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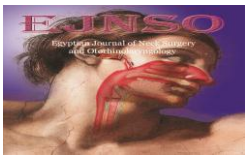

# Pediatric acute otitis media and serum calcium level: Is there a relation?

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## Abstract:

**Background:** Acute otitis media (AOM) is one of the most common disorders in children and commonest reason for antibiotic prescriptions. The aim of this study is to evaluate the link between acute otitis media and serum calcium levels in children.

**Patients and procedures:** Between December 2016 and March 2017, a prospective study was undertaken at Assiut University Hospital. A sample of ambulatory children with AOM and healthy controls were included in the study. A blood sample (5cm) was collected in the laboratory immediately after the diagnosis of AOM to measure serum calcium.

**Results:** The study comprised 79 children, 59 of whom were cases and 20 of whom were controls. The average age of the cases was 4.9 years, while the average age of the controls was 5.9 years. Furthermore, in the AOM group, 36 (60%) are boys and 23 (40%) were girls, compared to 10 (50%) boys and 10 (50%) girls in the control group. All children in the sick group had otalgia and a congested tympanic membrane. In addition, 70% had an upper respiratory tract infection prior to the AOM incident, and 33.3% had an AOM attack previously. Out of 59 children in the AOM group, 43 (73.3%) have abnormal serum calcium levels.

**Conclusion:** Children with recurrent AOM had significantly lower serum calcium levels than healthy children. So, Calcium can share in the treatment regimen of recurrent AOM to prevent further recurrence.

**Key words:** Pediatric, acute Otitis media, serum calcium level.

## Introduction

Acute otitis media (AOM) was defined by the presence of tympanic membrane that is visibly abnormal in terms of color, position, or mobility suggesting middle ear effusion; plus at least one of the following symptoms or signs of acute infection fever, earache, irritability, diarrhea, vomiting, acute otorrhea not caused by otitis externa and other symptoms of respiratory infection and it was caused by a mix of conditions that increases sensitivity to Eustachian tube dysfunction and recurrent respiratory tract infection.[1]

Chemotaxis, adhesion, and the release of pro- and anti-inflammatory cytokines are all controlled by calcium ions ($Ca^{2+}$), which is a ubiquitous second messenger in immune cells. [2] The specific role that elevations in serum $Ca^{2+}$ play in phagocytosis has been a matter of significant debate since the early research by Stossel [3], indicating that an increase in serum calcium may govern phagocytosis. The increases in serum

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calcium are essential for efficient phagosome maturation and influence the following steps in the phagocytic process. [2]

Increased susceptibility to infection has been linked to aberrations in phagocyte $Ca^{2+}$ homeostasis in numerous chronic diseases such as renal failure and diabetes. [3-6] Furthermore, intracellular infections like Mycobacterium TB and Leishmania alter $Ca^{2+}$-dependent mechanisms to avoid phagocytic killing. [7-8]

Although significant work has been made in identifying the mechanisms that encode and interpret phagocytic $Ca^{2+}$ signals, many molecular actors are still unknown. The role of $Ca^{2+}$ in phagocytosis has significant therapeutic implications. [2]

The goal of this study is to see if there's a link between acute otitis media and serum calcium level in children.

## Patients and methods:

**Study subject:**

This is a case-control study that was conducted at Assiut University Hospital's Department of Otorhinolaryngology between December 2016 and March 2017 after receiving institutional ethics committee permission and informed consent from the parents of all children in the study.

Children with AOM who ambulatory and healthy controls were made up the study group. Age of children from (1-13) years

1. Group A: Fifty-nine children diagnosed with acute otitis media by history, examination, otoscopic examination
2. Group B: Twenty healthy controls children free from any craniofacial abnormality or chronic diseases

**Inclusion criteria:**

- Bilateral AOM without tympanic membrane perforation
- The criteria of diagnosis of AOM are:
  1. Acute onset
  2. Symptoms (Otalgia, hearing loss, fever)
  3. Inflammatory signs (Tympanic membrane hyperemia).

