Investigating Zinc, Iron and 25-hydroxyvitamin D Levels alongside Hemoglobin status in Acute Myocardial Infarction (AMI) Patients

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Abstract:
Electrolyte abnormalities play a pivotal role in the context of cardiovascular emergencies, posing significant risks that contribute to morbidity and mortality worldwide. The aim of this study is to evaluate the impact of 25-hydroxy vitamin D levels on electrolyte concentrations (Zinc, and Iron) in patients with acute myocardial infarction (AMI), alongside examining the structural changes in the hemoglobin molecule (met-Hb, S-Hb, CO-Hb, and CO2-Hb) in these patients. This study was case-control, the population included 150 participants (60 ST-segment elevation myocardial infarction (STEMI) cases, 60 non-ST-segment elevation myocardial infarction (NSTEMI) cases and 30 control groups). Blood samples from the groups were taken on admission of patients and analyzed for electrolytes, 25-hydroxy vitamin D levels and hemoglobin derivatives. Our study reveals significant electrolyte and vitamin D deficiencies in STEMI patients, with less impact on NSTEMI patients. Additionally, STEMI patients showed the most profound changes in hemoglobin structure, indicating a severe alteration in blood chemistry following acute myocardial infarction. Monitoring electrolytes and vitamin D is crucial for managing acute myocardial infarction (AMI), which impacts hemoglobin properties and cardiac function. Future studies need to investigate how different vitamins and minerals affect AMI.

Keywords: Electrolyte, 25-Hydroxyvitamin D, Hemoglobin Derivatives, Myocardial Infarction.

Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMI</td>
<td>Acute Myocardial Infarction</td>
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<tr>
<td>NSTEMI</td>
<td>Non-ST-segment Elevation Myocardial Infarction</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST-segment Elevation Myocardial Infarction</td>
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<tr>
<td>HbA1c</td>
<td>Hemoglobin A1C</td>
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<tr>
<td>PPCI</td>
<td>Primary Percutaneous Coronary Intervention</td>
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<td>S-Hb</td>
<td>Sulphhemoglobin</td>
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<tr>
<td>met-Hb</td>
<td>Methemoglobin</td>
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<tr>
<td>CO-Hb</td>
<td>Carboxyhemoglobin</td>
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<tr>
<td>O2-Hb</td>
<td>Oxyhemoglobin</td>
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1- Introduction

with notable advancements in prognosis during the past decade (Reed et al., 2017). Myocardial infarction (MI) is defined as a condition of myocardial necrosis brought on by a sudden blockage of the coronary blood supply. The degree of occlusion, the length of the occlusion, and the existence or lack of collateral circulation all influence how severe the MI is. It is a component of the larger spectrum known as acute coronary syndrome, which also includes non-ST segment elevation myocardial infarction (NSTEMI) and unstable angina (STEMI), emphasizing how crucial it is to comprehend the physiological foundations of these disorders. A study conducted by Hariprasad and Basavaraj (2018) determined the possible effects of electrolyte imbalances on the development and severity of acute myocardial infarction (AMI) by examining the alterations in serum electrolytes, mainly potassium and sodium levels, in patients with AMI and comparing these alterations to those in healthy individuals.

The significance of fluid and electrolyte balance extends beyond the context of AMI, as recent studies have illuminated the association between electrolyte imbalances and increased morbidity and mortality among critically ill patients. This underscores the necessity for health care providers to be well-versed in the principles of fluid and electrolyte physiology and pathophysiology to deliver optimal care (Bulloch et al., 2024). The heart's healthy operation critically depends on the normal levels of calcium, magnesium, potassium, and sodium. These electrolytes are integral to regulating the heart's electrical activity and contraction, with zinc also playing a pivotal role in gene expression, cell proliferation, and maintaining cellular integrity (Huang et al., 2022; Ma et al., 2018). Moreover, vitamin D's role in maintaining electrolyte homeostasis emerges as a crucial element in preventing cardiovascular diseases, with vitamin D insufficiency becoming a common nutritional disorder with far-reaching implications for cardiovascular health (Forrest and Stuhldreher, 2011). The interrelation between serum electrolyte levels and anemia further complicates the context of cardiovascular health. Specifically, iron deficiency anemia's impact on HbA1c levels and serum electrolytes in a non-diabetic adult population illuminates the intricate interactions between blood components and electrolyte balance (Patil et al., 2023).

