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Mindray Semi-Auto Analyzer versus Atomic Absorption Spectrometer: A Comparative Study for Measuring Calcium, Magnesium, and Zinc in Iraqi Women with Osteoporosis

Zainab Hussein Kadhum, Saadiyah Ahmed Dhahir, Layla Othman Farhan*

Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq.

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Abstract

Osteoporosis, a progressive skeletal disorder characterized by low bone mass and deterioration of bone microarchitecture, increases the risk of fractures. Deficiencies in serum calcium (Ca), magnesium (Mg), and zinc (Zn) are key contributing factors. This study aimed to evaluate and compare the effectiveness of the Mindray Semi-Auto and Atomic Absorption Spectrometry (AAS) in determining serum concentrations of Ca, Mg, and Zn in Iraqi women with osteoporosis. A total of 120 serum samples were collected from Al-Wasiti Teaching Hospital and analysed at the College of Science for Women, University of Baghdad. Participants were divided into two groups: 80 patients with osteoporosis and 40 healthy controls, aged 48–56 years. Mindray Semi-Auto and AAS. The results for all osteoporosis patients ($\text{Ca}=7.40\pm0.557$ mg/dL, $\text{Mg}=1.82\pm0.153$ mg/dL, $\text{Zn}=0.073\pm0.0215$ mg/dL) were low compared to the healthy individuals (HI). The same samples analyzed by AAS yielded slightly lower values in osteoporotic patients ($\text{Ca}=4.54\pm1.049$ mg/dL, $\text{Mg}=1.04\pm0.211$ mg/dL, $\text{Zn}=0.21\pm0.033$ mg/dL). Analytical performance was assessed through relative standard deviation (RSD%) and recovery (Rec%) rates. The Mindray device showed an RSD of 2.68% and a recovery of 102.0%, while AAS showed superior precision (RSD: 0.99%) and recovery (99.2%). Both methods met acceptable recovery criteria (95–105%). In conclusion, the study confirmed that serum levels of calcium, magnesium, and zinc were significantly lower in osteoporotic women, highlighting the importance of early mineral assessment. Technically, AAS demonstrated higher precision and reproducibility compared to the Mindray Semi-Auto analyser, indicating its superiority as a reference method.

Keywords: Osteoporosis, Calcium, Magnesium, Zinc, Mindray Semi-Auto and AAS

جهاز التحليل شبه الآلي من مايندراي مقابل مطياف الامتصاص الذري: دراسة مقارنة لقياس الكالسيوم والمغنيسيوم والزنك لدى النساء العراقيات المصابات بهشاشة العظام

زينب حسين كاظم، سعدية أحمد ظاهر، ليلى عثمان فرحان*

*قسم الكيمياء، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.

الخلاصة

*Email: laylaof_chem@cs.w.uobaghdad.edu.iq

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هشاشة العظام، اضطراب هيكلي تدريجي يتميز بانخفاض كتلة العظام وتدهور بنيتها الدقيقة، يزيد من خطر الإصابة بالكسور. يُعد نقص الكالسيوم (Ca) والمغنيسيوم (Mg) والزنك (Zn) في المصل من العوامل الرئيسية المساهمة. هدفت هذه الدراسة إلى تقييم ومقارنة فعالية جهاز Mindray شبه الآلي ومطيافية الامتصاص الذري (AAS) في تحديد تركيزات الكالسيوم والمغنيسيوم والزنك في مصل النساء العراقيات المصابات بهشاشة العظام. جُمعت 120 عينة مصل من مستشفى الواسطي التعليمي، وُحلت في كلية العلوم للنبات بجامعة بغداد. قُسمت المشاركات إلى مجموعتين: 80 مريضة مصابة بهشاشة العظام و 40 امرأة سليمة، تتراوح أعمارهن بين 48 و 56 عامًا. جُهاز Mindray شبه الآلي ومطيافية الامتصاص الذري. وكانت النتائج لجميع مرضى هشاشة العظام (كالسيوم = 0.557 ± 7.40 ملغ / ديسيلتر، ملغ = 1.82 ± 0.153 ملغ / ديسيلتر، زنك = 0.0215 ± 0.073 ملغ / ديسيلتر) منخفضة مقارنة بالأفراد الأصحاء (HI). أظهرت نفس العينات التي تم تحليلها بواسطة AAS قيمًا أقل قليلًا لدى مرضى هشاشة العظام ($Ca = 4.54 \pm 1.049$ ملغ / ديسيلتر، $Mg = 1.04 \pm 0.211$ ملغ / ديسيلتر، $Zn = 0.21 \pm 0.033$ ملغ / ديسيلتر). تم تقييم الأداء التحليلي من خلال معدلات الانحراف المعياري النسبي (RSD) ومعدلات الاسترداد (Rec%). أظهر جهاز Mindray انحراف معياري نسبي بنسبة 2.68% واسترداد بنسبة 102.0%، بينما أظهر AAS دقة فائقة (RSD: 0.99%) واسترداد (99.2%). استوفت كلتا الطريقتين معايير الاسترداد المقبولة (95-105%). وفي الختام، أكدت الدراسة أن مستويات الكالسيوم والمغنيسيوم والزنك في المصل كانت أقل بكثير لدى النساء المصابات بهشاشة العظام، مما يسلط الضوء على أهمية التقييم المبكر للمعادن. من الناحية الفنية، أظهر AAS دقة أعلى وقابلية إعادة إنتاج مقارنة بجهاز التحليل شبه التلقائي Mindray، مما يشير إلى تفوقه كطريقة مرجعية.

