



Studying the Relationship between Mg and Lymphocytes in Elderly People in Saudi Arabia

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Abstract

Magnesium (Mg) has become a common area of interest for many researchers due to its central role in the immune system. This research studied Mg's impact on the immune system functionality in different age groups in the Saudi population. The influences on the immune system on the ageing process were evident in the production of B and T cells being reduced in the thymus and bone marrow and the lowered mature lymphocytes' function in the secondary lymphoid tissues. Therefore, immuno-senescence was associated with elderly individuals in which their response to immune challenges was not as drastic as with the younger population. Studies have suggested that the impaired immune function and diminished resistance to infection could be nutritional intercession as this may reverse the effects associated with ageing. Two hundred participants of young (25-35 years) and older (55-70 years) Saudi adults of both genders were recruited from Jeddah city, Mg was measured in standards, plasma samples, and blanks (sample diluent) by using inductively coupled plasma-atomic emission spectroscopy. T cell, B cell, and natural killer cell levels were determined by flow cytometry. From the results, our data proved that Mg levels were lower in elderly people compared to the younger group, and had significant effects on the T cell and B cell activity in elderly people.

Disciplinary: Bioscience & Biochemistry, Medicine & Health Science (Immunology), Nutrition Science.

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1 Introduction

As the second most abundant cation in cellular systems, Mg carries out many and varied biological functions, including a controlling role in enzyme inhibition or activation, complexing with the negatively charged groups (phosphates in nucleic acids), and regulatory roles by

modulating cell cycle progression, differentiation, and cell proliferation. Several studies in the nutritional immunology research area have reported that significant deficiencies in macronutrients have significant impacts on the immune system functionality. These deficiencies were also linked to the high morbidity and mortality among patients suffering from infectious diseases (Pae et al. 2012). In elderly people, an imbalance of Mg can be linked to defective membrane functions, stress susceptibility, diabetes, and immune dysfunction, inflammation, and cardiovascular diseases (Rayssiguier et al, 1993). Within elderly people, it is usual to find reduced nutrient intake, particularly for vitamin D and Mg. Studies have shown significant interactions for these nutrients, and that they play an important role in the immune system, which have been linked to interactions with other biosystems such as calcium (McCoy & Kenney, 1996). Deficiencies in these essential elements interfere with fundamental immune system processes, such as effects on enzymatic and hormonal actions, cell cycle regulation, cell transformation, reactive oxygen species production, and nuclear DNA/chromatin stabilization. Many *in vitro* and *in vivo* studies have reported that there are Mg-dependent functions involved in immune cell activity, release, and synthesis. For example, in animals Mg deficiency is associated with impaired cell-mediated and humoral immunity function. This can also affect the activity and production during adhesion of mononuclear phagocytes and granulocytes. Furthermore, Mg deficiency has been linked to reductions in the immunoglobulin G (IgG) level as well as increases in IgE. This observation indicates there is a role for Mg in the immediate hypersensitivity response. Interestingly, Mg was found to play a crucial role in the alternative pathway complement activation and maybe also by the classical pathway (specific immunity) via the antigen-antibody complexing to trigger the immune response (Tam et al. 2003).

Additionally, immuno-senescence which is a case where ageing altered the adaptive and innate immune cells can be influenced by several factors including environment, genetics, nutritional status, and lifestyle of individuals. During aging. It has been suggested that the humoral immune response mediated by B cells is compromised during ageing. The function of T helper cells undergoes age-related impairment, which contributes to the observed reduced immune response of the humoral system, and there is also evidence that B cells form intrinsic defects during ageing. Studies have shown that in aged mice there are substantial decreases in the numbers of pre-B cells and pro-B cells that are exported from the bone marrow (Tam et al., 2003; Frasca et al., 2011), whereas peripheral B cell numbers remain at a relatively constant level which is similar to the pattern of T cell homeostasis seen during ageing. These results indicate that the peripheral B cells have increased longevity (Tam et al., 2003). Other studies have shown Ig blood concentrations remained increased or unchanged generally, but there was also evidence that the antibody (Ab) specific response decreased during ageing (Wagner et al., 2018). For elderly people, the higher non-specific Ab levels, lower affinity and levels of specific Ab, and vaccination resulting in decreased IgG isotype class switching show how the less optimal humoral immune response can be characterized (Tam et al., 2003; Wagner et al., 2018). Furthermore, as age increases specific Ab

response declines, and autoantibody levels increase (Coquette et al., 1986), which could explain why there is an increased risk for the elderly of developing autoimmune diseases (Galland 1988; Tam et al., 2003; Pae et al., 2012).

