RESEARCH PAPER

# A Case-Control Study Evaluation of Serum Calcium and Magnesium Levels as Potential Biomarkers for Tinnitus

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**ABSTRACT**

**Introduction:** Tinnitus refers to the experience of hearing sounds in the ear or head without any actual external noise. It is often described as a ringing, buzzing, hissing, or clicking noise that may fluctuate in volume and can be ongoing or sporadic. Although it is a common condition, the exact causes of tinnitus remain largely unclear. In fact, no definitive cause has been established so far. Given the subjective nature of tinnitus and the complexity of the auditory system, researchers are actively studying its underlying mechanisms to develop more effective treatments. Recent research emphasizes the importance of biochemical components, especially calcium and magnesium, in the onset of tinnitus. Magnesium plays a vital role in the functioning of the nervous system and manages calcium channels that are crucial for the transmission of auditory signals. Magnesium supplementation might aid in maintaining and restoring the functionality of the auditory nerve following hearing loss, which could help reduce tinnitus symptoms. The proper functioning of the inner ear relies on the regulation of calcium, and tinnitus has been linked to increased serum calcium levels and disrupted calcium signaling.

**Objective of Study:** To study the relationship between Serum Calcium and Magnesium and Subjective Tinnitus.

**Materials and methods:** 36 patients presenting to the department of Otorhinolaryngology and Head and Neck surgery who are presenting with complaints of Subjective Tinnitus for any reasons satisfying the inclusion and exclusion criteria were grouped into the research group and groups of 36 individuals without tinnitus were selected to be the control group. Informed consent is taken from the patient, with their complete details and a comprehensive history in compliance with the set inclusion and exclusion criteria. After which the patient is asked to fill a Tinnitus Severity Index (TSI) form. Patient are kept on overnight fast after which blood samples are taken. Data obtained is analyzed using IBM SPSS software.

**Results:** While both groups exhibited some variation, the graphical depiction indicates that cases generally exhibit higher calcium levels compared to controls. The average serum calcium concentration was elevated in cases (10.09 mg/dl) in comparison to controls (9.23 mg/dl), which was found to be statistically significant. The average serum magnesium level was reduced in cases (1.70 mg/dl) in contrast to controls (1.86 mg/dl) however it was not found to be statistically significant.

**Conclusions:** The findings underscore a disruption in calcium and magnesium balance in tinnitus patients, with increased calcium being a more significant observation. Patients with tinnitus showed elevated mean serum calcium levels relative to controls, indicating a possible link between increased calcium levels and tinnitus, potentially influenced by changes in cochlear or neuronal excitability. While lower serum magnesium levels were noted in cases, the significant variability between groups hindered conclusive statements about statistical importance.

**Keywords:** Tinnitus, Tinnitus Severity Index, Serum Calcium levels, Serum Magnesium levels.

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## INTRODUCTION

Tinnitus is the perception of sound in the human ear or head without any external auditory input. A ringing, buzzing, hissing, or clicking sound that can vary in volume and be either intermittent or continuous is commonly used to describe tinnitus.[1,2] An estimated 15% of people worldwide have tinnitus, and the prevalence rises with advancing age. Approximately one-third of persons aged 60 or older are reported to have tinnitus.[1,3] There is still little known about the exact causes of tinnitus despite it being such a common condition.[4] In over 80% of cases, there appears to be no clear reason for the development of tinnitus.

### The Pathophysiology of Tinnitus

The cause of Subjective Tinnitus is thought to originate from damage or faulty functioning within the Auditory System affecting primarily the cochlear hair cells, the auditory nerves and the pathways to the Central Nervous System.[3] The inner ear has hair cells that convert sound waves into electrical signals that are interpreted by the brain. Damaged hair cells may send Auditory Signals back to the brain either incorrectly or inappropriately as a result of the original damage; in most cases, resulting from the ageing process, exposure to loud noises or taking ototoxic medications. The brain may interpret these incorrect signals as sound in the absence of any actual noise. Additionally, abnormal activity along the auditory cortex and/or the Central Nervous System may result in excessive auditory signal perception due to amplification of the signals by the brain's auditory cortex [7]

