excretion in response to changes in nutritional status(16). We note that most of the studies conducted using this technique measured the desired elements Study it using statistical programs for various tests to determine the differences in averages between the studied groups and whether statistical significance exists or not.

4. Conclusion

We've highlighted the importance of atomic absorption devices in aiding research across various fields. To further enhance the capabilities of atomic absorption devices, you've mentioned the need for ongoing development, specifically in terms of speed and the ability to detect more elements. It's clear that technological advancements in laboratory methods, particularly in atomic absorption techniques, play a crucial role in understanding the role of trace elements in health and disease. It's important to note that the detection and analysis of trace elements are essential for addressing deficiencies or intoxications, and the continuous refinement of laboratory methods contributes to the early identification of pathological consequences related to these elements.

In conclusion, our review emphasizes the significance of trace elements, the role of advanced techniques in detection, and the potential for further development in atomic absorption devices to enhance the speed and scope of trace element analysis in various scientific fields.

Compliance with ethical standards

Acknowledgments

Thanks the laboratories of the Faculty of Science of Kufa University, Iraq is especially appreciated by the authors for assisting with the completion of this work.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Leckenby J, Li H. Scholar (14). 1997.

[2] Mardan BR, Kadhim SA. comparative study a Copper and Zinc for colon cancer patients. Int J Health Sci (Qassim). 2022;(June):614–21.

[3] Harjan AH, Kadhim SA. Monitoring of cadmium and iron concentrations in healthy and prostate cancer patients in the Middle Euphrates Cancer Center, Najaf, Iraq. Int J Health Sci (Qassim). 2022;(October):2303–8.

[4] Alshebly SAK, Hussain HH, Trier SH, Kadhim BA. Serum levels of lead, cadmium and silver in patients with breast cancer compared with healthy females in Iraq. AIP Conf Proc. 2019;2086.

[5] Koirtyohann SR. A history of atomic absorption spectroscopy from an academic perspective. Anal Chem [Internet]. 1991 Nov 1;63(21):1024A-1031A. Available from: https://doi.org/10.1021/ac00021a001

[6] Neilson B, Rossiter N. Scholar (16). From Precarity to Precariousness and Back Again: Labour, Life and Unstable Networks. 2005. p. 5.

[7] Smith JC, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clin Chem. 1979;25(8):1487–91.

[8] Willis JB. The determination of metals in blood serum by atomic absorption spectroscopy-III. Sodium and potassium. Spectrochim Acta. 1960;16(5):551–8.

[9] Elements T, Christian GD. Medicine , and Atomic Absorption Spectroscopy.

[10] Ayres JS. The Biology of Physiological Health. Cell [Internet]. 2020;181(2):250–69. Available from: https://doi.org/10.1016/j.cell.2020.03.036

[11] Mungmunpuntipantip R, Wiwanitkit V. LIBS as a diagnostic tool in pediatrics beta-thalassemia. Lasers Med Sci. 2022;37(2):1371.

[12] Şahin A, Er EÖ, Öz E, Yıldırmak ZY, Bakırdere S. Sodium, Magnesium, Calcium, Manganese, Iron, Copper, and Zinc in Serums of Beta Thalassemia Major Patients. Biol Trace Elem Res. 2021;199(3):888–94.

[13] Kadhim KA, Baldawi KH, Lami FH. Prevalence, Incidence, Trend, and Complications of Thalassemia in Iraq. Hemoglobin [Internet]. 2017;41(3):164–8. Available from: https://doi.org/10.1080/03630269.2017.1354877

[14] Yalwa LM, Durumin-iya SG, Abdulhamid AM, Uzair M, Adamu SB, Garba I. INVESTIGATION OF HEAVY METALS CONTAMINANTION LEVEL IN THE LOCALLY CULTIVATED RICE USING ATOMIC ABSORPTION SPECTROSCOPY (AAS). 2023;18(3):356–60.

[15] Menzies AC. A Study of Atomic Absorption Spectroscopy. Anal Chem. 1960;32(8):898–904.

[16] Mohammad AR, Hussein HA, Kadhim SA, Hashoosh MH, Hassan ES. A Comparative Study of Lead and Cadmium Concentrations in Serum Samples of Diabetic Patients in Al-Najaf Governorate. Iran J War Public Heal. 2022;14(3):317–21.