Low hemoglobin levels have been independently linked to mortality, cardiovascular events, and major bleeds in individuals with stable coronary artery disease, signifying the prognostic value of hemoglobin and its derivatives in cardiovascular health (Kalra et al., 2017). The determination of hemoglobin derivatives through spectrophotometric and multi-component spectrophotometric analysis provides essential insights into the biochemical pathways influencing cardiovascular disease and its outcomes (Kaewprayoon et al., 2020; Huang et al., 2021; Attia et al., 2015). Therefore, this study aims to unravel the complex interplay between electrolytes, vitamin D, and hemoglobin derivatives, shedding light on the multifaceted mechanisms underpinning cardiovascular health and disease. Understanding these intricate relationships offers a promising avenue for developing more effective strategies to combat cardiovascular diseases, enhancing patient care and outcomes in this critically important area of medical research.

2- Materials and Methods

2.1. Study design and population

Our research adopted a case-control study design, focusing on patients who presented with myocardial infarction and were admitted to the coronary care unit of the cardiology department at
Aswan University Hospital, affiliated with Aswan University. To compare, we selected an age-matched control group from individuals attending the outpatient clinic of cardiology who had no myocardial disease or systemic illnesses influencing electrolytes. The study encompassed a diverse age range of participants, from 25 to 70 years old, including a subset of up to 30 healthy individuals within the same age bracket for a balanced comparison. Overall, our study comprised 150 participants, divided into 60 ST-segment elevation myocardial infarction (STEMI) cases, 60 non-ST-segment elevation myocardial infarction (NSTEMI) cases, and 30 controls. The STEMI patients were primarily identified in the emergency room and subsequently referred to the Aswan Heart Center for primary percutaneous interventions, ensuring a comprehensive inclusion of myocardial infarction presentations within our research scope.

2.2. Patients and sample collection
For our research, each participant, including both patients with myocardial infarction and healthy controls, underwent comprehensive initial clinical and laboratory evaluations. These evaluations encompassed a detailed medical history and physical examination, resting electrocardiograms (ECG), echocardiography, and a thorough electrolyte assessment, which included serum levels of iron and zinc. Blood samples were meticulously collected from patients diagnosed with non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI) prior to undergoing Primary Percutaneous Coronary Intervention (PPCI), as well as from the healthy control group for comparative purposes. It was crucial to confirm that patients had experienced a recent acute myocardial infarction, specifically within a timeframe of less than 120 minutes before the percutaneous intervention, to accurately analyze coronary circulation outcomes and determine the necessity for coronary vessel stenting. Venous blood samples, ranging from 3 to 5 ml, were drawn from both patient groups and healthy controls using strict aseptic techniques. These samples were then transferred to sterile plain test tubes without anticoagulants to facilitate serum separation. Following a period for clot formation, the blood was subjected to centrifugation at 3000 rpm for 10 minutes.

Before being included in the study, every participant gave written informed consent, fully understanding that their information would be kept private. This investigation was ethically approved by the Aswan University Faculty of Medicine, following the guiding principles of the World Medical Association's Helsinki Declaration.

2.3. Methods for determinations
In our methodology, for the quantification of zinc in serum, we employed a spectrophotometric determination method facilitated by an Elabscience Biotechnology Kit (Tokimoto et al., 2003), whereas iron concentrations were measured using a kit from QUÍMICA CLÍNICA APLICADA S.A. (Tobacco et al., 1981), employing a similar spectrophotometric approach. Vitamin D levels were assessed through the Cobas e 411 analyzer, which leverages electrochemiluminescence (ECL) technology for immunoassay analyses, another innovation from Roche Diagnostics Ltd. (Roach Diagnostic company, Forrenstrasse Rotkreuz, Switzerland).