1. Introduction

Osteoporosis is an aging related, systemic skeletal disorder where bone density declines and there is a subsequent increase in fracture risk, and systemic complications of the skeleton [1-3]. The bone weakening process continues over time in this progressive condition until the bone becomes so weak that a fracture can occur with minimal stress [1-3]. Osteoporosis is most often caused by a natural drop in estrogen, that is normally secreted by the ovaries, after the age of 35-40, but will also result from surgically removing the ovaries (oophorectomy), causing the rapid bone loss after menopause [4-6]. Bone Mineral Density (BMD) is typically assessed by using a central dual energy X-ray absorptiometry (DXA) measurement to diagnose osteoporosis [7]. BMD is considered the gold standard for detecting both osteopenia and osteoporosis because DXA measures the actual mineral content of bone tissue [8]. A major factor leading to osteoporosis is low calcium levels in the body [9]. Low levels of zinc, an essential trace element with antioxidant properties, are associated with decreased immune response, increased inflammation, and ultimately bone damage [10]. Trace minerals including copper, selenium, and manganese, support the structure of bones through their involvement in collagen synthesis, antioxidant activity, and bone matrix formation [41]. Magnesium is an essential mineral for many of the physiological functions of the body and a direct correlation has been identified between magnesium intake and bone density [11]. Modern advances in clinical laboratory technology enable multiple analytical instruments to be housed in a single clinical laboratory setting [12]. The Mindray Automated Clinical Chemistry and Immunoassay System is a recent innovation in clinical chemistry and immunoassay systems developed to meet the demands of high throughput testing for the determination of a wide variety of biomarkers including liver and kidney function tests, electrolyte panels, cardiac marker panels and hormone panels [13]. Atomic Absorption Spectrometry (AAS) is one of the most commonly employed elemental analysis techniques in all types of clinical, pharmaceutical, environmental and industrial laboratories, especially in its flame (FAAS) and electrothermal (ETAAS) modes [14]. Based on the studies of Schulz et al., no statistical difference was noted in serum concentrations of Ca,

Mg and Zn between patients diagnosed with osteoporosis and control subjects, regardless of whether the measurements were obtained with either the Mindray Semi-Auto Analyzer or by AAS [15]. On the other hand, Côté et al. propose an alternative hypothesis that suggests that both the biological and instrumental factors may cause significant variations in serum mineral concentrations between osteoporotic and non-osteoporotic patients and that variations in methodologies, i.e., the colorimetric method and AAS, may affect sensitivity and precision and thus impact clinical interpretation [13]. The main goal of this study is to compare the accuracy and sensitivity of two analytical techniques, the Mindray Semi-Auto Chemistry Analyser and Atomic Absorption Spectroscopy (AAS) for measuring these elements.

2. Materials and Methods:

The study included serum samples taken from the College of Science for Women, University of Baghdad, along with Al-Wasiti Teaching Hospital. The 120 serum samples collected from 120 female participants aged between 48 and 56 years old were made up of 80 women who had been clinically diagnosed with osteoporosis and 40 healthy women. From each participant 5 mL of blood was collected in a tube containing gel activator, allowed to coagulate and then spun at 3000 r.p.m./min for 10 min. Sera was isolated from the blood after spinning and placed at -20°C until the time that they were to be tested. Concentrations of Ca, Mg and Zn in sera were determined by measuring the concentration of these elements in sera using the Mindray BA-88A Semi-Auto Chemistry Analyser (Mindray, China). The method of analysis used by this instrument was based upon the principle of colorimetry, using assay kits for Ca and Mg supplied by Biosystems (Spain) and an assay kit for Zn supplied by Spectrum Diagnostics (Egypt). In addition, all samples were run through the Mindray Semi-Auto analyser after running internal quality control samples prior to each sample run to test the analytical precision of the semi-auto analyser. Each of the same samples was also analyzed by Atomic Absorption Spectroscopy (AAS) using a Shimadzu AA-7000 Spectrometer (Japan), with an air-acetylene flame. The Mindray Semi-Auto analyzer was chosen as the primary analyzer due to the fact that it is commonly employed in clinical laboratories for routine biochemical testing, whereas AAS was chosen as the reference "gold standard" because of its superior sensitivity and specificity. Calibration standards for Ca, Mg and Zn were prepared from certified reference materials, prior to analyzing the samples by AAS. The analytical wavelength for Ca was 422.7 nm; Mg was 285.2 nm; and Zn was 213.9 nm. The samples were diluted 1:10 with deionised water prior to analysis. A five-point calibration curve using certified standards was applied, and detection limits were 0.01 mg/dL Ca, 0.005 mg/dL Mg, and 0.002 mg/dL Zn.

3. Statistical Analysis

Statistical analysis was performed using SPSS (Version 26; SPSS Inc., Chicago, U.S.A.), and continuous data are expressed as mean $\pm$ standard deviation (SD). To evaluate the relationships between variables, the Pearson correlation coefficient (r) was calculated, and the statistical significance of r was tested. Differences between independent groups were analysed using appropriate statistical tests, including the independent-samples t -test and one-way depending on the number of groups compared. Agreement between the quantitative measurements obtained by the Mindray Semi-Auto and the AAS methods for Ca, Mg, and Zn was assessed using the Bland–Altman analysis. A p -value < 0.05 was considered statistically significant, while $p > 0.05$ was regarded as non-significant.