This study aimed to investigate the relationship between aging, immune cells, and Mg in elderly people in the Saudi population.

2 Materials and Methods

2.1 Participants

Two hundred male and female participants who were younger adults (aged 20-30 years) and older adults (55-75 years) were recruited from King Abdul Aziz University students, King Abdul Aziz University Hospital. The criteria for exclusion included the presence of cancer, liver or kidney diseases, non-Saudis, and other age groups. Based on their age, the participants were divided into two groups (51 young males, 51 young females, 49 older males, and 49 older females). The study was approved by the Ethical Committee, Faculty of Medicine, King Abdul Aziz University, and the Health Affairs of Jeddah city, and all participants gave written informed consent.

2.2 Preparation of Plasma Samples

Blood 3 ml were collected in trace-element free tubes according to the instruction provided by IZiNCG (2007a). Plasma samples were separated and stored at -80°C for later use. A 1:10 dilution of plasma samples was prepared by adding 0.5 ml of plasma to 4.5 ml of sample diluent. Sample diluent was prepared by adding 8.34 ml of ultra-pure HCl in a 2 L volumetric flask and the volume was completed to the mark by adding MilliQ water. Sample diluent was used as blank.

2.3 Analytical Method

Mg was measured in standards, plasma samples, and blank (sample diluent) by using inductively coupled plasma-atomic emission spectroscopy (Magnesium 279.077).

2.4 Analysis of T cells, B cells, and Natural Killer by Flow Cytometry

Fresh blood 3 ml was used to separate the buffy coat which coat was then washed with normal saline and centrifuged at 300 g for 10 min to form the cell pellet. Then, 1 ml of freezing media (50 % RPI 1640 + 40 % fetal calf serum + 10 % DMSO) was added to the 47 pelleted leukocytes and the sample was stored at -80°C for later immunophenotyping of leucocytes by flow cytometry. At the time of analysis, buffy coat samples were thawed at 37°C, washed with 3 ml of PBS, and centrifuged at 1600 rpm for 10 min at 4°C. The cell pellet was then resuspended in 200 µl PBS. 30-40 µl of each sample (~500,000 cells as counted by VI-CELL cell viability analyzer, Beckman Coulter) was added equally to 3 flow cytometry tubes. The first tube contained 20 µl of cocktail monoclonal antibody (CD3/CD16/CD56 antibody, FITC-PE conjugate) (Thermo Scientific). The second tube contained 20 µl of another cocktail monoclonal antibody (CD3/CD19/CD45 antibody, FITC-PE, Cy5-PE) (Thermo Scientific). The third tube contained 10 µl of each of the following antibodies: CD14 PE, CD15 APC, and CD45 FITC (Thermo Scientific). The mixture was incubated in a refrigerator for 30 min, then washed and centrifuged at 1600 rpm for 7 min, and the pellet was

resuspended in 1 ml of PBS. Lastly, 10 μ l of 7AAD dye (BioLegend) were added to tubes 1 and 3 to exclude dead cells from analysis. The samples were analyzed using a Navios Flow Cytometer (Beckman Coulter). The instrument was calibrated before each run by Flowcheck Pro Fluorospheres (Beckman Coulter) to check the laser. CD3⁺ cells were measured for T cell levels, CD56⁺ cells for natural killer cell levels, and CD19⁺ cell levels for B cells.

