Zinc Supplementation Improves Anticancer Activity of Monocytes in Type-2 Diabetic Patients with Metabolic Syndrome

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Abstract. *Background: Transmembrane tumor necrosis factor (TNF)-α, found on monocytes, is a body's key defense against cancer. In patients with type 2 diabetes mellitus (DM) and metabolic syndrome, immunity is suppressed, resulting in a high risk of several inflammatory disorders and cancer. Patients and Methods: Seventeen patients with type 2 DM and metabolic syndrome were supplemented with either 30 mg of elemental zinc/day or placebo for eight weeks. Transmembrane TNF-α-expressing monocytes and lymphocytes, and plasma TNF-α levels were analyzed before and after supplementation. Results: The present study revealed that zinc supplementation significantly increased the proportion of monocytes expressing transmembrane TNF-α. While the plasma TNF-α levels and TNF-α expressing lymphocytes were not significantly altered in the zinc-treated and placebo groups, higher proportion of TNF-α bound monocytes were observed in the zinc-treated group. Conclusion: Because functional transmembrane TNF-α was shown to be implicated in defense mechanisms, these findings suggest that zinc supplementation may benefit immune response against cancer in patients with DM and metabolic syndrome.*

It is well-documented that tumor necrosis factor (TNF)-α is an important pro-inflammatory cytokine playing a role in the pathogenesis of several inflammatory diseases (1). TNF-α is primarily produced by immune cells and adipocytes (2) like transmembrane TNF-α, which can be proteolytically cleaved by the TNF-α converting enzyme (TACE) to soluble TNF-α (3). In general, transmembrane TNF-α exerts biological functions through cell-to-cell contact and cell-type-specific manners, while the soluble form of TNF-α mediates its functions through less specific endocrine actions (4). Focusing on cancer, the function of transmembrane TNF-α in inhibition of cancer progression by eliminating cancer cells but preserving normal cells is accepted as being an important mechanism of the defense machinery against cancer (5, 6).

Importantly, *in vivo* evidence indicates that transmembrane TNF-α leads to death of cancer cells by a safer and more retainable antitumor activity than that of secreted TNF-α (7). Impaired mitogen-stimulated TNF-α production from immune cells in patients with diabetes mellitus (DM) has been proposed (8), and this alteration may be a possible mechanism for increased risk of cancer in these patients (9). A strategy which increases patients' functional transmembrane TNF-α would be beneficial in the attenuation of infections as well as the risk of cancer.

Metabolic syndrome is known to increase the risk of diabetes, and the presence of such metabolic abnormalities in patients with DM has been shown to lead to complications, especially cardiovascular disease and cancer (9, 10). It is interesting that an increased production of TNF-α by adipose tissue was frequently found in those with metabolic syndrome and DM (2); however, the body's overall defense function in such patients was poor (8, 11). Together with the evidence indicating that plasma TNF-α could render insulin resistance in patients with DM (12), and the membrane-bound form of TNF-α was shown to have a more potent role in eliminating an infection and unwanted cancer cells (7), investigations into the levels of transmembrane TNF-α and soluble TNF-α in patients with DM and metabolic syndrome would benefit a better understating of the roles of such a cytokine in pathology.

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Several nutrients and natural substances have been shown to possess potential immunomodulating properties (13, 14). Among those, zinc, an essential trace mineral, which plays a vital role in growth and immune function, has garnered the most attention. Deficiency of zinc results in growth retardation, loss of appetite, and impaired immune function (15-18). Interestingly, zinc supplementation has been shown to reverse immune-related problems in many diseases (19, 20). A previous study revealed that the treatment of human peripheral blood mononuclear cells (PBMCs) with zinc resulted in an increased production of several immune-related cytokines, namely interleukin (IL)-6, IL-1β, and TNF-α (21). Moreover, an *in vitro* study showed that treatments of monocytes with zinc induced cytokine production in response to lipopolysaccharide (22).

The aforementioned information leads to the notion that zinc supplementation might benefit the immune function of patients with DM and metabolic syndrome. Therefore, the present study evaluated the effects of zinc supplementation on transmembrane TNF-α-expressing monocytes and lymphocytes and on circulating TNF-α levels in such patients.