Neurophysiological and functional imaging studies have revealed that tinnitus is a disorder of brain function characterised by abnormal processing of auditory signals by the brain, rather than simply a peripheral hearing deficit [3,4] . People suffering from tinnitus show increased activation in the prefrontal cortex (PFC), limbic system and auditory cortex, which indicates that double sensory (auditory) as well as emotional components are present in those experiencing tinnitus; this may account for the distress experienced by those with tinnitus. [16]

Damage to cochlear hair cells and abnormalities in neuronal and central auditory pathways are linked to subjective tinnitus.

Recent studies highlight the role of biochemical elements, particularly calcium and magnesium, in the development of tinnitus. The central nervous system relies on magnesium to function, and controlling calcium channels is necessary for hearing signals.[15,17,22] Magnesium supplementation may help to restore and maintain auditory nerve health after hearing loss, thereby helping relieve symptoms of tinnitus. [18,19,20] Proper inner ear function depends on calcium regulation; tinnitus has been associated with elevated serum calcium levels and disrupted calcium signalling. [10,11,12,13,14,21]

Magnesium, a vital mineral involved in over 300 enzymatic processes- many occurring in the brain and nervous system- is essential for neurotransmission, ion channel regulation, and neuronal protection from excitotoxicity caused by overstimulation.[7,14,16] It controls calcium channels essential for neural communication in the auditory system, thereby maintaining the electrical stability required for signal transmission. Magnesium deficiency may increase neuronal excitability, activate calcium channels excessively, and cause cellular damage.[14,19,20] Research suggests that magnesium supplementation may help preserve and repair auditory function. It may aid the regeneration of inner-ear nerve cells following noise-induced hearing loss or sudden sensorineural hearing loss.[17,18,19] Magnesium also reduces inflammation and oxidative stress caused by loud sounds, thereby protecting cochlear function and possibly preventing or lessening tinnitus.[14] Studies have found that individuals with persistent tinnitus tend to have lower serum magnesium levels than those without tinnitus.[14,15,22] This supports the potential use of magnesium in tinnitus treatment, particularly in cases associated with hearing loss. Magnesium may reduce abnormal activity in auditory pathways by stabilising calcium channels and decreasing neuroinflammation, thus alleviating tinnitus symptoms.[18,19,20]

### Calcium Regulation in the Inner Ear and Its Impact on Tinnitus

The functioning of sensory cells in the hearing system relies on calcium, a vital element.[10,12,13] Calcium ions enter inner ear hair cells via voltage-dependent channels, binding to otoferlin, which is crucial for neurotransmitter release at hair cell and auditory nerve synapses. This process is essential for transmitting sound signals from the ear to the brain.

A precisely controlled mechanism regulates calcium levels in the inner ear, and any imbalance can impair synaptic transmission, leading to hearing loss.[12,13,21] Research links higher serum calcium levels with a

greater risk of subjective tinnitus. As with magnesium deficiency, excess calcium can overactivate channels, thereby damaging neurons and hair cells. Studies are investigating calcium channel blockers for the treatment of tinnitus and hearing loss by reducing excitotoxicity and stabilising nerve function.[12,13] However, not yet standard, these drugs support the importance of calcium balance in maintaining healthy hearing and suggest that calcium dysregulation may contribute to tinnitus. [12] In addition, abnormal calcium control within the cochlea has been linked to the development of noise-induced hearing loss. Excessive amounts of sound create too much calcium to come into the cell, leading to metabolic stress and damage to the hair cells. This excessive influx of calcium is believed to contribute to the development of tinnitus following acoustic trauma. [21]

Hearing Loss from Noise Exposure

Noise exposure and ototoxicity increase calcium influx into hair cells.