4. Results and Discussion

4.1 Results

Table 1 indicates that there is no significant age difference between the patients and HI groups, with ages ranging from 48 to 68 years ($p=0.358$). However, both the T-score, and Z-score differ

significantly between patients and HI groups with ($p=0.001$). The Z-score reflects the deviation of bone density deviates from the average range for individuals of the same age and sex, and the results reveal a clear reduction in bone density among osteoporosis patients compared to HI, indicating a substantial difference between the two groups. The diagnosis of osteoporosis in the first group relative to the healthy group is confirmed by the T-score, which quantifies the degree to which bone density deviates from the ideal density of young, healthy bones and likewise shows a substantial, significant difference.

Table 1: Comparison of age and bone density scores between osteoporosis patients and HI*.

Variables	Osteoporosis patients	Healthy individuals	P- value
	(N=80)	(N=40)	
Age(year)	52 ± 1.948	51.98 ± 2.178	0.358
z-scor	-1.155 ± 0.8510	0.480 ± 0.7198	0.001
T-scor	-2.255 ± 0.7388	-0.217 ± 1.0345	0.001

*HI: Healthy individual

Accuracy and Precision

Table 2. presents the accuracy, precision, and recovery performance of the Mindray Semi-Auto method for detecting standard solutions, with each standard solutions for Ca, Mg, and Zn were measured 5 times. The Table includes various analytical validation parameters such as mean $\pm$ standard deviation (SD), relative standard deviation (RSD%), relative error (Erel%), recovery percentage (Rec %), average recovery (Av Rec %), and 95% confidence intervals.

The Ca concentrations tested were 4, 6, and 8 mg/dL recoveries ranged from 103.45% to 104.99%, with a mean of 104.4%, indicating a relatively high accuracy, although there was a slight tendency for over-measurement. RSD% values ranged from 2.77% to 3.76%, indicating acceptable repeatability. Confidence intervals were narrow (e.g., 4.14 ± 0.001), indicating reliable measurements. Mg concentrations tested were 1.5, 1.7, and 1.9 mg/dL Recovery rates showed excellent values ranging from 98.66% to 100.52%, with an average of 99.7%, reflecting very high accuracy. Very low RSD values were observed (between 0.35% and 1.50%), confirming the excellent repeatability of measurements. Zn concentrations tested were 0.08, 0.09, and 0.1 mg/dL. Recovery rates were near perfect, recording 100 % and 105%, with an average of 102 % RSD% was also low (1.23–2.46%), reflecting the accuracy and consistency of the results A highly neutral, semi-precise Mindray method for measuring Ca, Mg, and Zn elements was developed, achieving excellent performance and recovery with good specificity, which led to the practice of accurate laboratory analyses of these elements.

Table 2: The Precision and accuracy of all metal (Ca, Mg and Zn) mg/dl by the Mindray Semi- Auto method(n=5).

Metal	Concentration mg/dl		Mean $\pm$ SD*	RSD% (N =5)	Erel %	Rec %	Av Rec %	Confidence intervals (95%)
	Taken	Found						
Ca	4	4.14	4.14 ± 0.0079	3.76	3.5	103.45	104.4	4.14 ± 0.001
	6	6.29	6.29 ± 0.0088	2.77	4.8	104.90		6.29 ± 0.011
	8	8.40	8.40 ± 0.0143	3.40	5	104.99		8.40 ± 0.018
Mg	1.5	1.48	1.48 ± 0.0056	1.50	-1.3	98.66	99.7	1.48 ± 0.007
	1.7	1.70	1.70 ± 0.0015	0.35	0	100		1.70 ± 0.002
	1.9	1.91	1.91 ± 0.0059	1.23	0.5	100.52		1.91 ± 0.008
Zn	0.08	0.084	0.084 ± 0.0257	5.94	5	105	102	0.084 ± 0.032
	0.09	0.091	0.091 ± 0.0112	2.46	1.1	101		0.091 ± 0.014
	0.1	0.1	0.1 ± 0.0075	1.45	0	100		0.1 ± 0.009

*SD: standard deviation

Table 3. illustrates the accuracy, precision, and recovery performance of the AAS method in detecting standard solutions, where the standard solutions were repeated 5 times for Ca , Mg and Zn .

Ca Concentrations tested were 4, 6, and 8 mg/dL. Recoveries ranged from 97 % to 97 %, with a mean of 97.7%, indicating a relatively high accuracy, although there was a slight tendency for over-measurement. RSD% values ranged from 0.62% to 0.26%, indicating acceptable repeatability. Confidence intervals were narrow (e.g., 1.94 ± 0.0005), indicating reliable measurements. Mg Concentrations tested were 0.4, 0.6, and 0.8 mg/dL Recovery rates showed excellent values ranging from 103 % to 99 %, with an average of 101%, reflecting very high accuracy. Very low RSD values were observed (between 1.08% and 0.39%), confirming the excellent repeatability of measurements. Zn Concentrations tested were 0.4, 0.6, and 0.8 mg/dL. The recovery rates for the three metals were all very good, ranging from a best of 95-100 percent and averaging 99.6 percent; similarly, the RSD values were quite small, at 1.41-1.83 percent, demonstrating that the data collected by this study are very reliable and consistent. Variability in recovery and precision of these three metals can be due to the physical/chemical properties of each metal, the effect of the matrix of the sample on each element's concentration determination, and the unique sensitivity of each metal under the specific conditions of the AAS analysis including the flame temperature, atomization efficiency, and wavelength selected. Additionally, the sample dilution factor (1:10) and potential adsorption or loss during handling may contribute to minor deviations. Overall, the AAS method demonstrated excellent performance in terms of accuracy, precision, and repeatability, making it a robust and reliable technique for the quantitative determination of Ca, Mg, and Zn in biological samples.

