Original Article

Study of Serum Zinc Level and Its Effect on CD4/CD8 Ratio in the Elderly before and After Zinc Supplementation.

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Abstract:

Objective: The interaction between immunity, infection, and mortality in older people is of considerable scientific and clinical significance. Proper nutrition, including adequate dietary zinc intake or supplementation, could play an important role in the prevention or reduction of infectious diseases in this population. The aim of this work is to estimate the plasma zinc level in a group of healthy elderly and to find out its effect on CD4/CD8 ratio before and after zinc supplementation. **Methods:** This study was carried out on 20 healthy subjects of old people and 10 healthy young adults as a control group. In the elderly group serum zinc and CD4/CD8 ratio were measured before and after zinc supplementation. **Results:** Significant statistical decrease in serum zinc and CD4/CD8 ratio in old compared to young before zinc supplementation (p1<0.001, p1=0.005). After one month of zinc supplementation, there was significant statistical increase in serum zinc and CD4/CD8 ratio in old subjects (p2<0.001, p2<0.001). Conclusion: Ageing is associated with decrease in serum zinc and CD4/CD8 ratio. These conditions can be reversed by zinc supplementation.

Keywords: zinc, CD4/CD8, Elderly.

Introduction:

Many micronutrients affect immunity and suboptimal nutritional supply can cause an impaired immune response.[1] This is especially true for zinc, given its essential role in many immunological processes. In many elderly, the required supply of zinc is not met.[2] A multitude of influencing factors has been suggested, which include physiological, social, psychological, and economic factors. For example, reduced mobility leads to a decrease in energy requirements. The resulting consumption of smaller quantities of food also means consuming lower amounts of trace elements, including zinc. In addition, decreased intestinal absorption, which in part depends on the composition of the food, and medication like diuretics, could cause a negative zinc balance, even if there is sufficient intake. All these factors together can result in insufficient nutritional supply with trace metals in the elderly.[3] Finally, some diseases that occur with increased frequency in older people, such as diabetes, are also accompanied by zinc deficiency.[3-5]

One major mechanism by which zinc affects immunity is its role as a signaling ion (figure 1). The intracellular concentration of free zinc is regulated by three mechanisms. One is transport through the plasma membrane.[6] Another mechanism involves storage in and release from vesicles, so-called zincosomes, in which zinc is stored as a complex with multiple ligands.[7] Finally, zinc binds to metallothionein (MT). Through its 7 binding sites with different affinities, MT buffers zinc in the pico- to nanomolar range, and can additionally be controlled by release of zinc by oxidation of zinc-binding cysteine thiol residues.[8]

Zinc signals, i.e. changes in the intracellular concentration of free zinc mediated by these three mechanisms, act on immune cell signal transduction.[9] The first example was protein kinase C (PKC), which has been identified as a molecular interaction partner for zinc in T cells.[10] Its N-terminal regulatory domain contains four Cys3His zinc binding motifs. Zinc treatment stimulates PKC kinase activity, its affinity to phorbol esters, and binding to the plasma membrane and cytoskeleton. Furthermore, zinc chelators inhibit the induction of these events by physiological activators of PKC.[9] The lymphocyte protein tyrosine kinase (Lck), a Src-family tyrosine kinase, is an example for a different mechanism by which zinc acts on signal transduction. Zinc ions promote activation of Lck and its recruitment to the T cell receptor complex by linking two protein interface sites. The N-terminal region of Lck is recruited to the intracellular domains of the membrane proteins CD4 or CD8 by a'zinc clasp' structure.[11-13]

Aging of the immune system, also referred to as immunosenescence, describes the age-related changes in immune function that lead to increased susceptibility of older people to infectious diseases, autoimmunity, and cancer. The capacity of the immune system to mount an adequate response decreases with age, starting around 60, but several factors such as lifestyle and underlying disease can significantly affect the onset in each individual.[14] As it could be expected from the decline in immune function, aged patients suffer from an augmented incidence and mortality of infectious diseases such as pneumonia [15] and tuberculosis [16], and re-infections with herpes zoster.[17] The frequency of autoimmune diseases is augmented with age, too, accompanied by an increase in autoantibodies, which is, interestingly, not observed in centenarians.[18,19] A prominent feature of immunosenescence is thymic involution. This leads to a decrease in the generation of new T cells, finally resulting in a lower number of naïve (CD45RA+) and a higher number of memory (CD45R0+) T cells.[20]