**Exclusion criteria:**

1. Tympanic membrane perforation
2. Children with unilateral AOM.
3. Chronic medical diseases
4. Craniofacial anomalies
5. Rickets

The case and control groups are matched for age and gender

Every case was subjected for the following

**1) History taking:**

- Concerning symptoms related to AOM: Pain, fever, irritability, crying, lack of sleep & decrease appetite
- Upper respiratory tract infections (URTIs)
- History of similar attacks of acute otitis media

**2) Clinical examination:**

- Full ENT examination
- Ear examination: (Electric & pneumatic otoscopy):
  - Tympanic membrane is (congested- bulging)
  - Another otolaryngologist re-performed visual otoscopy on the patient whose otoscopic examination revealed nonuniformity or contradiction or feeling persisted.
  - The following criteria must be met for AOM to be diagnosed: acute onset, inflammatory signs, or symptoms (otalgia, severe tympanic hyperemia), - Appointment records that include the child's name, the date of the visit, and

symptoms gathered from the parents.

**3) Laboratory investigation:**

- Blood sample (5cm) was taken immediately after the diagnosis of AOM in the laboratory for measuring serum calcium.
- Blood sample taken by venipuncture into plastic tubes and avoiding hemolysis and separated from the cell as soon as possible.

**4) Principle of the test:**

- In an alkaline solution, cresolphtalein complexone (CPC) combines with calcium ion to produce a violet color.
- The intensity of the violet color in the sample is related to the calcium concentration.
- The addition of 8-hydroxy quinolone eliminates magnesium interference.
- According to Biotecnica Instruments S.P.A., the normal calcium level is between 8.7-10.7 mg/dl.

## Statistical analysis:

For data analysis, IBM's statistical program for social science version 16 (IBM, USA) was utilized.

The qualitative variable will be expressed as a number and a percentage, whereas the quantitative variable will be expressed as a mean and standard deviation.

A paired sample independent t-test was used to compare the results between the two groups, and qualitative variables were evaluated using Chi-square and Fishers exact tests. Significant was defined as a P value of less than 0.05.

## Results

This study comprised 79 children, 59 of whom were cases and 20 of whom were controls. The average age of the cases was 4.9 years, while the average age of the controls was 5.9 years. Furthermore, in the AOM group, 36 (60%) where boys and 23 (40%) where girls, compared to 10 (50%) boys and 10 (50%) girls in the control group.

The 59 children who were diagnosed with bilateral AOM all complained of significant discomfort in the afflicted ear's side, and their tympanic membranes were all highly congested on examination. In addition, 70 % had URTIs prior to the AOM assault, and 33.3% had an AOM infection before.

**Table (1): Clinical presentation of AOM group:**

| | AOM | |
|---|---|---|
| | No. | % |
| **Pain** | 59 | 100.0 |
| **Fever** | 25 | 43.3 |
| **URTI** | 41 | 70.0 |
| **Recurrence** | 20 | 33.3 |
| **TM (congested)** | 59 | 100.0 |

**Table (2): Values of Ca2+ and their mean ± SD in AOM group:**

| | **AOM** | | **Normal** |
|---|---|---|---|
| | Range | Mean ± SD | |
| **Ca2+** | 7.1 - 9.2 | 8.14 ± 0.67 | 8.7-10.7mg/dl |

**Table (3): Values of Ca2+ and their mean ± SD in the control group are:**

| | **Control** | | **Normal** |
|---|---|---|---|
| | Range | Mean ± SD | |
| **Ca2+** | 7.09–9.02 | 8.22 ± 0.61 | 8.7-10.7mg/dl |

It was noted that hypocalcemia is statistically insignificant in AOM group & the control group as, 43 (73.3%) out of 59 children of AOM group have low serum $Ca^{2+}$ and 7 (70%) out of 10 children of the control group have low serum $Ca^{2.+}$

Comparison between normal and abnormal value (hypo & hypercalcemia) of $Ca^{2+}$ in AOM and control group in table (4) show that abnormal value of $Ca^{2+}$ is statistically insignificant in AOM group & the control group as shown in Figure (1)