Table 3: The Precision and accuracy of all metal (Ca, Mg and Zn) mg/dl by the AAS method(n=5) .

Metal	Concentration mg/dl		Mean $\pm$ SD	RSD% (N =5)	Erel %	Rec %	Av Rec %	Confidence intervals (95%)
	Taken	Found						
Ca	2	1.94	1.94 ± 0.00038	0.62	-3	97	97.7	1.94 ± 0.0005
	3	2.98	2.98 ± 0.00021	0.22	-0.7	99		2.98 ± 0.0003
	4	3.88	3.88 ± 0.00032	0.26	-3	97		3.88 ± 0.0004
Mg	0.4	0.413	0.413 ± 0.0018	1.08	3.3	103	101	0.413 ± 0.0023
	0.6	0.609	0.609 ± 0.0022	0.87	1.5	101		0.609 ± 0.0027
	0.8	0.794	0.794 ± 0.0013	0.39	-0.8	99		0.794 ± 0.0016
Zn	0.4	0.38	0.38 ± 0.00055	1.41	-5	95	99.6	0.38 ± 0.0007
	0.6	0.60	0.60 ± 0.00128	2.24	0	100		0.60 ± 0.0015
	0.8	0.80	0.80 ± 0.00142	1.83	0	100		0.80 ± 0.0018

Table 4. illustrates the accuracy, precision, and recovery performance of the Mindray Semi-Auto method in detecting Where the patents Serum were repeated 3 times for Ca , Mg and Zn .

The tested concentrations showed very close measured means to the expected values Ca 7.37 ± 0.153 , 6.77 ± 0.153 , 7.33 ± 0.252 . The recovery percentages ranged from 99.5% to 105%, with an average of approximately 101.5%, indicating good accuracy with a slight positive bias. RSD% values ranged from 2.07% to 3.43%, suggesting acceptable precision. For Mg, the measured means closely matched the added concentrations, with values of 1.77 ± 0.025 ,

1.61±0.017, and 1.85±0.036. Recovery rates ranged between 95.8% and 103%, averaging approximately 100.3%, showing reliable accuracy. RSD% ranged from 1.2% to 2.2%, reflecting good precision. For Zn, tested concentrations yielded results of 0.075±0.04, 0.057±0.075, and 0.062±0.054. Recovery percentages were between 99.5% and 101.9%, with an average of 100.4%, demonstrating high accuracy. RSD% values ranged from 0.54% to 2.47%, reflecting very good precision. The Mindray Semi-Auto method has demonstrated high efficiency in measuring Ca, Mg and Zn in serum, achieving excellent accuracy and reproducible results, proving its reliability in clinical and diagnostic applications.

Table 4: The Precision and accuracy Assessment of patients Serum Ca, Mg and Zn Measurements Using Mindray Semi-Automated Analyzer.

Metal	Mean±SD	RSD% (N=5)	Erel %	Rec %
Ca	7.37±0.153	2.07	5.2	105
	6.77±0.153	2.26	-0.4	99.5
	7.33±0.252	3.43	0.4	100
Mg	1.77±0.025	1.4	3.5	103
	1.61±0.017	1.1	-4.3	95.8
	1.85±0.036	1.9	2.2	102
Zn	0.075±0.000404	0.54	1.4	101.9
	0.057±0.000751	1.30	-0.02	99.5
	0.062±0.0015	2.47	1.61	101

Table 5. illustrates the accuracy, precision, and recovery performance of the AAS method in detecting where the patients' Serum was repeated 3 times for Ca ,Mg and Zn.

The tested concentrations showed very close measured means to the expected values Ca 3.6079±0.00015, 5.7824±0.0004, 2.9760±0.0001. The recovery percentages ranged from 100% to 100%, indicating good accuracy with a slight positive bias. RSD% values ranged from 0.0042% to 0.003%, suggesting acceptable precision. For Mg, the measured means were consistent with the added concentrations 0.9462±0.00013, 1.1956±0.00037, 1.1956±0.00037. Recovery values ranged between 99.9% and 101.3%, showing reliable accuracy. RSD% ranged from 0.014% to 0.921%, indicating good precision. For Zn, tested concentrations yielded results of 0.2095±0.00015, 0.1871±0.00021, and 0.1991±0.00015. Recovery percentages were between 99.9% and 99.8%, demonstrating high accuracy. RSD% values ranged from 0.0729% to 0.0767%, reflecting very good precision. The results demonstrate that the AAS analysis is highly precise and reproducible, with RSD values (0.003-0.9%) being extremely low. All values were rechecked to confirm accuracy. The AAS method shows high efficiency in measuring Ca, Mg, and Zn concentrations in serum, providing perfect accuracy, excellent repeatability, and near-perfect recovery, confirming its high reliability in clinical analyses.

Table 5: The Precision and accuracy Assessment of patients Serum Ca, Mg, and Zn Measurements Using AAS .