Patients and Methods

Patients. Outpatients with type 2 DM and metabolic syndrome were recruited from the Public Health Center 66, Health Department, Bangkok Metropolitan. Metabolic syndrome was defined based on the International Diabetes Federation criteria (23). The 17 patients who agreed to participate in the study gave their written informed consent after they received information about the experimental procedures. This study was approved by the Ethics Committee for Researches involving Human Subjects, Bangkok Metropolitan Administration (Registered Number: 041.54).

Study design. This study was a randomized, placebo-controlled trial. The patients were randomly assigned into either the experimental group or the placebo group. The patients in the zinc-treated group were supplemented with zinc sulfate (one capsule containing 66 mg zinc sulfate equivalent to 15 mg elemental zinc), while the patients in the placebo group received placebo (66 mg corn starch). The patients in both groups were assigned to take two capsules of the supplement daily for eight weeks. At the beginning and the end of the study, blood samples were collected for analysis of transmembrane TNF-α on monocytes and lymphocytes and of plasma TNF-α level.

Isolation of PBMCs. Blood samples were drawn from antecubital vein, and PBMCs were isolated by Histopaque density gradient (Histopaque 1077; Sigma-Aldrich, St. Louis, MO, USA). The isolated PBMCs (1×10^6/ml) were finally resuspended in 1 ml of 1% bovine serum albumin in phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 10 min. After washing with PBS, the cells were incubated with fluorescein-conjugated monoclonal antibody against TNF-α (Sigma-Aldrich, St. Louis, MO, USA) and phycoerythrin-conjugated monoclonal antibody to cluster of differentiation 14 (CD14) (Sigma-Aldrich, St. Louis, MO, USA) for an analysis of transmembrane TNF-α on monocytes by flow cytometry.

Biochemical analysis. Blood samples were collected at baseline and after eight weeks of zinc/placebo supplementation to determine complete blood count and plasma levels of zinc, fasting blood glucose and glycated hemoglobin (HbA1c). Plasma TNF-α level was also analyzed by sandwich enzyme-linked immunosorbent assay (ELISA) using human TNF-α Quantikine® HS ELISA kit (R&D systems, Minneapolis, MN, USA). The mean minimum detectable dose according to the manufacturer protocol was 0.106 pg/ml.

Statistical analysis. The data are presented as the mean±standard deviation (SD). An independent sample *t*-test was used for a statistical comparison of data between the zinc-treated group and the placebo group. The comparisons of the data within each group were made using paired sample *t*-test. Statistical significance was considered for those with a *p*-value less than 0.05.

Results

The characteristics of the patients are presented in Table I. At baseline, our results indicated no significant differences in patients' characteristics, including plasma zinc and TNF-α levels, between the placebo and the zinc-treated groups.

Blood cell and plasma TNF-α levels. In order to investigate the effect of zinc supplementation on immune cells after zinc/placebo supplementation for eight weeks, the number of monocytes and lymphocytes, and the levels of plasma TNF-α were determined. Even though our results indicate that the level of plasma zinc in the zinc-treated group were significantly increased from baseline ($p<0.05$), and such levels were significantly higher than those in the placebo group ($p<0.05$), no significant alteration was found in terms of the number of monocytes and lymphocytes after 8-week zinc supplementation (Table II). In addition, the results revealed that no significant change in plasma TNF-α levels in either the placebo or zinc groups, in comparison to the corresponding baseline took place.

Expression of transmembrane TNF-α on monocytes and lymphocytes. Figure 1 shows the percentages of transmembrane TNF-α-expressing monocytes and lymphocytes. The results indicate that transmembrane TNF-α-expressing monocytes and lymphocytes at baseline were not different between placebo and zinc-treated groups. Interestingly, after 8-week zinc supplementation, the percentage of transmembrane TNF-α-expressing monocytes of patients in the zinc-treated group significantly increased from that at baseline ($p<0.05$), while no change of those in the patients of the placebo group was observed. In addition, the percentage of transmembrane TNF-α-expressing monocytes, week 8, of patients in the zinc-treated group was significantly higher than their placebo counterpart ($p<0.05$). However, zinc supplementation caused no significant effect on the percentage of transmembrane TNF-α-expressing lymphocytes of the patients in either group after eight weeks of study.