3 Results

The results of this study showed Mg levels (Figure 1) were decreased significantly in elderly people ($p < 0.0001$), and that T cells populations (Figure 1) were significantly lower in the elderly groups compared with young adults ($p < 0.0001$). However, B cells populations and NK cells (Figure 1) were not significantly changed between the two groups. There was a significantly positive correlation between T cell numbers and Mg levels ($p < 0.005$). However, there was no correlation between B cell levels and Mg levels (Figure 2).

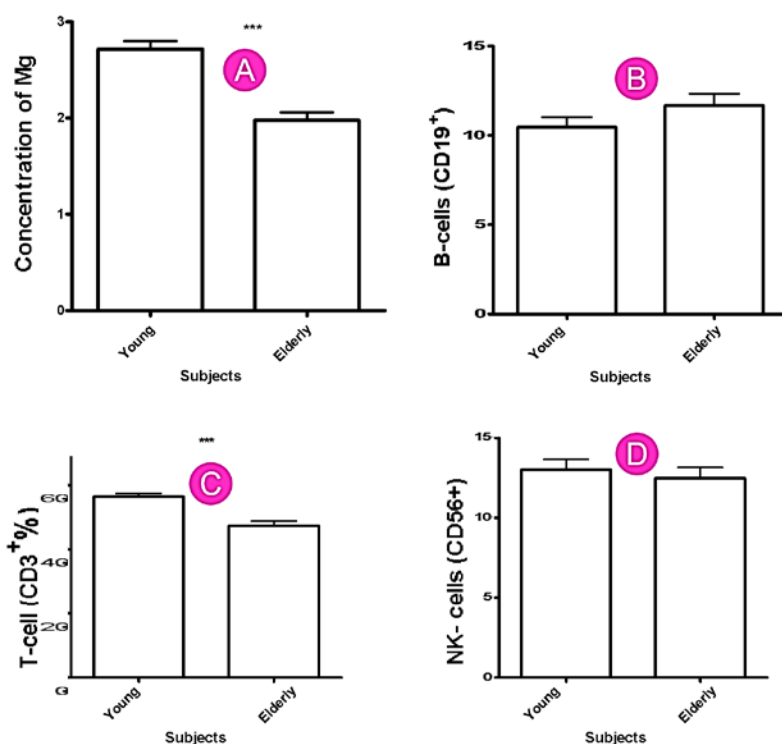


Figure 1: Test results comparison between young and elderly people.

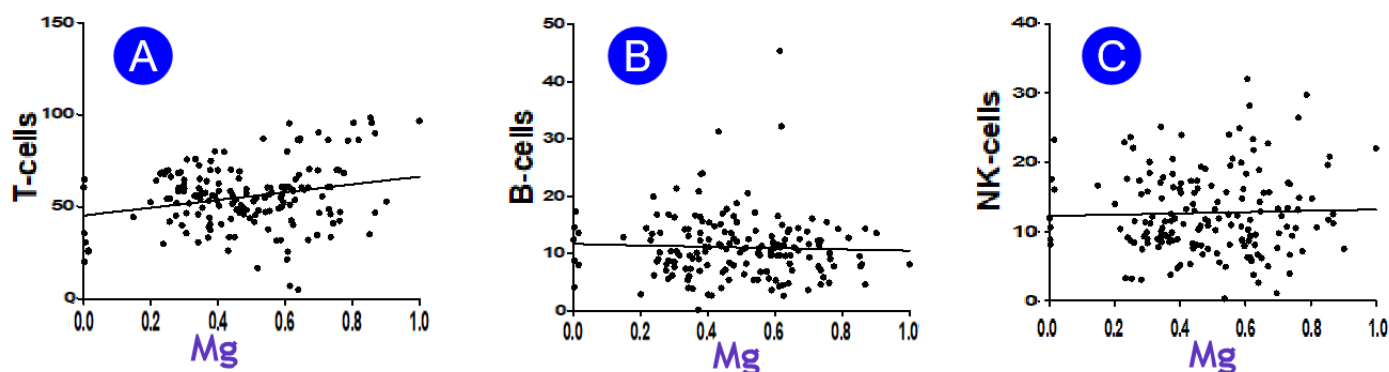


Figure 2: Scatter plots between Mg and T-cells, B-cells, and NK cells.