This increased calcium influx leads to:

1. Increased levels of reactive oxygen species (ROS)
2. Oxidative stress and damage to hair cells
3. Decreased output from the cochlea, which contributes to tinnitus

Magnesium Acts:

1. Blocks NMDA receptors to reduce excitotoxicity
2. Stabilises calcium channels
3. Acts as an antioxidant to decrease ROS
4. Increases blood flow to the cochlea

Reduced hair cell damage and hyperactivity of neurons decreases the risk and severity of tinnitus.

## METHODOLOGY AND METHODS-

### Study design –

The study included a group of 72 individuals which was divided into two groups of thirty six participants each. The Case Group contained 36 patients with subjective tinnitus recruited from the otorhinolaryngology Department and Head and Neck Surgery department who met by both inclusion and exclusion criteria. The Control Group contained 36 consenting adults without subjective tinnitus. Mean age was 43.58±15.88, in the Case Group, 22 were male, and 14 were female, among the Controls, 19 were male and 17 were female. Each subject signed an Informed Consent document, completed a Participant Information Sheet, and provided their medical history according to the criteria established for this study. A Tinnitus Severity Index (TSI), measure for tinnitus severity, was completed by all subjects. Blood was collected after an 8-hour fasting period. The data was analyzed via the IBM SPSS program.

**Inclusion Criteria included:**

- Age between 18 and 65 years.
- Patients presenting to the ENT OPD with complaints of Tinnitus.

**Exclusion Criteria included:**

- Patients with a history of acoustic trauma or barotrauma in the last four weeks
- Patients with a history of ototoxic drug use.
- Patients with a history of temporomandibular joint dysfunction.
- Patients with a history of Neurotrauma.
- Patients below the age of 18 years at the time of presentation.

**Serum Calcium and Magnesium-** Blood samples from patients and controls were taken during the morning after 12 hours of fasting. Serums were obtained by centrifuging blood samples (3000 rpm/10 min). Atomic Absorption Spectrophotometry measured serum levels of Calcium and Magnesium.

### Statistical Analysis

The results obtained for the two groups, namely the research and control groups, were statistically evaluated. The IBM SPSS Statistics for Windows version 20.0 (IBM Corporation, Armonk, NY, USA) was used to analyse the data. The means ± standard deviation were used to represent continuous variables. The chi-square test was applied to compare qualitative data, and a two-tailed t-test was used to compare quantitative data on the demographic characteristics. The study group and control groups' magnesium and calcium levels were compared using two-tailed t tests. It was decided that statistical significance was indicated by a p-value <0.05.

## RESULTS

The following observations were made and tabulated as follows-

**I) Comparison of Tinnitus severity index, Serum magnesium levels (mg/dl) and Serum calcium levels (mg/dl) in cases and controls using Shapiro Wilk Test**

| Parameters | Groups | Shapiro-Wilk | df | p-value |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Tinnitus severity index | Cases | 0.9600 | 36 | 0.0587 |
| | Controls | 0.9770 | 36 | 0.6290 |
| Serum magnesium levels (mg/dl) | Cases | 0.9850 | 36 | 0.9030 |
| | Controls | 0.9550 | 36 | 0.1450 |
| Serum calcium levels (mg/dl) | Cases | 0.9660 | 36 | 0.3250 |
| | Controls | 0.9660 | 36 | 0.3280 |

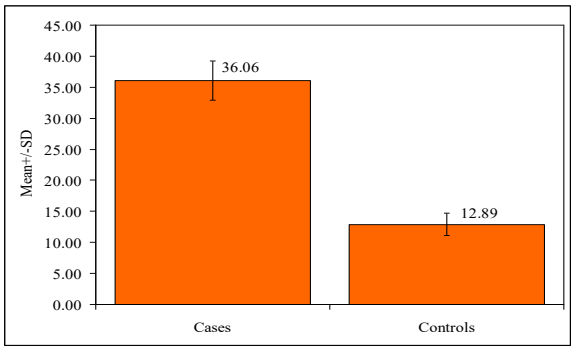
Table 1: Shapiro Wilk Test applied to check Normality of study group