Metal	Mean $\pm$ SD	RSD% (N =5)	Erel %	Rec %
Ca	3.6079 $\pm$ 0.00015	0.0042	0	100
	5.7824 $\pm$ 0.0004	0.007	0.003	100
	2.9760 $\pm$ 0.0001	0.003	0.003	100
Mg	0.9462 $\pm$ 0.00013	0.014	-0.06	99.9
	1.1956 $\pm$ 0.00037	0.031	-0.03	99.9
	0.4168 $\pm$ 0.00384	0.921	1.3	101.3
Zn	0.2095 $\pm$ 0.00015	0.0729	-0.004	99.9
	0.1871 $\pm$ 0.00021	0.1113	-0.001	99.8
	0.1991 $\pm$ 0.00015	0.0767	-0.001	99.8

Table 6 presents the statistical analysis of the serum concentrations of Ca , Mg, and Zn in two groups: osteoporosis patients (n=80) and healthy individuals (n=40). These mineral levels were measured using the Mindray Semi-Auto biochemical analyzer for minerals. The results demonstrated highly significant differences ($p<0.001$) between the two groups (osteoporosis and healthy individuals) for all measured parameters in serum Ca, Mg, and Zn .

Table 6: Statistical comparison in Mindray Semi- Auto of serum Ca ,Mg and Zn, levels between osteoporosis patients and healthy individuals.

Variables	Osteoporosis patient Mean $\pm$ SD (N=80)	Healthy individuals Mean $\pm$ SD (N=40)	p-value
Ca mg/dl	7.40 $\pm$ 0.557	8.530 $\pm$ 0.460	0.001
Mg mg/dl	1.82 $\pm$ 0.153	1.940 $\pm$ 0.110	0.001
Zn mg/dl	0.073 $\pm$ 0.0215	0.088 $\pm$ 0.0133	0.001

Table 7 presents the statistical analysis of the serum concentrations of Ca, Mg and Zn. Measurements were conducted using the (AAS). The results demonstrated highly significant differences ($p<0.001$) between the two groups (osteoporosis and healthy individuals) for all measured parameters in serum Ca, Mg, and Zn.

Table 7: Statistical comparison in method 2 of serum (Ca ,Mg ,and Zn) levels, AAS between osteoporosis patients and healthy individuals.

Variables	Osteoporosis patient Mean $\pm$ SD (N=80)	Healthy individuals Mean $\pm$ SD (N=40)	p-value
Ca mg/dl	4.54 $\pm$ 1.049	11.059 $\pm$ 1.996	0.001
Mg mg/dl	1.04 $\pm$ 0.211	0.932 $\pm$ 0.194	0.005
Zn mg/dl	0.21 $\pm$ 0.033	0.288 $\pm$ 0.781	0.001

Table 8 presents a statistical comparison of serum levels of Ca, Mg and Zn in osteoporosis patients, as measured by two different analytical techniques: the Mindray Semi-Auto device and AAS. The comparison includes mean $\pm$ SD, F and T values, p-values, mean differences, and the 95% confidence intervals of the differences. Ca: The mean Ca level measured by the Mindray device was (7.40 ± 0.557 mg/dl), while the AAS method reported a significantly lower value (4.54 ± 1.049 mg/dl). This difference was statistically significant ($P = 0.001$), with a mean difference of 2.864 and a 95% confidence interval ranging from 2.602 to 3.127. This indicates that Ca levels appear lower when measured by AAS compared to Mindray. Mg: The Mg concentration was also significantly different between the two methods. The Mindray device reported (1.82 ± 0.153 mg/dl), while AAS showed (1.04 ± 0.211 mg/dl), with a statistically significant difference ($P = 0.000$), suggesting methodological variation in Mg detection. Zn: A highly notable discrepancy was observed in Zn measurements. The Mindray device showed (0.073 ± 0.0125 mg/dl), whereas AAS reported only (0.21 ± 0.033 mg/dl), with a statistically significant mean difference of -0.136 ($P = 0.000$). These differences could be due to the variation in analytical principles. The Mindray system uses colorimetric reactions, which are more susceptible to interference from components in the serum matrix, whereas AAS measures elemental concentrations directly through light absorption, offering higher specificity but increased sensitivity to dilution, flame stability, and trace-level detection limits.

Table 8: Statistical comparison of biochemical analysis in osteoporosis patients results between the Mindray Semi- Auto and AAS.

Variables	Mindray Semi-Auto	AAS	F	P-value	T	Mean Difference	95% Confidence Interval of the Difference	
	Mean $\pm$ SD	Mean $\pm$ SD					Upper	Lower
Ca mg/dl	7.40 $\pm$ 0.557	4.54 $\pm$ 1.049	44.846	0.001	21.572	2.864	3.127	2.602
Mg mg/dl	1.82 $\pm$ 0.153	1.04 $\pm$ 0.211	7.882	0.001	26.821	0.780	0.838	0.723
Zn mg/dl	0.073 $\pm$ 0.0215	0.21 $\pm$ 0.033	41.71	0.001	-34.49	-0.136	-0.126	-0.141

Table 9 provides a statistical comparison of serum Ca, Mg and Zn levels in healthy individuals, as measured by two different analytical techniques: the Mindray Semi-Auto system and AAS. The data includes the mean $\pm$ standard deviation (SD), F and t-test values, p-values, mean differences, and the 95% confidence intervals. Ca: The Mindray system recorded a mean of (8.53 ± 0.461 mg/dl), while the AAS method showed a significantly higher value (11.06 ± 1.996 mg/dl), with a statistically significant difference ($P = 0.000$). The mean difference was -2.529, with a 95% confidence interval from (-3.174 to -1.884). Mg: A significant difference was noted between the two methods: Mindray measured (1.94 ± 0.111 mg/dl) versus (0.93 ± 0.195 mg/dl) with AAS, with a significant p-value ($P = 0.001$). Zn: There was a substantial difference between methods, as Mindray reported (0.088 ± 0.0133 mg/dl), while AAS measured a higher concentration of (0.29 ± 0.078 mg/dl), again with a highly significant p-value ($P = 0.001$).

