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Improved Immunity Elderly after Receiving Zinc

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Abstract
Advanced age is one of the population groups that are at risk to changing immune function. Changes in the body's immune function primarily in cell-mediated immune system. In accordance with increasing age resulted in the immune system of the elderly decreased immune response against infectious and non-infectious diseases. Based on that caused the elderly easy to develop diseases such as infectious diseases, hypertension, coronary heart disease, cancer, autoimmune disease, and other chronic diseases. The increasing number of elderly is one indicator of the success of development as well as challenges in development. The purpose of this study was to analyze the increase in elderly immunity after getting zinc. This research is a pre-experimental research in the treatment group were given a sample that zinc supplements. We then measured IL-2, IFN-γ and IL-10 in each group. After that the measurement results analysis to determine differences in the expression of immune aging. Analysis of normality and homogeneity test for determining the parametric or non-parametric statistics with the Shapiro-Wilk test. Furthermore, if eligible, the parametric analysis used in this research is to use before after t test to see the effects of each intervention group (pre-post test) and if it does not qualify using nonparametric statistics were similar. The results showed that zinc supplementation has the potential to raise the profile of IL-2, IFN-γ and IL-10 were significantly (p <0.05) in the elderly. Zinc supplementation would increase IL-2, IFN-γ and IL-10 so as to maintain the health of the elderly. Suggestions elderly should consume zinc to maintain immunity to stay healthy.

Keywords*: Elderly, zinc, immune, IL-2, IFN-γ and IL-10*

I. INTRODUCTION

Based on data from the Central Statistics Agency (BPS) showed that the number of elderly people in Indonesia increased starting in 2004 amounted to 16,522,311, and in 2006 amounted to 17,478,282, and in 2008 amounted to 19,502,355 which is 8.55% of the total population amounting to 228 018 900, while in 2020 predicted that the number of elderly increased by about 28 million people. This is a very large number so that if there is no effort to improve the welfare of elderly from now would cause problems and could be a big problem in the future. The tendency of these types of problems is also marked with numbers corresponding Susenas elderly dependency BPS 2008 amounted to 13.72%. Figures dependency high and the population will be felt by the population of productive age when coupled with the number dependence of population aged less than 15 years, where the current population of less than 15 years amounted to 29.13% (Martono, 2010).

Meanwhile, life expectancy (UHH) increasing human Indonesia where the National Medium Term Development Plan (RPJMN) The Ministry of Health in 2014 is expected to occur in life expectancy of 70.6 years in 2010 to 72 in 2014 which will lead to changes in the age structure population. Based on projections Bappenas number of senior citizens 60 years and older will increase from 18.1 million in 2010 to double to 36 million people in 2025 (BPS, 2010). According to the Basic Health Research (RISKESDAS) in 2007 showed that urban elderly shows morbidity of 27.42, 33.35 for the rural elderly and morbidity rate amounted to 31.11 towns and villages. From these data indicate a trend in morbidity in the elderly has increased from year to year. Diseases in the elderly suffer the most was followed by a joint disorders hypertension, cataracts, stroke, emotional mental disorders, heart disease and diabetes mellitus. In addition it is the cause of death at the age of 65 years in men are stroke (20.6%), chronic lower respiratory disease (10.5%), Tuberculosis (TB) (8.9%), hypertension (7.7%), NEC (7.0%), ischemic heart disease (6.9%), other heart diseases (5.9%), diabetes mellitus (4.9%), liver disease (4, 4%) and pneumonia (3.8%). Sementara pada perempuan penyebab kematian terbanyak adalah stroke (24,4 %), hipertensi (11,2 %), NEC (9,6 %), penyakit saluran pernafasan bawah kronik (6,6 %), diabetes mellitus (6,0 %), penyakit jantung iskemik (6,0 %), penyakit jantung lain (5,9 %), TB (5,6 %), pnemonia (3,0 %) dan penyakit hati (2,2 %) (Balitbangkes, 2007).