The main changes of aging affect the T cell system. T cells from elderly subjects show decreased proliferation in response to T cell receptor (TCR) stimulation or mitogens [21], an altered CD4/CD8 ratio, and higher expression of CD95 and the pro-apoptotic BAX combined with a decrease in BCL-2 and p53, which leads to increased apoptosis.[22] The expanded subsets were primarily CD8 positive whereas CD4 populations remained unchanged. Monoclonal expansion has been found for T cells from elderly subjects. The expanded subsets can make up a large fraction of T cells, but no signs of malignant transformation have been reported.[23] However, T helper cells are also affected by aging, showing a decreased TH1/TH2 ratio in the elderly, measured by CCR4/CCR5 surface expression.[24] The TH1 cytokines IFN-γ, IL-2, and sIL-2R are reduced. In contrast, TH2 cytokines IL-4 and IL-10 are increased, resulting in a shift toward TH2 cytokines.[25,26] The effects of ageing on T cells are summarized in figure 2.

Subjects and Methods:

After approval of local ethics committee, this study was conducted on thirty subjects who were divided into two groups, the first was the elderly group which was divided into before and after zinc supplementation. The second group was 10 healthy young adults as a control.

Subjects with the following criteria were excluded from the study; patients who had diabetes mellitus, chronic hepatic diseases, collagenic diseases, subjects under immunosuppressive therapy or drugs affecting immunity and subjects taking vitamin and/or mineral supplements in the last 6 months. All subjects were subjected to thorough history taking and clinical examination, routine laboratory investigations including: CBC, fasting postprandial blood sugar, tests to asses lipid profile, tests to asses renal functions: Creatinine clearance (modified equation) and complete urine analysis, tests to asses liver functions: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum albumin.

Specific investigations: Estimation of serum zinc and CD4/CD8 ratio before and after 1 month of zinc supplementation (zinc sulphate 50 mg/day) in elderly. Estimation of serum zinc and CD4/CD8 ratio was done to the young control group.

Results:

Table I shows that among cases, the mean lymphocytes count was 26.84±4.42 (ranged between 18-34) and 32.52±2.45 (ranged between 29.6-37) in the control group with significant statistical decrease in lymphocyte count in old compared to young ($p<0.001$).

Table II shows that the mean serum zinc among cases before zinc supplementation was 60.4±13.73 (ranged between 43 and 90) and after zinc supplementation was 92.1±9.4 (ranged between 78 and 107). Among subjects of the control group, the mean serum zinc was 111.8±21.05 (ranged between 85 and 149). Significant statistical decrease in serum zinc in old compared to young before zinc supplementation ($p1<0.001$). After one month of zinc supplementation, there was significant statistical increase in serum zinc in old subjects ($p2<0.001$).

Table III shows that among cases, the mean CD4% was 33.05±7.01% (ranged between 20 and 50) before zinc supplementation and after zinc supplementation was 51.55±6.02% (ranged between 40 and 61). Among subjects of the control group, the mean CD4% was 46.9±12.03 (ranged between 30 and 68). Significant statistical decrease in CD4% in old compared to young before zinc supplementation ($p1<0.006$). After one month of zinc supplementation, there was significant statistical increase in CD4% in old subjects ($p2<0.001$).

Table IV shows that among cases, the mean CD8% was 42.7±7.83 (ranged between 34 and 60) before zinc supplementation and 37.35±5.73 (ranged between 30 and 50) after zinc supplementation. Among subjects of the control group, the mean CD8% was 28.4±6.57 (ranged between 19 and 40). Significant statistical increase in CD8% in old subjects compared to young before zinc supplementation ($p1<0.001$). After one month of zinc supplementation, there was significant statistical decrease in CD8% in old subjects ($p2=0.002$).