The Shapiro–Wilk test revealed that the p-values for tinnitus severity index, serum magnesium levels, and serum calcium levels exceeded 0.05 in both cases and controls. This suggests that the data for all examined parameters follow a normal distribution in both groups therefore parametric tests can be applied for further analysis and for comparing cases and controls. (Table 1 )

**II) Comparison of Tinnitus severity index scores among cases and controls**

| Groups | n | Mean | SD | SE | t-value | P-value |
|---|---|---|---|---|---|---|
| Cases | 36 | 36.06 | 3.17 | 0.53 | | |
| Controls | 36 | 12.89 | 1.77 | 0.29 | 38.2815 | 0.0001* |

Table 2: Comparison of Tinnitus Severity Index in case and control group using Independent t-test



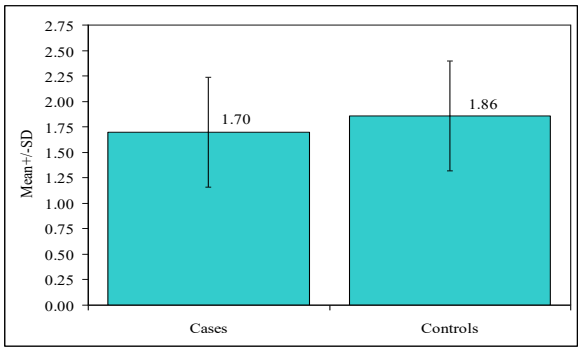

Graph 1: Bar Chart showing Tinnitus Severity Index Score in cases and control groups

The Tinnitus Severity Scores in the cases are much higher than those in the controls. This suggests that the tinnitus group has severe symptoms, including increased perceived noise, discomfort, and impairment in day-to-day functioning. The difference with respect to the Tinnitus Severity Index indicates statistical and clinical significance.
(Table 2 and Graph 1)

**III) Comparison of Serum Magnesium among cases and controls**

| Groups | n | Mean | SD | SE | t-value | P-value |
|---|---|---|---|---|---|---|
| Cases | 36 | 1.70 | 0.54 | 0.089 | | |
| Controls | 36 | 1.86 | 0.54 | 0.090 | -1.2162 | 0.2280 |

Table 3: Comparison of Serum Magnesium levels (mg/dl) in case and control group using Independent t-test



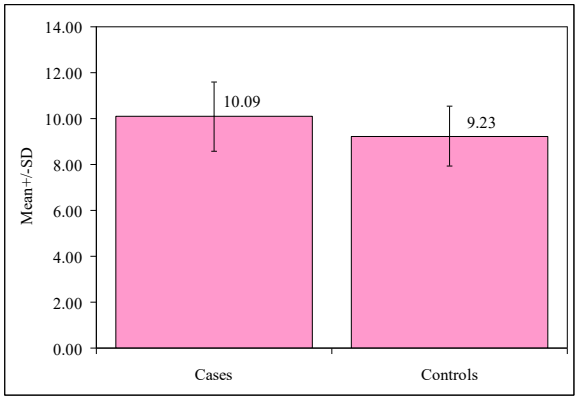

Graph 2: Bar Chart showing Serum Magnesium levels in cases and control groups

The average value of the Serum Magnesium is lower in cases (1.67 ± 0.55) as compared to controls (1.87 ± 0.39) signifying that low magnesium levels may be a risk factor for development of Tinnitus however, it was not found to be statistically significant. (Table 3 and Graph 2)

**VII) Comparison of Serum Calcium among cases and controls**

| Groups | n | Mean | SD | SE | t-value | P-value |
|---|---|---|---|---|---|---|
| Cases | 36 | 10.09 | 1.52 | 0.25 | | |
| Controls | 36 | 9.23 | 1.30 | 0.22 | 2.5864 | 0.0118* |