Table I. *Characteristics of the patients at baseline*.

Parameter	Placebo group (n=9)	Zinc-treated group (n=8)
Age (years)	57.67±10.05	58.75±10.53
Body mass index (kg/m^2)	25.83±1.68	26.59±1.91
Fasting blood glucose (mmol/l)	7.96±1.91	7.78±3.36
HbA1c (%)	7.41±1.03	7.51±1.93
Plasma TNF-α (pg/ml)	1.54±0.72	1.32±0.39
Plasma zinc (μmol/l)	11.15±0.69	11.51±0.55

Data are expressed as the mean±standard deviation (SD). HbA1c: Glycated hemoglobin; TNF-α: tumor necrosis factor-α.

Table II. *The effects of zinc supplementation on plasma zinc levels, the number of monocytes and lymphocytes and plasma tumor necrosis factor (TNF)-α levels*.

Parameter	Placebo group (n=9)		Zinc-treated group (n=8)	
	Baseline	Week 8	Baseline	Week 8
Plasma zinc (μmol/l)	11.15±0.69	11.17±0.69	11.15±0.55	13.16±0.48*#
% Lymphocytes	35.83±6.99	37.91±8.44	36.18±5.63	39.69±7.11
% Monocytes	6.16±1.78	6.02±2.17	6.88±1.90	6.08±1.37
Plasma TNF-α (pg/ml)	1.54±0.72	1.52±0.84	1.32±0.39	1.16±0.38

Data are expressed as the mean±standard deviation (SD). TNF-α: tumor necrosis factor-α. Significant difference at $p<0.05$. *from baseline. #between groups at week 8.

Discussion

This study aimed to investigate the possible role of zinc supplementation in modulating immune functions of patients with DM and metabolic syndrome by characterizing the effects of such a supplementation on transmembrane TNF-α produced by PBMCs and on circulating TNF-α. Notably, the baseline plasma zinc levels of the patients in this study were found to be low compared to those in healthy individuals (24, 25). Abnormal zinc homeostasis in diabetic patients could be explained by impaired absorption or high urinary excretion (26). Deprived zinc status was associated with diabetic complications (27). Maintaining plasma zinc levels within the normal range could be accomplished with zinc supplementation and may be beneficial in optimizing zinc function and thus preventing complications in these patients. This study found that 8-week zinc supplementation resulted in improved plasma zinc levels in type-II diabetic patients with metabolic syndrome.

It is well-accepted that circulating TNF-α is associated with impaired glucose tolerance and increased insulin resistance (12), while transmembrane TNF-α was shown to be implicated in defense response against infections and cancer (7). In fact, monocytes and macrophages exhibit cytotoxic activity against tumor cells by the production of several toxic molecules, including reactive oxygen species (ROS), reactive nitrogen species, and TNF-α (7, 28). TNF-α, especially the transmembrane form has been shown to have a dominant impact on the inhibition of cancer progression (7). Our data indicate that administration of zinc at a dose of 30 mg/day for eight weeks resulted in the increase of transmembrane TNF-α but had no effect on circulating TNF-α, implying that such supplementation could have a positive effect on patient health with a lesser effect on insulin-resistant status.

DM is a group of common metabolic disorders resulting in high levels of glucose in the blood. Many factors contribute to hyperglycemia, including reduced insulin secretion, decreased glucose utilization, and increased glucose production. With metabolic syndrome, patients with type 2 DM were demonstrated to be at higher risk for complications such as cardiovascular disease compared to individuals without type 2 DM and metabolic syndrome (29). The role of transmembrane TNF-α in diabetes and metabolic syndrome is largely unknown but is currently receiving great attention. It is generally known that the impaired immune function often found in patients with DM leads to increased infections and a higher incidence of cancer (30). In case of infection, causes seem to be multi-factorial and complicated. One accepted explanation is that in DM patients this is due to defects of TNF-α expression from monocytes (30). A previous study reported an impaired activity in response to toll-like receptor (TLR)