4 Discussion

Studies of immunology and nutrition have shown that there is a key role for Mg in the immune response, working as a co-factor for immune cell adherence, immunoglobulin synthesis, antibody-dependent cytotoxicity, T helper–B cell adherence, macrophage response to lymphokines, and IgM lymphocyte binding (Galland, 1988). During aging, the level of Mg may be reduced for two main reasons: changes in Mg metabolism or insufficient intake. It has also been shown that a deficiency in Mg may be involved in the aging process itself, contributing to age-related disease vulnerability (Rayssiguier et al, 1993). Our data showed higher mean plasma Mg concentrations in younger adults when compared to older adults. Interestingly, there was a strong negative correlation between Mg levels and the T cell population. This confirms the importance of Mg concentration as a crucial contributor in the regulation of the thymus gland function, which helps in regulating the activity of the cell-mediated response in young subjects. This agreed with previous studies that indicated Mg plays a role in the function and proliferation of lymphocytes, including adhesion-recognition during the reaction of cytolytic T lymphocytes.

According to the literature, thymus involution has been linked to Mg deficiency. For the effects of Mg deficiency within the human body, one of the most significant results is the increased apoptosis level found in the thymuses of h-deficient rats when compared with the control group (Malpuech-Brugere et al., 1999; Tam et al., 2003). Rats fed an Mg deficient diet for 8 days also showed signs of inflammation clinically, such as splenomegaly and leukocytosis. In Mg deficient rats the spleen cell suspensions contained higher numbers of adherent cells, which confirms previous studies that have suggested in the spleens of these rats there are increased numbers of macrophages (Malpuech-Brugere et al., 1998). Another study presented very interesting findings concerning the role of Mg deficiency at the early stages of development as they linked it to gene expression in rat thymocytes (Petrault et al., 2002).

Regarding lymphocytes concentrations, the results presented in this research showed decreased numbers of T cells in elderly people compared with young subjects. Unlike the T cells, B cells didn't show any significant changes between the two age groups. The aging process affects both humoral and cell-mediated immune responses. Research in humans and animal models showed that the largest alterations are observed in T cells (Tam et al., 2003; Pae et al., 2012). Aging affects the early development of T cells in the thymus as well as the peripheral lymphoid tissue where their function, expansion, and differentiation are affected. Aging leads to thymic involution, which reduced T cell differentiation and maturation and resulted in a significant failure in the output of new T cells (Coquette et al. 1986; Pae et al., 2012). These findings agree with our findings in this research. Despite the limitations in the T cell production and function in elderly people, evidence from the literature suggests there is a positive role for higher levels of antigen-experienced memory T cells compared to the naive T cells (Roberts-Thomson et al., 1974; Nagel et al. 1988; Wayne et al., 1990). Although it's a matter of debate, however, it is believed that despite drastic thymic involution, the production and peripheral T cell levels are well-maintained during

aging, apart from there is a moderate reduction in the numbers of naive T cells. According to some studies, the naive T cells must last longer to stabilize the T cell population, but there may be an accumulation of defects in these naive cells which would make their function less optimal (Swain, Clise-Dwyer et al., 2005; Jones et al., 2008) also cause a reduced T cell receptor diversity (Thoman and Weigle, 1981; Adolfsson et al., 2001, Larbi et al., 2004; Goronzy and Weyand, 2005; Goronzy et al., 2007; Pae et al., 2012).

5 Conclusion

Mg was measured in standards, plasma samples, and blanks (sample diluent) by using inductively coupled plasma-atomic emission spectroscopy. T cell, B cell, and natural killer cell levels were determined by flow cytometry. Results: our data proved that Mg levels were lower in elderly people compared to the younger group, and had significant effects on the T cell and B cell activity in elderly people.

This study presents Mg as a vital nutrient for the optimal cellular-immune function, as it has a direct impact on the activity of thymocytes, which is the site of maturation of T cells rather than on the production of T cells from the bone marrow. Further study is to investigate the effect of Mg on the different subpopulations of T cells and their cytokines secretion.

6 Availability of Data and Material

Data can be made available by contacting the corresponding author by email.

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