This condition should certainly get the attention of various parties. Elderly ailing would be a burden for the family, the community and even the government, so it would be a burden in development. The process of population aging will have an impact on the emergence of a variety of life issues such as: social, economic, cultural, educational, health mainly due to the increasing age of the organ function will decrease either due to natural or due to illness. If it is not anticipated at this time, it is probable that the development process will encounter a

variety of obstacles. Therefore, the problem of the elderly should be the concern of all parties, not only the government, public institutions and society it self. The mindset that had been there that the elderly population is a vulnerable community to be borne by the family, society and the state, to be changed. Elderly should be an opportunity nation must continue to be empowered. To be elderly healthy, productive and independent, must be at the start with a healthy lifestyle and preparing for elderly times better (Hidayat, 2010). Numerous studies show that the prevalence of malnutrition among the elderly is very high and often only realized when the elderly had to be hospitalized. A study in Jakarta showed that about two-thirds of the elderly suffer from a deficiency of thiamin (www.bkkbn.go.id. Up date 11/12/2015).

Immune function also decreases with age, resulting in increased incidence of infections and malignancy (cancer). Research on immune function in the elderly introduced the idea that the immune system in the elderly have specific characteristics, the immune system not only decreases with increasing age, but the regulation of immune system disorders will be progressive throughout his life. Early changes occur in the immune system humoral than cellular, the evolution of the immune system associated with reduced function of the thymus. Nutritional factors play an important role of the immune response in healthy elderly. The aging process also leads to a decrease in immune function (immunosenescence), which can increase the tendency to hyporesponsiveness vaccination and infectious diseases and non-communicable. Immunosenescence characterized by a decrease in the number of circulating CD3 T cells, increased production of interleukin-6 (IL-6), IL-1 and tumor necrosis factor-α (TNF-α) by peripheral blood mononuclear cells, cell activity decreased as both phagocytosis and natural killer (NK) (Aspinall and Andrew, 2004).

Nutritional deficiencies are often found in developing countries and a zinc deficiency found in many types of diseases. Almost every cell in the human body there are zinc and is vital to the approximately 300 types of enzymes that are useful in a variety of biochemical processes in the body. Zinc is known to play an important role on the immune system of various types of infectious and degenerative diseases. Zinc also serves to catalyze the energy metabolism, carbohydrate and fat, degradation / protein synthesis, nucleic acid synthesis, synthesis of heme, transport CO2. Zinc is also a component of cell membranes, stabilize the function of RNA, DNA and ribosomes, complex stabilizes the hormone and its receptor, as well as its role in regulating the polymerization of tubulin. In addition, the physiological functions of zinc is important for the growth of cells and tissues, repklikasi cells, bone formation, skin integrity, cell mediated immunity, the operation of various hormones. The impact of zinc deficiency in a person would increase vulnerability to various types of pathogens (Prasad, 2007). In addition, it also showed the symptoms of wound healing to be late, infectious diseases become easier, taste and smell acuity systems to be down, infections and malignancies (Keenan and Morris, 1993 in Hidayat 2010).

Problems in the elderly is the importance of enhancing the role of the levels of IL-2 as a cytokine for the proliferation of T lymphocytes, IFN-γ is a proinflammatory cytokines and IL-10 as a cytokine antiinflammatory on the immune response, the study will focus on "enhanced immunity elderly after receiving zinc supplements ".

II. MATERIAL AND METHODS

A. Study design

This type of research is the study of pre experimented with zinc supplements. We then measured IL-2, IFN- γ, and IL-10 to see the effect of supplementation on the expression of immune zinc elderly.

B. Location and Time Research

Studies zinc supplementation location on the immune response of elderly will be conducted in Posyandu Elderly in Puskesmas Mangasa in Makassar. Zinc supplementation on samples carried out for 3 months starting in November 2015 through to February 2016 (90' days), with weight control and nutrition of the elderly.

C. Population, Sample and Sampling

The population studied is the entire elderly in Puskesmas Mangasa Makassar. The samples are aged between the age of 60-70 years and become active participants in Elderly Posyandu Puskesmas Mangasa Makassar, clinically healthy. Sample size is the numbers of the population to be sampled. The sample size of each treatment group was calculated using the following formula (Kuntoro, 2010): $n = (\frac{(Z_{1-\alpha}+Z_{1-\beta}).^2\sigma^2}{\Delta^2})$

Where : n = number of sample
Z = the value of z
α = level of significance 5%
β = power of test 10%
σ^2 = variant of IL-2 (assumed to be) the same on each-each group with 3,24
Δ = difference differences between the mean increase in IL-2 (assumed) every month between treatment groups zinc treatment was 1,8.