[17] Hashoosh MH, Kadhim SA. Study of variation in zinc concentration of serum, nails and hair samples of diabetic patients in Iraq (Najaf). In: AIP Conference Proceedings. AIP Publishing; 2022.

[18] Hussein HH, Alsabari EK, Kadhim BA, Hatif KH, AL-Khafaji QS, Hamidi SAK. Study the Impact of the Trace Elements between the Healthy Females and who take Chemotherapy for samples of Sera. Res J Pharm Technol. 2017;10(10):3323–5.

[19] Ogunfowokan AO, Adekunle AS, Oyebode BA, Oyekunle JAO, Komolafe AO, Omoniyi-Esan GO. Determination of Heavy Metals in Urine of Patients and Tissue of Corpses by Atomic Absorption Spectroscopy. Chem Africa [Internet]. 2019;2(4):699–712. Available from: https://doi.org/10.1007/s42250-019-00073-y

[20] Uddin AH, Khalid RS, Alaama M, Abdualkader AM, Kasmuri A, Abbas SA. Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. J Anal Sci Technol [Internet]. 2016;7(1). Available from: http://dx.doi.org/10.1186/s40543-016-0085-6

[21] Willis JB. Determination of Lead and Other Heavy Metals in Urine by Atomic Absorption Spectroscopy. Anal Chem. 1962;34(6):614–7.

[22] Reader, T. W., Flin, R., Mearns, K., & Cuthbertson BH. Scholar (17). 2009.

[23] Roviyanti, Leckenby J, Li H, Negus K, Pickering M, Adorno T, et al. Scholar (18) [Internet]. Vol. 3, Why We Need the Journal of Interactive Advertising. 2011. p. 45. Available from: http://www.sciencedirect.com/science/article/pii/S0160738315000444

[24] Mahyar A, Ayazi P, Pahlevan AA, Mojabi H, Sehhat MR, Javadi A. Zinc and copper status in children with beta-thalassemia major. Iran J Pediatr. 2010;20(3):297–302.

[25] Keikhaei B, Badavi M, Pedram M, Zandian K, Rahim F. Serum zinc level in Thalassemia Major. Pakistan J Med Sci. 2010;26(4):942–5.

[26] Oztas Y, Durukan I, Unal S, Ozgunes N. Plasma protein oxidation is correlated positively with plasma iron levels and negatively with hemolysate zinc levels in sickle-cell anemia patients. Int J Lab Hematol. 2012;34(2):129–35.

[27] Mashhadi MA. Copper status in patients with thalassemia major in Zahedan, Iran. Int J Hematol Stem Cell Res. 2013;7(3):20–3.

[28] Mashhadi MA, Heidari Z, Sepehri Z, Bakhshipour AR, Karimkoshte O. The selenium status in thalassemia patients in south east of Iran. Int J Hematol Stem Cell Res. 2014;8(4):1–4.

[29] Sultan S, Irfan SM, Kakar J, Zeeshan R. Effect of iron chelator desferrioxamine on serum zinc levels in patients with beta thalassemia major. Malays J Pathol. 2015;37(1):35–8.

[30] Bucak IH, Almis H, Benli S, Turgut M. The Assessment of Skin Color and Iron Levels in Pediatric Patients with β-Thalassemia Major Using a Visual Skin Color Chart. Hemoglobin [Internet]. 2017;41(2):120–3. Available from: https://doi.org/10.1080/03630269.2017.1337033

[31] Zekavat OR, Bahmanjahromi A, Haghpanah S, Ebrahimi S, Cohan N. The zinc and copper levels in thalassemia major patients, receiving iron chelation therapy. J Pediatr Hematol Oncol. 2018;40(3):178–81.

[32] Sharif Y, Irshad S, Tariq MH, Qadeer MI, Sharif S. Correlation of serum trace elements (copper and selenium) in patients with beta thalassemia major in Pakistan . Trace Elem Electrolytes. 2020;37(01):17–22.

[33] A GR, S GS, S BS, C KA. Alteration in Serum Zinc and Copper Concentrations and Effect of Oral Therapeutic Supplementation of Zinc on Transfusion Dependant Beta Thalassemia Major Patients. Int J Clin Biomed Res. 2020;10–2.

[34] Sudha K, Prabhakaran N, Sowndarya K, Rao D. Metalloprotein status in Indian patients with beta thalassemia trait. Biomed. 2022;42(3):474–7.