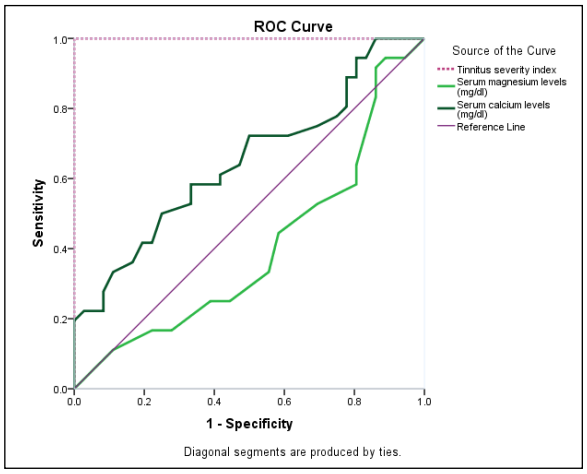
*p<0.05, Table 4: Comparison of Serum Calcium levels (mg/dl) in case and control groups using Independent t-test




Graph 3: Bar Chart showing Serum Calcium levels in cases and control groups

The mean serum calcium level was higher in cases (10.30 ± 1.79) than in controls (9.25 ± 1.22), a difference that was statistically significant, suggesting a potential link between high Calcium levels and the occurrence of Tinnitus. (Table 4, Graph 3)

**VIII) Receiver Operating Characteristic curve for accuracy of prediction of cases by Tinnitus Severity Index, Serum magnesium levels (mg/dl) and Serum calcium levels (mg/dl)**




Graph 4: Receiver Operating Characteristic curve for the Study Groups

Receiver operating characteristic (ROC) Curves are commonly used as a statistical tool to assess how well diagnostic tests or biomarkers distinguish between two populations (e.g., healthy vs. sick). The Y-Axis (Sensitivity) indicates the proportion of true positives (causes accurately identified by the test) relative to the total number of actual positive cases. The X-Axis (1-Specificity) shows the percentage of false positives (test incorrectly indicating that disease is present) as compared to the total number of individuals that test positive when no disease exists. A line through the origin to one half of the distance through both axes represents a test showing no predictive value.

Serum Calcium Levels (pink line): This curve mostly stays above the reference line, indicating moderate sensitivity and specificity. It is the most effective biomarker, with the largest area under the curve (AUC) compared to others, suggesting higher calcium levels are a strong indicator of tinnitus.

Serum Magnesium Levels (dark blue line): This curve stays mostly below the reference line, pointing towards an inverse association. Lower magnesium levels are linked to tinnitus.

Tinnitus Severity Index (dashed blue line): With an AUC close to 1.0, this index shows nearly perfect diagnostic performance, acting as either part of the diagnostic criteria or a near-perfect predictor, and serving as a gold-standard measure for this dataset (Graph 4).

## DISCUSSION

Our study seeks to evaluate the serum Calcium and Magnesium levels in cases of Tinnitus. It was found in our analysis that cases had higher levels of Serum Calcium levels which was found to be statistically significant and lower Serum Magnesium levels however it was not statistically significant thus establishing a possible link between Serum Calcium levels and Serum Magnesium levels and occurrence of Tinnitus.

A study by Andrei Osman et al. suggests that oral calcium channel blockers and fluoride supplements may be a non-surgical approach to managing tinnitus in patients with otosclerosis, particularly those with mild symptoms. The significant improvement in THI scores in the mild tinnitus subgroup indicates that supplementation of Calcium Channel Blockers could be a safe early intervention thus proving possible role of Calcium in development of Tinnitus. Our study on the other hand did not include any intervention however Serum calcium and magnesium levels between patients with tinnitus and controls were collected and analysed which showed that patients with tinnitus consistently had higher serum calcium levels, suggesting a positive association.

Dawes et al. conducted a study in England with a sample size of over 34,000 adults that looked specifically at the relationships between diet, tinnitus, and hearing loss. After controlling for potential confounders, increased intakes of calcium, iron and fat were shown to have a positive correlation to increased risk of developing tinnitus. In our retrospective case-control study, we did not evaluate dietary intake; however we measured calcium and magnesium levels, both of which are essential nutrients from the diet.