Based on the sample size formula above, then obtained the required number of samples each is 9 person. To avoid the drop out of the sample, then use the formula Higgins = 1/(1-f) where f is 20% of the total sample. Furthermore, the sample size was n = 1/(1 to 0,2) x 9 = 11,25 ≈ 12. With the addition of the total sample now 12 people.

D. Data Collection

Primary data that IL-2, IFN-γ and IL-10 will be collected before and after the intervention ie in November 2015 to February 2016 (90 days). Secondary data are the characteristics of the samples collected at the same time.

E. Data analysis

Analysis of normality and homogeneity test for determining the parametric or non-parametric statistics with the Shapiro-Wilk test. Furthermore, if eligible, the parametric analysis used in this research is to use before after t test to see the effects of each intervention group (pre-post test). Furthermore, if the data are not normally distributed then used nonparametric statistics were similar.

F. Presentation of data

Data are presented in tabular form accompanied by narration to see the correlation between variables.

G. Ethical clearance

Conduct in this study reviewed and recommended by the Ethics Committee for Health Research Health Polytechnic Makassar.

III. RESULTS

A. Characteristics of respondents

This study uses 12 elderly people aged between 60-70 years with random allocation residing in Puskesmas Mangasa Makassar City RW 2 and RW 5 with the following characteristics: Makassar tribe, religion Islam, gender elderly person is 4 males and 8 females. Before and after the supplementation blood sample is drawn through the veins of each at 5 cc. Examination of blood samples carried out in the Laboratory Teaching Hospital (RSP) Unhas. Measurement of body weight and nutrition conducted every month during supplementation. Of the 12 elderly were given zinc supplement obtained from one of the pharmacies in the city of Makassar. Characteristics of respondents (sample) can be seen table 1. The results showed that the average weight for 3 months of interventioan 56,83±17,76 kg, average protein intake of 36,83±10,65 g and average zinc intake 3,65±1,4 mg. This suggests that this indicates that the weight factor and protein intake is not a factor that affected the results because it fits the needs of the elderly ($p > 0,05$) and than zinc intake were statistically significant ($p < 0.05$). Based on the fulfillment of its main nutrient intake of protein ranged between 56-66% zinc intake ranged from 28 to 36.5% dietary allowances. It shows that the nutritional intake is still lacking due to under 90% of the nutritional adequacy rate (ADI).

Table1: Characteristic of Respondents (sample) Elderly

	Zinc	p
Body weight	56,83±17,76 kg	0,058
Protein intake	36,83±10,65 g	0,058
Zinc intake	3,65±1,4 mg	0,031

B. Effect on immune response zinc IL-2

Based on Table 2 shows that an increase in the average levels of IL-2 before and after the intervention. According to the data analysis can concluded that there is significant influence Zinc supplementation on levels of IL-2 to increase, but still being dynamic.

Table 2 Characteristic of Response Zinc IL-2

	Zinc Supplementation		
	Before	After	p
Aveage IL-2	100,74±12,61	657,13±174,21	0,000

C. Effect on immune response zinc IFN-γ

Based on the analysis of the data shows an increase in IFN gamma before and after the intervention, but in a state of balance (homeostasis). Statistical analysis showed that zinc supplementation before and after was significant. Data can be seen in the table below:

Table 3 Characteristic of Response Zinc IFN- γ

	Zinc Supplementation		
	Before	After	p
Aveage IFN- γ	83,34±15,33	152,29±50,84	0,000

D. Effect on immune response zinc IL-10

Based on research results show that IL-10 increases before and after the intervention. According to the results of statistical analysis on zinc supplementation showed a significant ($p < 0.05$), but the increase in the limit immune balance.