Table V shows that among cases, the mean CD4/CD8 ratio was 0.8±0.17 (ranged between 0.4-1.2) before zinc supplementation and 1.39±0.2 (ranged between 1.1 and 2) after zinc supplementation. Among the subjects of the control group, the mean CD4/CD8 ratio was 1.79±0.86 (ranged between1-3.6) before zinc supplementation. Significant statistical decrease in CD4/CD8 ratio in old compared to young before zinc supplementation ($p1=0.005$). After one month of zinc supplementation, there was significant statistical increase CD4/CD8 ratio in old subjects ($p2<0.001$).

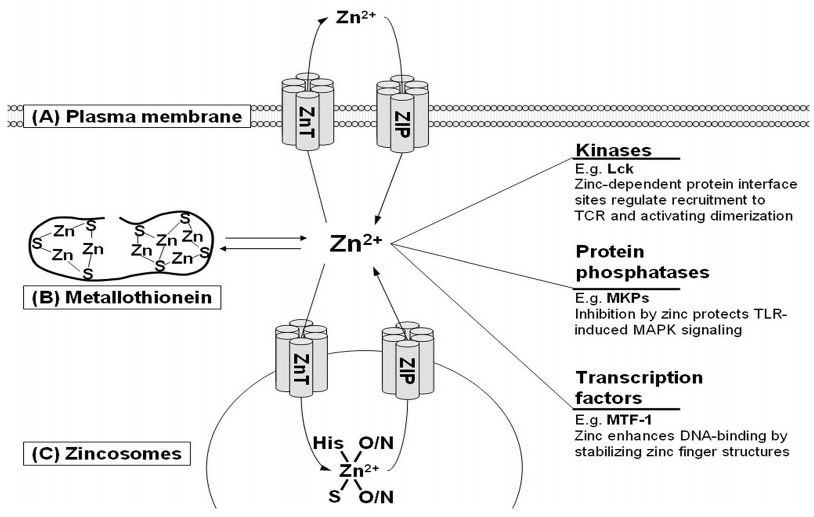

Figure 1: Zinc as a signal molecule for immune cells.

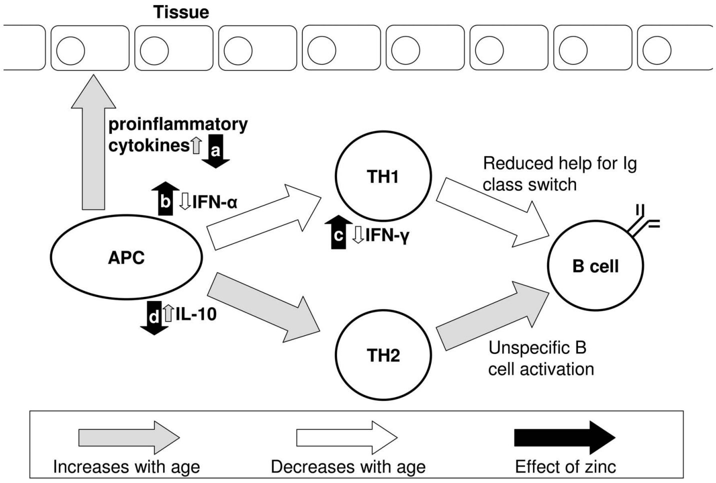

Figure 2: Influence of zinc on age-related changes of immune function.

Table (I): Comparison between the two studied groups according to WBCs picture differential count

	Control (n = 10)	Cases (n = 20)	t	p
Neutrophils				
Min. - Max.	49.0 – 61.50	49.0 – 68.0		
Mean ± SD.	57.34 ± 3.80	58.42 ± 4.75	0.621	0.540
Median	58.15	58.50		
Lymphocytes				
Min. - Max.	29.60 – 37.0	18.0 – 34.0		
Mean ± SD.	32.52 ± 2.45	26.84 ± 4.42	4.531*	<0.001*
Median	31.80	27.95		
Monocytes				
Min. - Max.	3.50 – 6.50	2.50 – 8.0		
Mean ± SD.	5.10 ± 1.04	4.81 ± 1.64	0.507	0.616
Median	5.20	4.45		