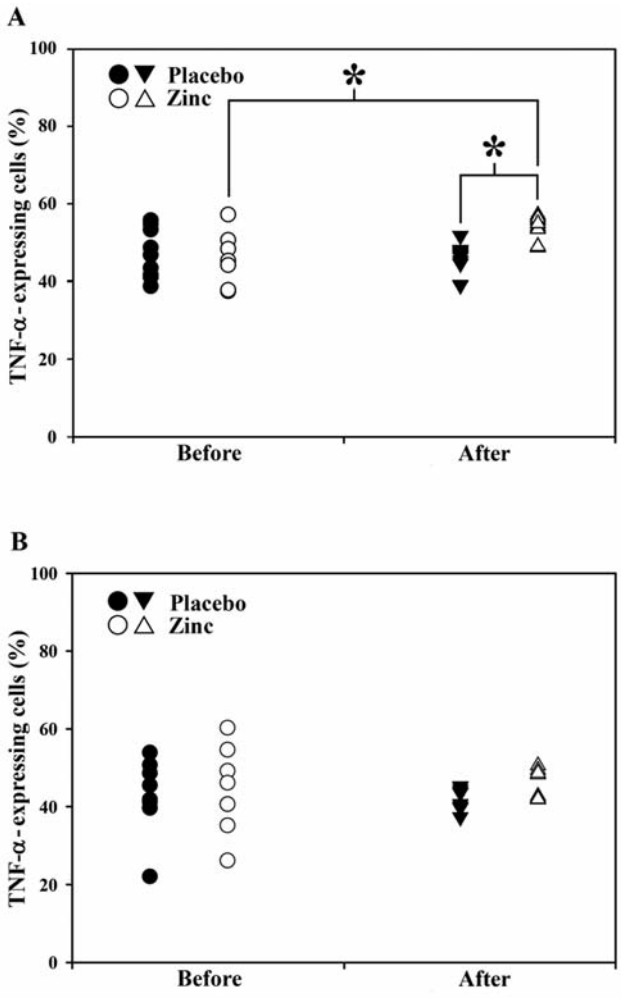

Figure 1. *The expression of transmembrane TNF-α on monocytes (A) and lymphocytes (B). *Significant difference at p<0.05.*

ligands and decreased phagocytotic activity in monocytes of type 2 diabetic patients (30). Decrease of monocyte-derived soluble TNF-α in response to stress, and abnormal PBMC function were demonstrated in patients with DM (11, 31). Consistent with the previous finding, our results support the role of zinc in enhanced TNF-α production in immune cells and further showed that monocytes are the most affected immune cells in such a case.

In conclusion, our study has provided evidence, as far as we are aware for the first time, that zinc supplementation in patients with DM and metabolic syndrome may be beneficial since it significantly enhances transmembrane TNF-α expression on monocytes while having only a minimal effect on lymphocytes and the circulating level of TNF-α. These data may support the use of zinc in patients with DM.

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References

1 Bradley JR: TNF-mediated inflammatory disease. J Pathol *214*: 149-160, 2008.

2 Xu H, Uysal KT, Becherer JD, Arner P and Hotamisligil GS: Altered tumor necrosis factor-α (TNF-α) processing in adipocytes and increased expression of transmembrane TNF-α in obesity. Diabetes *51*: 1876-1883, 2002.

3 Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ and Cerretti DP: A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature *385*: 729-733, 1997.

4 Horiuchi T, Mitoma H, Harashima S, Tsukamoto H and Shimoda T: Transmembrane TNF-α: Structure, function and interaction with anti-TNF agents. Rheumatology *49*: 1215-1228, 2010.

5 Van Horssen R, Ten Hagen TL and Errgermont AM: TNF-α in cancer treatment: molecular insights, antitumor effects, and clinical utility. Oncologist *11*: 397-408, 2006.

6 Calzascia T, Pellegrini M, Hall H, Sabbagh L, Ono N, Elford AR, Mak TW and Ohashi PS: TNF-α is critical for antitumor but not antiviral T cell immunity in mice. J Clin Invest *117(12)*: 3833-3845, 2007.

7 Li Q, Li L, Shi W, Jiang X, Xu Y, Gong F, Zhou M, Edwards III CK and Li Z: Mechanism of action differences in the antitumor effects of transmembrane and secretory tumor necrosis factor-alpha *in vitro* and *in vivo*. Cancer Immunol Immunother *55*: 1470-1479, 2006.