Additionally, our study utilized a multicomponent spectrophotometric method to simultaneously determine four hemoglobin derivatives, conducting absorbance measurements at four specific wavelengths with a Cary UV/VIS double-beam spectrophotometer from Agilent Technologies, Australia (Attia et al., 2015). The conductivity of the hemoglobin solution was determined using a digimeter L21/L21C aqualytic autotemp meter, with measurements made at a continuous sine wave frequency of 1500 Hz, providing insights into the solution's ionic mobility (Cole et al.,
Lastly, the viscosity of each hemoglobin solution was meticulously measured using an Ostwald viscometer, focusing on solutions with a concentration of 5% at 25 °C in distilled water (Bishay 2000; Anderson 1986), thus ensuring precise and reliable data for our biochemical analyses.

2.4. Statistical analysis
SPSS was used to gather and analyze the data (Statistical Package for the Social Science, version 24.0, IBM, Armonk, New York). While nominal data were expressed as frequency, continuous data were expressed as mean + SD or median (range) (percentage).

The chi²-test was used to compare the nominal data of various groups, and the student t test was used to compare the continuous data of various groups. Since the level of confidence was maintained at 95%, a P value of less than 0.05 was deemed significant.

3. Results and Discussion

3.1. Effect of electrolytes on patients
The provided figures depict a comparison of mean elemental levels—iron (Fe) and zinc (Zn)—across three groups: control, ST-Elevation Myocardial Infarction (STEMI), and Non-ST-Elevation Myocardial Infarction (NSTEMI).

The figure 1 Iron levels exhibit a considerable drop in the STEMI group (mean of 6.96) compared to the control group (mean of 20.93) (p≤0.001), with a partial rebound in the NSTEMI group (mean of 14.8) (p=0.99).
The figure 2 Zinc levels significantly decrease in the STEMI group (mean of 1.7) compared to the control group (mean of 4.5) (p ≤ 0.001), and although there is an increase in the NSTEMI group (mean of 3.1), it does not return to control levels (p=0.201).

The patterns observed in zinc levels in our study participants are affirmed by the extensive research on the cardiovascular implications of zinc. Yesmin et al. (2017), Yousif and Abdalla (2018), and Huang et al. (2018) all found decreased zinc levels in AMI patients, indicating that hypozincemia might contribute to myocardial tissue injury. The recommendation by El-Mahdy et al. (2019) and Quader et al. (2021) for the routine assessment of serum zinc levels underscores the importance of this trace element in the management of AMI.

Additionally, our research indicated a decrease in serum iron levels in myocardial infarction patients, which is in line with Hasan et al. (2019) and the prognostic studies by Reinhold et al. (2021). The latter suggests that iron deficiency in ACS may be indicative of a worse long-term prognosis, which is corroborated by other studies (Anker et al., 2009; van Veldhuisen et al., 2017; Gada et al., 2013). The potential therapeutic benefits of iron supplementation in improving outcomes in STEMI patients, as suggested by Ye et al. (2015) and Li (2017), underscore the need for further investigation into iron as a modifiable risk factor in myocardial infarction management.

The findings from Gonçalves and Abreu (2020) also contribute to the understanding of our results, indicating that higher potassium levels and a lower sodium-to-potassium ratio could be protective against CVD in older populations. Moreover, Schmitz et al. (2021) identified a link between low calcium levels at hospital admission and higher mortality post-AMI, which may necessitate a reevaluation of calcium management in the acute setting.

3.2. Effect of Vitamin D

This deficiency is not merely a biomarker but could be an active participant in the pathophysiological process leading to myocardial infarction, as it can influence various cardiovascular risk factors such as hypertension and diabetes, both of which have been associated with vitamin D insufficiency. The gender and age-related differences in vitamin D levels and
their association with the severity of coronary atherosclerosis, as noted by Dziedzic et al. (2016), further complicate the cardiovascular impact of vitamin D and highlight the need for demographic-specific research.