Table 9: Statistical comparison of biochemical analysis normal results between the Mindray Semi- Auto and AAS.

Variables	Mindray Semi-Auto	AAS	F	P-value	T	Mean Difference	95% Confidence Interval of the Difference	
	Mean $\pm$ SD	Mean $\pm$ SD					Upper	Lower
Ca mg/dl	8.53 $\pm$ 0.461	11.06 $\pm$ 1.996	16.440	0.001	-7.807	-2.529	-1.884	-3.174
Mg mg/dl	1.94 $\pm$ 0.111	0.93 $\pm$ 0.195	2.336	0.001	28.462	1.009	1.079	0.938
Zn mg/dl	0.088 $\pm$ 0.0133	0.29 $\pm$ 0.078	81.22	0.001	-15.8	-0.199	-0.174	-0.224

Figure 1 shows the Bland-Altman plot for comparing two methods (Method_1 (Mindray Semi-Auto) and Method_2 (AAS)) in measuring Ca , Mg and Zn for the patient group. Ca is indicated by the **a**. With limits of agreement ranging from 6.11% (95% CI: -2.34% to 14.56%) to 92.88% (95% CI: 84.42% to 101.33%), the data show a mean difference of 49.49% (95% CI: 44.57% to 54.42%, $p < 0.0001$). $y = 203.43 - 25.77x$ is the regression equation (both the intercept and the slope have $p < 0.0001$). **b** represents Mg. With limits of agreement ranging from 11.67% (95% CI: 3.06% to 20.28%) to 100.08% (95% CI: 91.47% to 108.69%), the results show a mean difference of 55.87% (95% CI: 50.85% to 60.89%, $p < 0.0001$). $y = 188.81 - 92.76x$ is the regression equation (both the intercept and the slope have $p < 0.0001$). Zn is indicated by **c**. With limits of agreement ranging from 198.35% (95% CI: 198.25% to 198.44%) to 199.34% (95% CI: 199.24% to 199.44%), the results show a mean difference of 198.84% (95% CI: 198.78% to 198.90%, $p < 0.0001$). $y = 197.8156 + 0.02796x$ is the regression equation ($p < 0.0001$ for both the intercept and slope). Concerns regarding the two approaches' interchangeability in clinical evaluations are raised by these results, which point to a statistically significant difference between them as well as significant variation in their agreement.

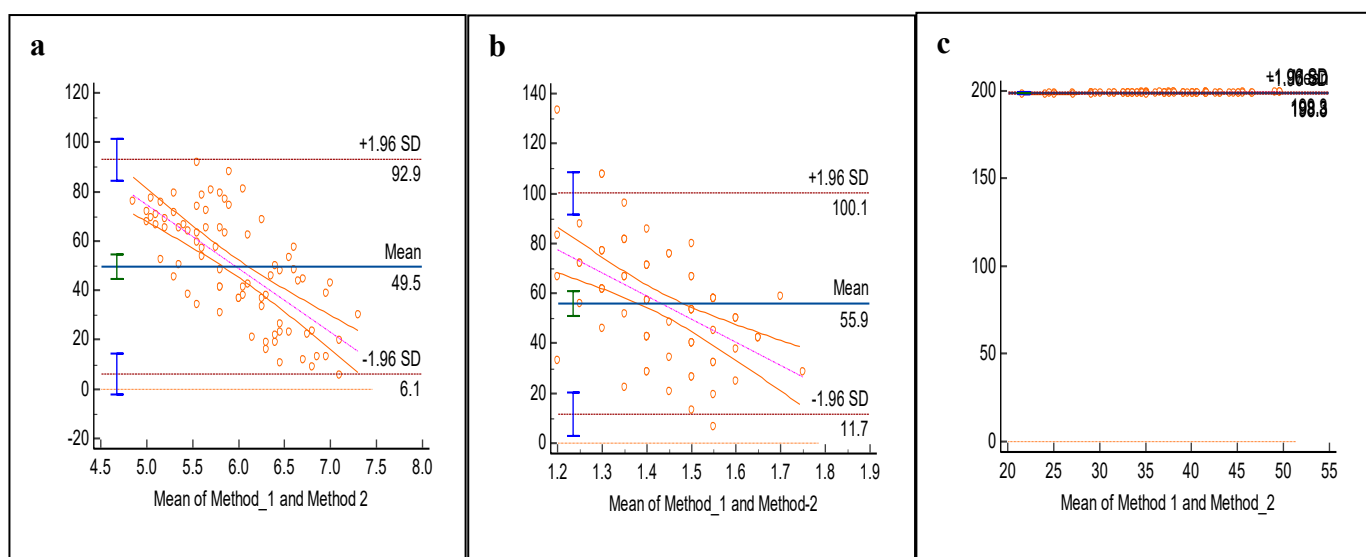
**Figure 1:** Bland-Altman plot for comparing two methods (Method_1 Mindray Semi- Auto and Method_2 AAS) in measuring Ca , Mg and Zn for patient.