Table (II): Comparison between the two studied groups according to serum zinc before and after zinc supplementation

	Control (n = 10)	Cases (n= 20)	
		Before	After
Serum zinc			
Min. - Max.	85.0 – 149.0	43.0 – 90.0	78.0 – 107.0
Mean ± SD.	111.80 ± 21.05	60.40 ± 13.73	92.10 ± 9.40
Median	108.0	56.50	91.50
p_1		<0.001*	0.017*
p_2		<0.001*	

Table (III): Comparison between the two studied groups according to CD4 before and after zinc supplementation

	Control (n = 10)	Cases (n= 20)	
		Before	**After**
CD4			
Min. - Max.	30.0 – 68.0	20.0 – 50.0	40.0 – 61.0
Mean ± SD.	46.90 ± 12.03	33.05 ± 7.01	51.55 ± 6.02
Median	45.50	33.0	51.0
p_1		0.006*	0.273
p_2		<0.001*	

Table (IV): Comparison between the two studied groups according to CD8% before and after

	Control (n = 10)	Cases (n= 20)	
		Before	**After**
CD8%			
Min. - Max.	19.0 – 40.0	34.0 – 60.0	30.0 – 50.0
Mean ± SD.	28.40 ± 6.57	42.70 ± 7.83	37.35 ± 5.73
Median	29.0	40.50	38.0
p_1		<0.001*	0.001*
p_2		0.002*	

Table (V): Comparison between the two studied groups according to CD4/CD8 ratio before and after

	Control (n = 10)	Cases (n= 20)	
		Before	**After**
CD4/CD8 ratio			
Min. - Max.	1.0 – 3.60	0.40 – 1.20	1.10 – 2.0
Mean ± SD.	1.79 ± 0.86	0.8 ± 0.17	1.39 ± 0.20
Median	1.55	0.80	1.35
p_1		0.005*	0.177
p_2		<0.001*	

Discussion:

In the current study plasma zinc was measured in a sample of elderly individuals. plasma zinc was found to be lower than normal in 60% of the cases in comparison to a sample of young adults suggesting age-related decrease in plasma zinc concentration (p1<0.001). After zinc supplementation (50 mg of zinc sulfate for one month) to elderly there was increase in plasma zinc (p2<0.001). This is summarized in table 2 and figure 4. Haase et al[27] reported that, in general, plasma zinc levels decrease with advancing age. Hotz et al [28] in the second NHANES revealed that serum zinc levels increase into the third decade of life and decline from that age. Andriollo-Sanchez et al [29] found that the decrease of zinc level in healthy elderly is detected in both men and women in comparison to mean concentrations in young adults. Such results substantiate the evidence that, in a healthy ageing body zinc content progressively declines, leading to a consistent loss of plasma zinc. For physiological, social, economic and psychological reasons, elderly subjects are at risk of zinc deficiency. This occurs in agreement with the results of previous studies as Blumberg et al,[30] McClain et al.[31] Sowers et al[32] suggested that the decrease in the serum zinc with ageing may be related to the nutritional status of the elderly people, because the principle sources of bioavailable zinc include more expensive food items e.g. meat and seafood. In addition, the increase intake of prescription and over-counter medications may contribute to a compromised zinc status as they decrease the absorption of zinc. Coudry et al [33] reported that after 3 months of zinc supplementation (at 15 or 30 mg/day), a beneficial effect on plasma zinc concentration was obtained in subjects aged over 55 years old.