Table 4 Characteristic of Response Zinc IL-10

	Zinc Supplementation		
	Before	After	p
Aveage IL-10	106,16±28,2	220,64±74,51	0,003

IV. DISCUSSION

A. Effect on immune response of zinc IL-2

Zinc deficiency in the elderly may impair zinc-dependent signaling, and thereby immune function. In one recently published study, peripheral blood mononuclear cells (PBMC) from zinc-deficient elderly showed impaired NF-κB activation and interleukin (IL)-2 production in response to stimulation with PHA, which was corrected by in vivo supplementation of zinc (45 mg/day as gluconate) for 6 months or ex vivo supplementation of zinc to PBMC (Prasad, Bao, Beck,Sarkar, 2006) indicating a link between zinc deficiency and the effect of zinc on NF-κB signaling. The results showed that zinc supplementation increases the levels of IL-2 were significantly ($p < 0.05$). based control of variable nutritional studies show that the intake of protein and zinc itself is less than the value of nutritional adequacy (ADI).

Thymulin is a thymus-specific hormone and it requires the presence of zinc for its biological activity to be expressed. Thymulin binds to high-affinity receptors on T cells, induces several T-cell markers, and promotes T-cell function, including allogenic cytotoxicity, suppressor functions, and interleukin-2 (IL-2) production. Because zinc deficiency affects IL-2 production and T-cell activation adversely, we have investigated the role of zinc on NF-κB (nuclear factor-κB) activation in HUT-78, a T helper 0 (Th0) human malignant lymphoblastoid cell line. We showed for the first time that, in zinc deficient HUT-78 cells, the activation of NF-κB was affected adversely (Prasad, 2008).

The production of interleukin-2 (IL-2) is a key and early event in the activation of T-lymphocytes. IL-2 triggers peripheral T-lymphocytes to enter the S phase of the cell cycle and to divide. This is probably a result of the suppressive effect of IL-2 on cell cycle inhibitors, which interfere with the activity of cyclin-dependent kinases (cdks) at checkpoints of the cell cycle. IL-2 also is involved in the differentiation of thymocytes, peripheral T- and B-lymphocytes and other cells of hematopoietic origin. A short segment of DNA, 275 base pairs in the promoter area of IL-2 gene, integrates numerous signaling pathways leading to IL-2 synthesis and the activation and proliferation of T-lymphocytes). Both the murine and human IL-2 promoters contain one binding site for genuine Rel/NF-κB factors. The sequence of this site, GGGATTTCAC, is identical for both promoters. NF-κB factors are induced rapidly by a variety of stimuli activating T cells. Almost every stimulus leading to T cell activation also activates NF-κB. Induction of IL-2Rαgene expression also is mediated by the induced nuclear expression of NF-κB (Prasad, 2008).

Zinc is a micronutrient essential for innate and adaptive immunity. Zinc deficiency affects both parts but mainly the adaptive immune system. Altered immune properties observed with age and zinc deficiency bear striking similarities in, for example, reduction of thymus and thymic hormone activity, pro-inflammatory status, T cell subpopulation imbalances, and decreased functionality of innate immunity. Many studies show an inadequate intake of zinc, but not a clear prevalence of zinc deficiency in the elderly. Although many of the elderly are not or only slightly zinc-deficient, faint changes in zinc are able to modify immune response. Hence, most studies reveal an improvement of the immune status of the elderly after zinc supply (Uciechowski & Lothar, 2015).

For more than 50 years, zinc is known to be an essential trace element, having a regulatory role in the immune system. Deficiency in zinc thus compromises proper immune function, like it is observed in the elderly population. Here mild zinc deficiency is a common condition, documented by a decline of serum or plasma zinc levels with age. This leads to a dysregulation mainly in the adaptive immunity that can result in an increased production of pro-inflammatory cytokines, known as a status called inflamm-aging. T cell activation as well as polarization of T helper (Th) cells into their different subpopulations (Th1, Th2, Th17, regulatory T cells (Treg)) is highly influenced by zinc homeostasis. In the elderly a shift of the Th cell balance towards Th2 response is observed, a

non-specific pre-activation of T cells is displayed, as well as a decreased response to vaccination is seen. Moreover, an impaired function of innate immune cells indicate a predominance of zinc deficiency in the elderly that may contribute to immunosenescence. This review summarizes current findings about zinc deficiency and supplementation in elderly individuals (Maywald and Lothar, 2014).