Figure 2 shows the Bland-Altman plot for comparing two methods (Method_1 (Mindray Semi-Auto) and Method_2 (AAS)) in measuring Ca, Mg and Zn for healthy individuals' group. Ca is indicated by the **d**. The mean difference and the bounds of agreement between the two approaches are shown in the graph. With limits of agreement ranging from -57.30% (95% CI: -66.55% to -48.05%) to 8.48% (95% CI: -0.77% to 17.73%), the results show a mean difference of -24.41% (95% CI: -29.78% to -19.04%, $p < 0.0001$). $y = 118.4763 - 14.5887x$ is the regression equation ($p < 0.0001$ for both the intercept and slope). **e** stands for Mg. With limits of agreement ranging from 36.12% (95% CI: 26.30% to 45.94%) to 105.95% (95% CI: 96.13% to 115.77%), the results show a mean difference of 71.03% (95% CI: 65.34% to 76.73%, $p < 0.0001$). $y = 238.4622 - 116.5243x$ is the regression equation ($p < 0.0001$ for both the intercept and slope). **f** shows Zn. With limits of agreement ranging from 197.92% (95% CI: 197.70% to 198.13%) to 199.45% (95% CI: 199.24% to 199.67%), the results show a mean difference of 198.69% (95% CI: 198.56% to 198.81%, $p < 0.0001$), ($y = 197.9030 + 0.01765x$) is the regression equation ($p < 0.0001$ for the intercept and $p = 0.0583$ for the slope). Concerns regarding the two approaches' interchangeability in clinical evaluations are raised by these results, which point to a statistically significant difference between them, as well as significant variation in their agreement.

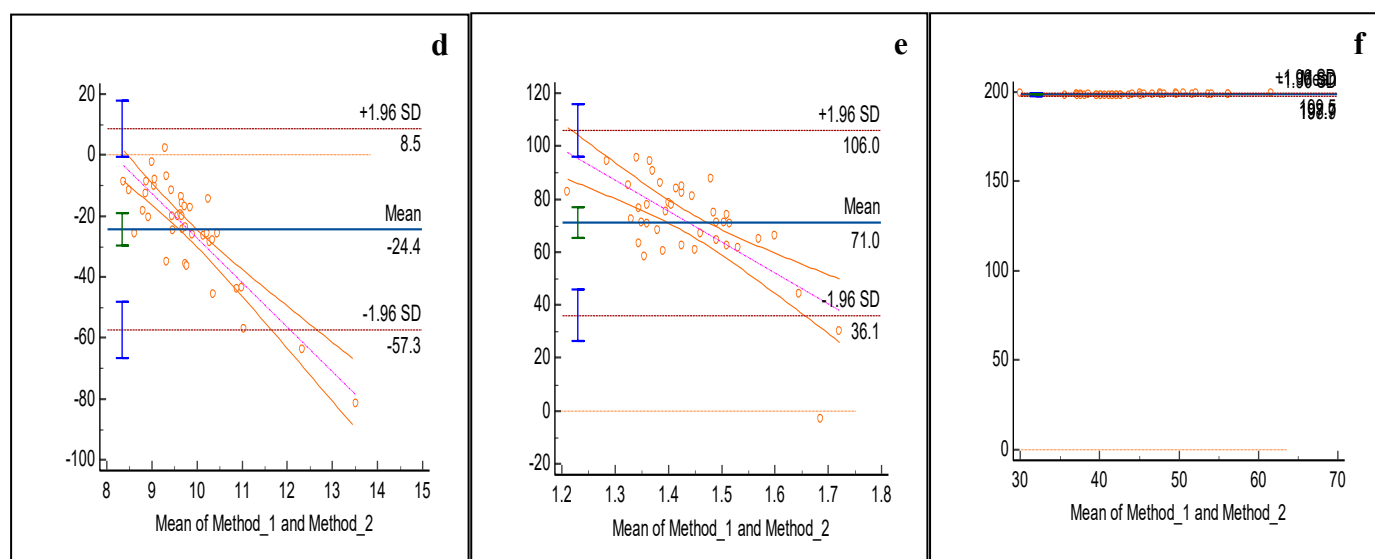


Figure 2. Bland-Altman plot for comparing two methods (Method_1 Mindray Semi- Auto and Method_2 AAS) in measuring Ca , Mg and Zn for

4.2 Discussion

Table 6 highlights the crucial role of Ca in Ca receptors in regulating bone formation and mineralization dietary or supplemental calcium intake is insufficient, the body compensates by extracting calcium from the bones to maintain stable blood calcium levels, which contributes to increased bone fragility in osteoporosis patients. [16,17]. One of the most important causes of Ca deficiency in the body is low blood Ca levels, which stimulates a series of physiological responses, such as the secretion of parathyroid hormone (PTH). The goal of this system is to raise blood Ca levels by removing Ca from the bones, sequestering it in the kidneys, and increasing its absorption from the intestine. However, with continued deficiency, it can cause chronic health problems such as osteoporosis and muscle weakness[18]. For healthy bones, Mg is a necessary mineral. Although the skeleton contains 50–60% of the body's Mg and skeletal