Figure 3 illustrates the mean Vitamin D levels across three groups: control, ST-Elevation Myocardial Infarction (STEMI), and non-ST-Elevation Myocardial Infarction (NSTEMI). The STEMI group has a mean vitamin D level of 13.95 ng/mL, significantly lower than the control group, which boasts the highest mean level at 20.1 ng/mL (p = 0.001). Similarly, the NSTEMI group displays a mean level of 15.38 ng/mL, which, while not significantly different from the STEMI group (p = 0.341), is significantly lower compared to the control group (p = 0.012). The study's outcomes, which resonate with the findings of Mohammed et al. (2015), emphasize the potential impact of vitamin D deficiency on cardiac health, particularly in STEMI patients. Vitamin D's regulatory influence over the renin-angiotensin-aldosterone system and myocardial matrix turnover may explain the heightened risk of myocardial infarction and adverse cardiac remodeling associated with its low serum levels.

Moreover, our findings suggest that vitamin D supplementation could have a therapeutic role, potentially improving cardiac cell therapy outcomes and aiding in myocardial repair, as posited by Hlaing et al. (2014). This therapeutic potential is underscored by the modulating effects of vitamin D on cardiac-derived cells and its implications for heart repair and regeneration.

The consistent association between vitamin D insufficiency and the increased incidence of acute myocardial infarction, as observed in various studies, underscores the potential of vitamin D levels as a modifiable risk factor in cardiovascular disease prevention strategies. This is particularly crucial given the heightened risk and morbidity associated with myocardial infarction.
Given the multifaceted role of vitamin D in cardiovascular health and disease, future studies should aim to delineate the mechanisms by which vitamin D exerts its effects on the cardiovascular system. Such studies could explore vitamin D's influence on endothelial function, its interplay with other hormonal regulators, and its direct impact on myocardial cells. There is also a pressing need for clinical trials to assess the efficacy of vitamin D supplementation in the prevention of myocardial infarction and the improvement of outcomes in patients with existing cardiovascular diseases.

3.3. Multicomponent spectrophotometric method for the simultaneous determination of four hemoglobin derivatives

Table 1: Hemoglobin derivatives among enrolled groups (Control, STEMI, and NSTEMI).

<table>
<thead>
<tr>
<th>Group</th>
<th>SHb (gm/dl)</th>
<th>SHb (%)</th>
<th>Met Hb (gm/dl)</th>
<th>Met Hb (%)</th>
<th>HbCO (gm/dl)</th>
<th>HbCO (%)</th>
<th>HbO2 (gm/dl)</th>
<th>HbO2 (%)</th>
<th>Total Hb (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I) (n= 30)</td>
<td>0.034 ± 0.0023</td>
<td>0.263</td>
<td>0.0313 ± 0.0034</td>
<td>0.243</td>
<td>0.02061 ± 0.0026</td>
<td>2.34</td>
<td>12.381 ± 0.027</td>
<td>97.09</td>
<td>12.752 ± 0.201</td>
</tr>
<tr>
<td>STEMI (II) (n= 60)</td>
<td>0.389 ± 0.0039</td>
<td>4.26</td>
<td>0.473 ± 0.0028</td>
<td>4.53</td>
<td>0.423 ± 0.0072</td>
<td>4.46</td>
<td>7.0621 **± 0.01</td>
<td>84.6</td>
<td>8.347 ± 0.43</td>
</tr>
<tr>
<td>NSTEMI (III) (n=60)</td>
<td>0.18 ± 0.005</td>
<td>2.52</td>
<td>0.25 ± 0.004</td>
<td>2.67</td>
<td>0.106 ± 0.003</td>
<td>3.19</td>
<td>9.52 ± 0.041</td>
<td>91.62</td>
<td>10.25 ± 0.24</td>
</tr>
</tbody>
</table>