B. Effect on immune response of zinc IFN-γ

The results showed that the zinc supplementation increases the levels of IFN gamma significantly. Zinc is an agent efficacy against defenses based homeostasis. As it is known that the function of trafficking zinc (zinc in these macrophages) is zinc in macrophages and T cells were very decisive, realize the inflammatory signal to build and improve inflammatory response in which the antimicrobial increased IFN-γ and IL-1 β increases. Zinc deficiency will result in reduced T-cell growth, inhibit cytokine production by T cells and inhibit the activity of B cells, T cell activity is highly dependent on the presence of zinc (Gruber, 1995). In addition to these zinc deficiency also resulted in cell mediated immunity does not function optimally, especially the T-cell response decreases, the ratio of Th1 / Th2 Th1 cells decreased which in this case is IFN-γ (Prasad, 2008).

IFN-gamma receptor expressed on the surface of virtually all cells. IFN-gamma binding to the receptor induces receptor oligomerization and activation, through trans-phosphorylation, receptor associated Janus kinase 1 and 2 (JAK1 and JAK2). Activated JAK phosphorylates intracellular domain of the receptor (eg, tyrosine 440 of human IFNGR1) that serves as a docking site for Signal transducer and activator of transcription 1 (STAT1). STAT1 is phosphorylated on tyrosine 701, undergo dimerization, translocates to the nucleus and regulate gene expression by binding to gamma-activated sequence (GAS) element in the IFN-gamma gene promoter (Subowo, 2010).

Several kinase can phosphorylate STAT1 on serine 727 (Ser727). This phosphorylation is not required for STAT1 translocation to the nucleus or to binding promoter. However, it is very important for the activation of transcription in full. Kinases are included in the delta Protein Kinase C (PKC-delta) and Calcium / calmodulin-dependent protein kinase II (MKNR II). The exact mechanism of activation of IFN-gamma-inducible kinase is not clear. However, it shows that IFN-gamma activate phosphatidylinositol 3-kinase (PI3K) / v-AKT murine thymoma viral oncogene homolog (AKT) signaling pathway, probably through an adapter Cas-Br-M ecotropic sequence of transformations retroviral (c-CBL) which binding subunit of PI3K (reg PI3K class 1A). PKC-delta is an effector of the PI3K pathway. Although the mechanism of PKC-delta activation of PI3K-dependent is not clear, PI3K dependent phosphorylation of PKC-delta by 3-phosphoinositide dependent protein kinase-1 (PDK (PDPK1)). IFN-gamma also induces c-CBL-mediated activation of v-CRK sarcoma viral oncogene homolog CT10 bird-like (CrkL). It provides a link between IFN-gamma receptor and Rap guanine nucleotide exchange factor 1 (C3G) and results in IFN-gamma -dependent activation of RAP1A, member of RAS oncogene family (Rap1A), a protein known to exhibit tumor suppressor activity and mediate IRF1 participate in the activation of suppressor of cytokine signaling-1 (SOCS-1). SOCS-1 protein is very important to inhibit IFN-gamma response (Schroder, Hertzog, Ravasi ,Hume, 2004).

Recent papers demonstrate an influence of zinc transporters on signal transduction. Zrt/Irt-like protein (ZIP)7 releases Zn from the ER, controlling tyrosine phosphorylation (Taylor et al, 2008), and lysosomal ZIP8 is required for zinc-mediated calcineurin inhibition and interferon (IFN)-γ expression in T cells (Aydemir, Liuzzi, McClellan, Cousins, 2009). Conversely, there also exist feedback mechanisms, which act on zinc homeostasis. The promoters of MT and of several zinc transporters are under the control of the metal-response element binding transcription factor (MTF)-1. In contrast to other transcription factors with zinc fingers that bind zinc constitutively, its DNA-binding is regulated by the stabilization of zinc finger motifs by free cellular zinc (Cousin, Liuzzi, Lichten, 2006; Laity & Andrews, 2007; Lichten & Cousins, 2009).