Abnormal levels of serum $Ca^{2+}$ in relation to age, sex, URTI & recurrence: it was found that abnormal levels of serum calcium were statistically

insignificant in both younger age group (1-6) years & the older age group (7-13) years and statistically insignificant relation to sex, but it was found that there is a highly statistically significant relation in children who have recurrent attacks of AOM and URTI. Table (5)

Table (4): Comparison between normal and abnormal value of $Ca^{2+}$ in AOM and control group

| $Ca^{2+}$ | Group | | | | P. value |
|---|---|---|---|---|---|
| | AOM | | Control | | |
| | No. | % | No. | % | |
| Abnormal | 43 | 73.3 | 14 | 70.0 | 0.838 |
| Normal | 16 | 26.7 | 6 | 30.0 | 0.132 |

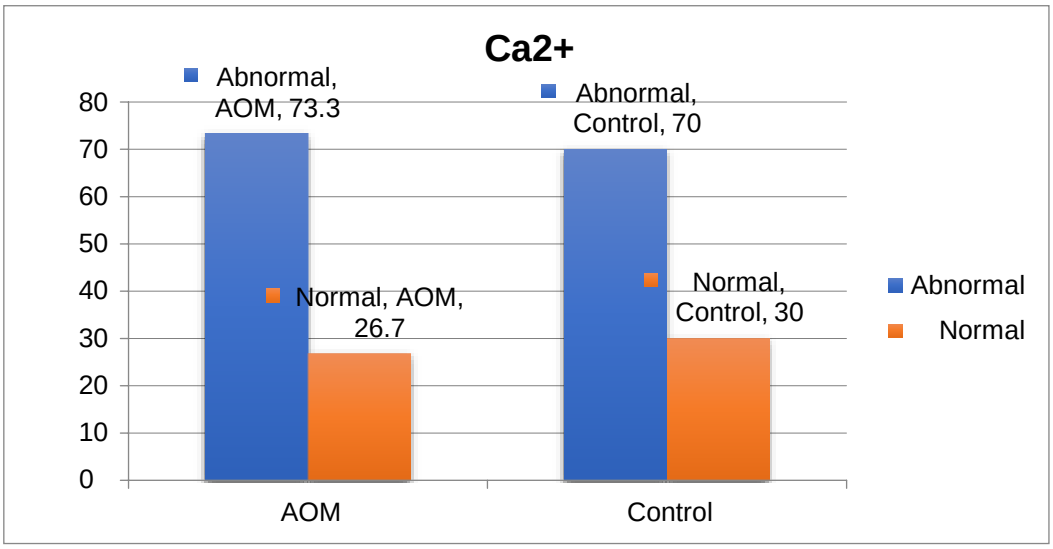


Fig. (1) The distribution of the normal and abnormal value of $Ca^{2+}$ in AOM and control group

Table (5): Abnormal serum level of $Ca^{2+}$ in relation to age, sex, and URTI & recurrence rate of AOM

| | Abnormal serum level of Ca2+ | | P. value |
|---|---|---|---|
| | AOM | | |
| | % | No. | |
| **Age** | | | |
| Younger age (1-6) | 77.3 | 33 | 0.118 |
| Older age (7-13) | 22.7 | 10 | |
| Mean ± SD | 0.373 | | 5.16 ± 3.04 |
| **Sex** | | | |
| Male | 59.1 | 26 | 0.307 |
| Female | 40.9 | 17 | |
| **URTI** | 72.7 | 32 | 0.061 |
| **Recurrence** | 36.4 | 16 | 0.001 |

## ***Discussion :***

In high-resource nations, AOM is one of the most common disorders in children and the most common reason for antibiotic prescriptions. AOM is currently recognized as a bacterial consequence of viral URTI, but viruses can cause AOM on their own. The high prevalence of AOM is due to the high prevalence of viral URTIs. About 35% of URTI episodes are complicated by AOM, occurring mainly within the first week of URTI onset. [9-10]

Because URTI assaults (viral or bacterial) enhance the risk of AOM, the risk of OM rises during the winter months, when URTIs are common. Colonizing microorganisms in the nasopharynx are a common source of AOM agents. Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis are the most common. In 10-40% of AOM instances, no agent can be isolated. These are hypothesized to be caused by viral agents. Syncytial virus, rhinovirus, adenovirus, and influenza virus are all important viral agents. When proper therapy is not delivered, life-threatening complications can occur.[11-12]