In the present study, we have found statistically significant decrease in CD4/CD8 ratio in old compared to young (p1=0.005) and after one month of zinc supplementation (50 mg of zinc sulfate), there was significant statistical increase in CD4/CD8 ratio in old subjects (p2<0.001). This is summarized in table 5 and figure 7. Olsson et tal [34] and Happert Fa et tal [35] reported that CD4/CD8 ratio decline with age. Hodkinson et al [36], the Zenith study, which aimed to investigate the effect of zinc supplementation on immune function in a total of 147 individuals, aged 55-70 years(77 women, 70 men). They describe no effect on some markers of immunity (natural killer cells) or inflammation (C-reactive protein), but only increased the ratio of CD4/CD8 T lymphocytes post zinc supplementation at month 6, suggesting that longer term Zn supplementation at a moderate dose (15 mg Zn/d) may result in maintenance of these lymphocyte subpopulations. Fortes et tal [37] in a study conducted on 209 healthy residents from Casa di Risopo Roma III, a home for older people (65 years of age or older) in Rome, Italy, reported that with zinc supplementation (25 mg as zinc sulfate for 3 months) there was statistically significant increase in the absolute number of CD4 cells and CD8 cells. Beck FWJ et al [38] and Prasad AS et al[39], in T cell subpopulation studies revealed that the CD4 to CD8 ratio was significantly related to zinc status. A decrease in this ratio was observed during zinc deficiency (for 6 weeks) but was corrected by zinc supplementation. Mocchegiani et al[40], Franceschi etal[41], Kahmann et al[42,43] and Chiricolo et al[44], in different studies reported that following zinc treatment (even if as zinc sulphate form) at the dose of 15 mg

Zn++/day for 1 month at alternating cycles in Down's syndrome subjects, in elderly and in old infected patients restores thymic endocrine activity, lymphocyte mitogen proliferative response, CD4(+) cell number, NK cell cytotoxicity, pro-inflammatory cytokine production and DNA repair. Bogden JD et al[45], Provinciali M et al[46] and Stewart-Knox et al[47] report that no effects on cell mediated and humoral immunity were observed when zinc was used at high doses and as zinc sulfate (90 mg/day for 3–6 months).

In the current study there was significant increase of CD8% in old subjects (p1<0.001) that decreases after zinc supplementation. (p2=0.002) as table 4 and figure 6 show. We also discover that there is a decrease in lymphocyte % in old subjects compared to young (table 1, figure 3). Chandra RK et al [48] found that the number of circulating T lymphocytes is slightly decreased. The number of CD4 is decreased, where as the number of CD8 cells is variously reported as normal, decreased, or increased. Wikby A et al[49] ,Strendhall J et al[50], Wikby A et al[51] and Pawelec G et al[52] in another recent sweedish studies, OCTO and NONA studies which aimed at identifying factors predicting 2, 4 and 6 year mortality rates and have resulted in the emerging concept of an Immune Risk Profile (IRP). The IRP defined from healthy octogenarians and nonagenarians, characteristically display (i) high levels of CD8+ and low level of CD4+ T-cells (an inverted CD4+:CD8+ ratio), (ii) an increase in the number of dysfunctional terminally differentiated memory T-cells (CD8+CD28-).

From all these studies, a physiological dose of zinc applied for a long period or high doses of zinc for short periods might induce limited effects on the immune response, perhaps due to zinc accumulation in various organs and tissues with subsequent toxic effect of zinc upon the immune functions.[53] In this context, it is also useful to remember that high doses of zinc trigger apoptosis of the immune cells in the presence of high-oxidative stress and inflammation.[54] Therefore, caution is advised for the management of zinc supplementation with the suggestion to perform the trial for short periods and on alternate cycles only.[53]

Conclusion:

Zinc ions are indispensable for immune function, especially for T cell mediated events, which are primarily affected in immunosenescence. The high prevalence of zinc deficiency in old subjects and the correlation between zinc status and immune function surely justifies zinc supplementation to these subjects to normalize zinc levels, and hereby restore important functions of the immune system. One central question remains: Should the decrease of zinc status with age be seen as a marginal zinc deficiency, which, in combination with multiple other factors, increases the susceptibility for infectious diseases and cancer, and should zinc be given to those with no clinical symptoms? From the results published so far, it looks like a moderate zinc supplementation that stays well below the limits for adverse effects could have substantial benefits. However, a rapid and reliable method for the assessment of zinc status would be helpful to identify those who would benefit most from zinc supplementation.

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