From this, we found that the patients diagnosed with tinnitus had significantly higher levels of calcium and lower levels of magnesium compared to individuals who did not have tinnitus.

A separate study conducted by Istvan et al, found that accurate intracellular calcium ($Ca^{2+}$) control is essential for a variety of cell functions, including hearing. In the cochlea, outer hair cells utilize $Ca^{2+}$ signalling in order to enhance the electromechanical feedback by changing the cortical cytoskeleton - a critical component of auditory perception - as well as improve both sound sensitivity and frequency discrimination. Any alteration in $Ca^{2+}$ signalling may cause over-stimulation to occur within the cochlear amplifier, which could lead to the onset of tinnitus in the cochlea. We have established a positive relationship between elevated levels of calcium in the body and subjective tinnitus, as well as evidence of statistical significance between the two, thereby establishing the correlation..

Jan et al.'s study noted that calcium was considered a fast, -low energy intracellular messenger which regulates the movement of neurotransmitters and enhances signal amplification as well as mediating synaptic transmission. Changes in the levels of calcium (i.e. due to exposure to loud sounds, head trauma, and/or due to the presence of toxic substances) will cause the activation of many distructive enzymes which lead to a loss of synapitic function, damage to hair cells and the eventual development of tinnitus, hearing losses and/or total deafness. Furthermore, in our study we found that there is a strong positive correlation of high serum calcium levels with the incidence of subjective tinnitus especially in those cases who have sensorineural hearing loss, potentially via effects on cochlear mechanics, synaptic transmission and/or neuronal excitability as it pertains to the auditory pathways.

Gowtham et al.'s study revealed that individuals with longstanding tinnitus had lower magnesium levels, also indicating that magnesium supplementation may confer therapeutic benefits in patients with persistent tinnitus. Our study, also showed the average serum magnesium level was lower in cases (1.67 mg/dL) than in controls (1.87 mg/dL) thus indicating a similar inference however, the difference in serum magnesium levels between the groups was not statistically significant.

According to study by Santosh et al, studies shows that inadequate consumption of magnesium has been hypothesized to affect auditory function adversely. The research performed in this study utilized a double-blind, randomized, placebo-controlled, crossover design with four testing events over approximately two months. During these testing events, participants' hearing and tinnitus were repeatedly assessed through the use of the Tinnitus Handicap Inventory in ascending order, with each subject receiving either 532 mg of magnesium or a placebo during alternating periods. There was some evidence to suggest that magnesium may have a positive effect on the severity of tinnitus symptoms; however, these findings were not statistically significant. In our study a trend toward a lower overall mean serum magnesium level in the tinnitus group as compared to that in the control group was noted, however there was no statistically significant difference.

## CONCLUSION

Results showed significant differences in serum mineral levels between patients with tinnitus and controls. Patients had higher serum calcium levels, suggesting a potential association between elevated calcium and tinnitus.

Conversely, serum magnesium levels were lower in cases than in controls, but the results were not statistically significant. This suggests that magnesium deficiency may be a contributing factor for development of Tinnitus in some individuals, but it is not a definitive distinguishing factor.

Overall, the findings indicate an imbalance in calcium and magnesium homeostasis among patients with tinnitus, with elevated calcium levels being more pronounced. These insights emphasise the need for larger, controlled studies to clarify the clinical and statistical significance of these biochemical differences and to explore their potential roles in diagnosing, treating, or preventing chronic tinnitus as well as use of Calcium Channel blockers and Magnesium supplements in treating patients of Tinnitus.

**Limitations of the Study**- Limitations of the study includes single centre study and a small sample size. Tinnitus is a subjective symptom; therefore, there might be some recall bias among patients.

**Ethical Clearance**- Approval and clearance from the institutional ethics committee were obtained from the "Ethical Clearance Committee" of KLE's Prabhakar Kore Hospital and MRC, Belagavi Ref. No. MDC/JNMCIEC/192, Dated 22/03/2024

**Conflict of Interest:** None declared.

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