Immune cells kill bacteria by engulfing them in the phagosome, a membrane- enclosed compartment. When foreign particles bind to receptors on phagocyte membranes, the $Ca^{2+}$ mobilizing second messengers inositol trisphosphate (InsP3) and sphingosine-1-phosphate (S1P) are produced intracellularly. The release of $Ca^{2+}$ from the endoplasmic reticulum (ER) causes store operated $Ca^{2+}$ entry (SOCE) channels in the plasma and/or phagosome membrane to open, resulting in continuous or oscillatory increases in cytosolic $Ca^{2+}$ concentration. Some, but not all, phagocytic receptors require cytosolic $Ca^{2+}$ elevations for efficient ingestion of foreign particles, and these elevations tightly control the ensuing phases in the formation of phagosomes. $Ca^{2+}$ is needed for the solubilization of the actin meshwork that surrounds newborn phagosomes, as well as the fusion of phagosomes with lytic enzyme granules, essential for the superoxide-generating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex's formation and activation. Furthermore, $Ca^{2+}$ entry occurs only at physiological voltages, necessitating the activation of proton channels to counterbalance the phagocytic oxidase's depolarizing effect. [2]

$Ca^{2+}$ signals have been found in immune system cells such as T and B cells, NK cells, mast cells, DCs, monocytes, and macrophages, where they aid in cell activation, effector activities, gene expression, and differentiation. About four decades ago, $Ca^{2+}$ signals were discovered to be critical for RNA production and mitotic cell division in leukocytes and thymocytes.[13]

The lack of TCR-mediated $Ca^{2+}$ signals results in decreased interleukin-2 (IL-2) production and T-cell proliferation in vitro, as well as inefficient TCR- mediated immune responses in vivo, according to a study of mutant T cells deficient for Ca2+ influx.[14]

In practically all eukaryotic cells, including immune system cells, $Ca^{2+}$ serve as a universal second messenger. $Ca^{2+}$ signals are required for lymphocyte activation, differentiation, and effector functions such as the establishment of a stable synapse between T cells and antigen-presenting cells (APCs), as well as vesicle exocytosis in cytotoxic T cells (CTLs). Following the interaction of immunoreceptors on the cell surface, an influx of $Ca^{2+}$ into the cytosol ensues. The principal method for increasing intracellular $Ca^{2+}$ concentrations in

lymphocytes is store-operated calcium entry (SOCE) through calcium release-activated calcium (CRAC) channels, which is required for T and B cell activation and cytokine gene production.[15]

Lymphocytic activation, proliferation, and effector activities are significantly hampered in the absence of persistent $Ca^{2+}$ input through CRAC channels.[13]

In Our study we found that hypocalcemia is statistically significant in the recurrent cases in AOM group than the control group. Also, we found that low level of serum $Ca^{2+}$ is statistically significant in AOM preceded by URTI infection. 43 (73.3%) out of 59 children of AOM group have low serum $Ca^{2+}$ and is statistically insignificant than the control group. Also, low levels of serum $Ca^{2+}$ were statistically insignificant in both younger age group (1-6) years & the older age group (7-13) years and statistically insignificant relation to sex.

In the literature, till now there is no study was done to evaluate the effect of low serum $Ca^{2+}$ on acute otitis media to compare our study results with it, however, in agreement with the important role of normal serum $Ca^{2+}$ for good immune system function, a relationship between recurrent acute otitis media and low level of serum $Ca^{2+}$ was found in this study. So, serum $Ca^{2+}$ play an important role in the recurrence of AOM in our children and can share in the regimen of treatment of AOM when it found low to prevent its recurrence and not to affect the quality of life of children.

## Conclusion:

Children with recurrent AOM had significantly lower serum calcium levels than healthy children. So, Calcium can share in the treatment regimen of recurrent AOM to prevent further recurrence.

**Conflict of interest**: There is no conflict of interest.

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