*P < 0.05  S                                ** P < 0.01 HS                               ***P < 0.001   VHS

Table 1 Show that a multicomponent spectrophotometric method to simultaneously quantify four hemoglobin derivatives: sulfhemoglobin (SHb), methemoglobin (MetHb), carboxyhemoglobin (HbCO), and oxyhemoglobin (HbO2) across three groups: control, STEMI, and NSTEMI. In patients with acute myocardial infarction (AMI), both those with ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI) exhibit elevated levels of sulfhemoglobin (SHb), methemoglobin (MetHb), and carboxyhemoglobin (HbCO), along with a notable decrease in oxyhemoglobin (HbO2), indicating altered hemoglobin functionality compared to the control group. However, STEMI patients demonstrate the most significant disruptions, with the highest increases in SHb, MetHb, and HbCO, and the lowest levels of HbO2, suggesting a more severe impact on hemoglobin's oxygen-carrying capacity in this subgroup of AMI patients. The findings of our study are in line with previous literature, indicating that AMI patients exhibit significant alterations in hemoglobin derivatives, specifically an increase in Meth-Hb, CO-Hb, and S-Hb, coupled with a decrease in O2-Hb when compared to a control group. This is consistent with Ghanem et al. (2010), who reported significant
variations in MetHb levels across different cardiac diagnoses and treatments, suggesting that these alterations could be attributed to the type of therapy received or an exacerbated hypoxic state in cardiac patients. Our results further support the hypothesis that the elevated levels of methemoglobin in cardiac patients, especially those with additional complications such as infection, could be indicative of an underlying exacerbated physiological stress or a response to specific therapeutic interventions. This aligns with findings from Walser et al. (2020) and Davutoğlu et al. (2006), which reported elevated methemoglobin levels in patients with sepsis, emphasizing the role of infection in altering hemoglobin dynamics in AMI patients.

3.4. Determination of viscosity

Table 2 contained the viscosity values among the different enrolled groups: control, STEMI, and NSTEMI. The STEMI group shows the highest viscosity values at 0.0698 ± 4.21x10^-5, significantly higher than both the control and NSTEMI groups, with a p-value of ≤0.001 when compared to the control. The NSTEMI group's viscosity is 0.038 ± 1.5x10^-5, which is significantly different from the STEMI group (p-value ≤ 0.05) but not significantly different from the control group (p-value = 0.152). The control group has the lowest viscosity at 0.0218 ± 2.36x10^-4. This demonstrates significant differences in viscosity between the groups, with the STEMI group showing the most marked increase.

Table 2: Viscosity Values among enrolled groups (Control, STEMI, and NSTEMI)

<table>
<thead>
<tr>
<th>Group</th>
<th>η</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I) (n= 30)</td>
<td>0.0218 ± 2.36x10^-4</td>
<td></td>
</tr>
<tr>
<td>STEMI (II) (n= 60)</td>
<td>0.0698 ± 4.21x10^-5*</td>
<td>I vs. II ≤ 0.001</td>
</tr>
<tr>
<td>NSTEMI (III) (n=60)</td>
<td>0.038 ± 1.5x10^-5*</td>
<td>II vs. III ≤ 0.05 and I vs. III = 0.152</td>
</tr>
</tbody>
</table>

Values of the viscosity coefficient η in poise among enrolled groups.

*ANOVA test was used to compare the mean difference between groups (P ≤ 0.001)

The findings from our study on blood viscosity in AMI patients provide critical insights into the rheological changes accompanying acute myocardial infarction, suggesting a potential link between increased blood viscosity and the severity of coronary artery disease. This observation is in harmony with the literature, highlighting alterations in hemoglobin structure and blood rheology in relation to the disease's activity and type. Particularly, our results align with studies by Caimi et al. (2018), which noted rheological profile impairment in AMI patients, and by Lowe et al. (1997) and Erbay et al. (2005), which associated higher blood and plasma viscosity with greater coronary artery disease severity. The deviation from spherical to non-spherical hemoglobin molecules, as reported in previous research, might contribute to the observed rheological changes, affecting blood viscosity. This study underscores the importance of considering blood viscosity and hemoglobin structure alterations as significant factors in the pathophysiology of AMI, potentially offering new avenues for prognosis and treatment strategies.
3.5. Electrical conductivity of Hb among enrolled groups

Table 3: Electrical conductivity of Hb among enrolled groups (Control, STEMI and NSTEMI)