IFN-gamma induces the expression of SOCS1 indirectly, by inducing the expression of IRF-1 transcription factor STAT1 through. IRF-1 in turn stimulates the transcription of SOCS-1 gene. Some of the STAT1 protein interacts with and modulates its transcriptional activity that CREB-binding protein (CBP and p300), minichromosome maintenance protein 5 (Mcm5) and breast cancer susceptibility gene 1 (BRCA1). CBP and p300 have a histone acetyl transferase activity and function as co-activators. Mcm5 and BRCA1 association with phosphorylated STAT1 and increase the activity of transcription (Schoenborn & Wilson, 2007).

Moreover, IFN-gamma may activate the JAK-STAT pathway-independent. Calcium-dependent tyrosine kinase protein tyrosine kinase 2 beta PTK2B (Pyk2 (FAK2)) is a substrate for JAK2. Pyk2 (FAK2) phosphorylates mitogen-activated protein kinase kinase kinase 4 (MEKK4). MEKK4 phosphorylated in turn phosphorylates mitogen-activated protein kinase kinase 6 (MEK6). Furthermore, MEK6 phosphorylate p38 MAPK phosphorylates and activates Activating transcription factor 2 (ATF-2). Protein-tyrosine phosphatase 2C (SHP-2) regulates this pathway by dephosphorylating MEKK4 and kinases that activate, Pyk2 (FAK2) (Schroder, Hertzog, Ravasi, 2004). Another pathway is stimulated by IFN-gamma involving mitogen-activated protein kinase kinase kinase 1 (MEKK1), mitogen-activated protein kinase kinase 1 (MEK1) and mitogen-activated protein kinase 1

and 3 (ERK1 / 2). MEKK1 / MEK1 / ERK1 / 2 cascade regulates the activity of CCAAT binding protein beta (C / EBP-beta) and C expression / EBP-beta-driven Interferon regulatory factor 9 (IRF9) gene. IRF9 is a subunit of the transcription complex ISGF3 participating in interferon signaling. The results show that zinc is the drug of choice because it is based on the efficacy of homeostasis (Akhavan and Rudikoff, 2008).

C. Effect on immune response of zinc IL-10

Results showed that zinc supplementation increases the levels of IL-10 was significantly ($p < 0.05$). The trace element zinc is essential for the immune system, and zinc deficiency affects multiple aspects of innate and adaptive immunity. There are remarkable parallels in the immunological changes during aging and zinc deficiency, including a reduction in the activity of the thymus and thymic hormones, a shift of the T helper cell balance toward T helper type 2 cells, decreased response to vaccination, and impaired functions of innate immune cells. Many studies confirm a decline of zinc levels with age. Most of these studies do not classify the majority of elderly as zinc deficient, but even marginal zinc deprivation can affect immune function. Consequently, oral zinc supplementation demonstrates the potential to improve immunity and efficiently downregulates chronic inflammatory responses in the elderly (Haase & Lothar, 2009).

In addition, alterations in the balance of TH1/TH2 cytokines occur that are similar to the effects observed during zinc deprivation. 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 (Uciechowski et al. 2008).

Proliferation and cytokine secretion in response to stimulation with PHA were analyzed in lymphocytes isolated from healthy elderly (70–85 y.) subjects with mean zinc intake and serum and erythrocyte levels within the normal range. There was a positive trend for a correlation between proliferation and serum zinc in male subjects. Furthermore, the production of IL-10 in response to PHA showed a negative correlation with erythrocyte zinc in males, while baseline and PHA-stimulated production of this cytokine were negatively correlated with serum zinc in females (Finamore et al, 2005).

V. CONCLUSION

Zinc supplementation in the elderly will increase IL-2, IFN-γ, and IL-10 so as to maintain the health of the elderly.

VI. SUGGESTION

To maintain the balance of the immune system of the elderly, it is advisable to take a zinc supplement every day. Further research to see differences in immune mechanisms in elderly men and women in urban and rural.

A. Conflict of Interest

Authors declare no conflict of interest within this research

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