Mg indicates Mg status, osteoporosis has been linked to low, normal, or high bone Mg content[19,20]. Because it helps regulate Ca balance within the bones. A large percentage of Mg is found in the bones, where it plays a role in bone stability and Ca absorption. Our findings suggest Mg deficit in osteoporosis patients is caused by elements that can impair the bones' capacity to efficiently store Ca, which can result in osteoporosis and a higher risk of fractures[21,22]. A notable decline in serum and lymphocyte Mg concentrations, along with a significant drop in bone mineral density in the lumbar spine and proximal femur, are the hallmarks of primary hypomagnesemia brought on by renal Mg loss. According to these findings, hypomagnesemia might be linked to a reduction in bone mass[23]. The function and proliferation of cells have been linked to Mg, according to studies. If Mg is absent from outside the cell, it will be unable to continue dividing as its ability to make DNA, RNA and proteins will be greatly decreased [24]. One of the most recent research studies indicated that Mg concentration outside the cell impacts the differentiation of the malignant cell line [25]. Although Mg is an important source of nutrition to all forms of life, it is a necessary component to all living organisms and acts as a key cofactor to many different enzymes; this includes DNA and RNA polymerase. On average the human body contains 1.5 – 3 grams of Zn, however, only .01% of that is lost every day, which means humans must obtain the rest through diet. There are several studies that confirm a link between Zn deficiency and delayed bone development, dermatitis, anemia and compromised immune systems [26, 27]. Numerous studies have also found that there is a direct correlation between Zn deficiency and bone loss due to osteoporosis. With the inability to create new bone and a higher likelihood of fractures, this may be one of the reasons why Zn deficiency leads to osteoporosis. Additionally, Zn influences the amount of hormones present in the body that regulate bone health (growth hormone and PTH), and a deficiency in Zn can cause unfavorable changes in those hormones and ultimately contribute to osteoporosis [28]. Zn may work in conjunction with other minerals, such as Ca and Mg, to improve bone health. Although Zn is not a substitute for Ca in bone building, it may enhance the effects of Ca on bones. Studies suggest that Zn deficiency may affect copper levels, another mineral essential for bone health. Therefore, mineral balance is vital. All of these reasons make Zn an important and vital element in the human body [29].

Table 7 shows a significant deficiency of Ca, Mg and Zn in patients with osteoporosis when measured using the SAA device. Similar deficiencies were observed with the Mindray Semi-Auto device, however, the differences between patients and healthy individuals were slight with Mindray, whereas the AAS device revealed a more pronounced difference. As previously mentioned, one of the causes of bone diseases, including osteoporosis, is a Ca deficiency, as it plays a vital role in the body. When Ca levels decrease, the body may begin to withdraw Ca from the bones to maintain its other functions, leading to a deterioration in bone health. Here, we must maintain Ca levels through diet (such as milk, yogurt, cheese, leafy vegetables, and fatty fish) or nutritional supplements if necessary [30,31]. Mg is an essential mineral for many vital functions in the body. Maintaining healthy Mg levels can support bone, heart, muscle, nervous system health, and more [32]. One of the most important causes of Mg deficiency in the blood is a diet that lacks foods containing Mg, such as nuts, whole grains, leafy vegetables, and legumes. This may lead to low Mg levels in the blood. Problems with Mg absorption, the most important of which is that people who have undergone surgery on the small intestine or have had part of it removed, may suffer from problems with Mg absorption. All of these cause Mg deficiency, which is one of the causes of osteoporosis. Our results are consistent with similar studies[33]. A Zn deficiency can have a number of detrimental effects on bone health. Low Zn levels can cause bones to weaken and become more prone to breaking. Zn helps preserve bone density. Weak bones can also result from a Zn deficit since it increases the activity of osteoclasts, which break down bones, in comparison to osteoblasts, which form bones. Zn is a cofactor in numerous enzymatic activities that aid in the formation of collagen, according

to studies. Enzymes involved in the formation of collagen require Zn as a necessary component [34]. For instance, Zn is necessary for the effective operation of enzymes like proline hydroxylase and lysine hydroxylase, which are involved in the conversion of amino acids (such as proline and lysine) into the modified forms required for the production of collagen. A Zn shortage impairs the production of collagen in bones, which increases the risk of osteoporosis, causes skeletal weakness, and reduces bone density. Additionally, it may impede the recovery of bones following fractures or traumas [35].

Table 8 shows that calcium and magnesium levels were lower when measured using the AAS compared to the Mindray Semi-Auto analyzer, whereas zinc levels were higher with the AAS. The automatic analyzer determines calcium, magnesium, and zinc concentrations based on the chemical reaction between each element and the reagent, by measuring the light absorption of the resulting colored complex. Therefore, the discrepancies in mineral concentrations observed between the Mindray Semi-Auto Chemistry Analyzer and Atomic Absorption Spectroscopy (AAS) can be attributed to several analytical and pre-analytical factors. First, the Mindray analyzer relies on colorimetric endpoint reactions, which are susceptible to interferences such as hemolysis, lipemia, and icterus—factors still regarded as major sources of analytical error in clinical chemistry, as recent studies confirm their impact on over 10 assays in modern analyzers [36,37]. These sample matrix effects can introduce systematic bias, leading to skewed absorbance readings compared to more specific methods. In contrast, AAS employs element-specific light absorption by free atoms in a gaseous state, offering higher specificity and sensitivity and reducing optical interference [38]. Moreover, pre-analytical variables, including sample matrix composition and calibration standard discrepancies, contribute further to measurement variation. Optimized thresholds for hemolysis and lipemia have been shown to differ between platforms, sometimes exceeding $\pm 10\%$ from reference methods [39]. Which is substantial enough to impact clinical interpretation. Therefore, although both methods effectively identify mineral deficiencies, AAS provides greater analytical robustness, emphasising the importance of validating and harmonizing techniques across platforms to ensure consistent and clinically meaningful results [40].

Conclusion

This study demonstrates that serum levels of Ca, Mg, and Zn are significantly lower in women with osteoporosis compared to healthy individuals, highlighting the critical importance of these minerals in bone health and the pathogenesis of the disease. The comparative analysis between the Mindray Semi-Auto Chemistry Analyzer and AAS revealed that although both techniques produced consistent deficiency trends, AAS exhibited superior analytical performance in terms of precision and repeatability, as evidenced by lower RSD values and acceptable recovery rates. These findings highlight the importance of early mineral screening in osteoporosis management and emphasize the need for accurate, validated analytical methods to ensure reliable clinical assessment and decision-making.

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