<table>
<thead>
<tr>
<th>Group</th>
<th>Conductivity Ms/cm</th>
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</thead>
<tbody>
<tr>
<td>Control (I) (n= 30)</td>
<td>37.548 ± 0.732</td>
</tr>
<tr>
<td>STEMI (II) (n= 60)</td>
<td>80.46 ± 0.983</td>
</tr>
<tr>
<td>NSTEMI (III) (n=60)</td>
<td>65.412 ± 1.363</td>
</tr>
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</table>

Table 3 presents data on the electrical conductivity of Hb among different enrolled groups. The STEMI group exhibits the highest electrical conductivity of hemoglobin (Hb) at 80.46 ± 0.983 Ms/cm, significantly greater than both the NSTEMI and control groups, with a p-value of ≤0.001 against the control and 0.032 against the NSTEMI. Subsequently, the NSTEMI group has a conductivity level of 65.412 ± 1.363 Ms/cm, which, while lower than STEMI, is significantly higher than the control (p-value ≤ 0.001). The control group shows the lowest conductivity at 37.548 ± 0.732 Ms/cm, underscoring significant differences when compared with the disease groups.

The pronounced increase in electrical conductivity of Hb in acute myocardial infarction (AMI) patients, as observed in our results, aligns with the hypothesis that unfolding of Hb exposes new hydrophobic groups on the protein's surface, altering its hydrophobic/hydrophilic ratio. This finding corroborates the proposed mechanism, where changes in the tertiary structure of the Hb molecule led to increased electrical conductivity. The significant differences in conductivity between the control, STEMI, and NSTEMI groups further suggest that the degree of Hb unfolding and, consequently, the changes in electrical properties may correlate with the severity of AMI. These alterations in Hb's electrical properties could reflect underlying molecular and structural changes associated with the pathophysiology of AMI, offering potential insights into the disease mechanism and its impact on Hb functionality.

Finally, low blood levels of vitamin D have been associated with an increased risk of CVDs. It is unclear, nonetheless, if low vitamin D levels are the primary cause or an effect of these disorders. Although some studies suggest that taking supplements of vitamin D may lower the risk of cardiovascular disease (CVD), further study is needed to fully understand the relationship between vitamin D and cardiovascular health (Haider et al., 2023). Numerous observational studies have revealed that VD may shield individuals from stroke (Manson et al., 2019). The body responds to vitamin D in a way similar to hormones, and vitamin D deficiency has been linked to altered disease progression. Deficiency worsens prognosis and raises death rates, particularly in conditions where an inflammatory process is involved in the disease's progression (Dini and Bianci, 2012). Numerous chronic disorders, including diabetes, hypertension, heart failure, and cardiopulmonary ailments, have been demonstrated to be impacted by it. Numerous investigations have demonstrated that elevated vitamin D levels positively impact Hb synthesis and elevate Hgb. Furthermore, it has been demonstrated to boost erythrocyte production in the bone marrow; as a result, severe deficiency inhibits erythropoiesis.
which results in anemia. Ergenç discovered a strong correlation between minerals, vitamin D, and hemoglobin levels. It's important to track and assess the patients' vitamin D levels in addition to their therapy and clinical exams. Hematological and biochemical alterations should also be evaluated (Ergenç et al., 2023).

**Conclusion**

The electrolyte variance is very important in cardiac disease, especially in the follow-up of acute myocardial infarction (AMI) patients and its effect on hemoglobin derivatives, viscosity, and conductivity. So, it is very important to evaluate the electrolyte concentrations, notably hemoglobin related minerals such as zinc and iron, in AMI patients to control the severity of the disease. Vitamin D can help improve cardiac function in patients with myocardial infarction (heart attack).

In summary, our study reaffirms the significance of monitoring electrolyte levels in AMI patients. The observed alterations in iron and zinc levels could potentially serve as biomarkers for myocardial injury and prognosis. The role of magnesium and zinc in cardiovascular health further emphasizes the potential of these elements as therapeutic targets. Future research should aim to confirm these findings in larger cohorts and to explore the mechanisms by which these electrolyte disturbances contribute to the pathophysiology of AMI. Additionally, the potential benefits of supplementation with these electrolytes, as part of the management strategy for AMI patients, warrant further clinical trials.

**Acknowledgment**

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**References**