8 Zykova SN, Svartberg J, Seljelid R, Iversen H, Lund A, Svistounov DN and Jenssen TG: Release of TNF-alpha from *in vitro*-stimulated monocytes is negatively associated with serum levels of apolipoprotein B in patients with type 2 diabetes. Scand J Immunol *60*: 535-542, 2004.

9 Braun S, Bitton-Worms K and LeRoith D: The link between the metabolic syndrome and cancer. Inter J Biol Sci *7(7)*: 1003-1015, 2011.

10 Grundy SM, Brewer HB Jr., Cleeman JI, Smith SC Jr., Lenfant C, American Heart Association, National Heart, Lung, and Blood Institute: Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation *109*: 433-438, 2004.

11 Geerlings SE, Brouwer EC, Van Kessel KC, Gaastra W, Stolk RP and Hoepelman AI: Cytokine secretion is impaired in women with diabetes mellitus. Eur J Clin Invest *30*: 995-1001, 2000.

12 Miyazaki Y, Pipek R, Mandarino LJ and DeFronzo RA: Tumor necrosis factor α and insulin resistance in obese type 2 diabetic patients. Int J Obes Relat Metab Disord *27*: 88-94, 2003.

13 Grimble RF: Nutritional modulation of immune function. Proc Nutr Soc *60*: 389-397, 2001.
14 Maggini S, Wintergerst ES, Beveridge S and Hornig DH: Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Br J Nutr *98(Suppl 1)*: S29-S35, 2007.
15 Shay NF and Mangian HF: Neurobiology of zinc-influenced eating behavior. J Nutr *130(5S Suppl)*: 1493S-1499S, 2000.
16 Umeta M, West CE, Haidar J, Deurenberg P and Hautvast JG: Zinc supplementation and stunted infants in Ethiopia: a randomised controlled trial. Lancet *355*: 2021-2026, 2000.
17 Bao B, Prasad AS, Beck FW and Godmere M: Zinc modulates mRNA levels of cytokines. Am J Physiol Endocrinol Metab 285: E1095-E1102, 2003.
18 Cole CR and Lifshitz F: Zinc nutrition and growth retardation. Pediatr Endocrinol Rev *5*: 889-896, 2008.
19 Bao B, Prasad AS, Beck FWJ, Snell D, Suneja A, Sarkar FH, Doshi N, Fitzgerald JT and Swerdlow P: Zinc supplementation decreases oxidative stress, incidence of infection, and generation of inflammatory cytokines in sickle cell disease patients. Transl Res *152*: 67-80, 2008.
20 Baum MK, Lai S, Sales S, Page JB and Campa A: Randomized, controlled clinical trial of zinc supplementation to prevent immunological failure in HIV-infected adults. Clin Infect Dis *50(12)*: 1653-1660, 2010.
21 Ibs KH and Rink L: Zinc-altered immune function. J Nutr *133*: 1452S-1456S, 2003.
22 Haase H, Ober-Blobaum JL, Engelhardt G, Hebel S, Heit A, Heine H and Rink L: Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. J Immunol *181*: 6491-6502, 2008.
23 Alberti KG, Zimmet P and Shaw J: IDF Epidemiology Task Force Consensus Group. The metabolic syndrome-a new worldwide definition. Lancet *366*: 1059-1062, 2005.
24 Brown KH: Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries. Am J Clin Nutr *68(suppl)*: 425S-429S, 1998.
25 Al-Maroof RA and Al-Sharbatti SS: Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type 2 diabetics. Saudi Med J *27(3)*: 344-350, 2006.
26 Chausmer AB: Zinc, insulin and diabetes. J Am Coll Nutr *17*: 109-115, 1998.
27 Soinio M, Marniemi J, Laakso M, Pyörälä K, Lehto S and Rönnemaa T: Serum zinc level and coronary heart disease events in patients with type 2 diabetes. Diabetes Care *30*: 523-528, 2007.
28 Martin JH and Edwards SW: Changes in mechanisms of monocyte/macrophage-mediated cytotoxicity during culture. Reactive oxygen intermediates are involved in monocyte-mediated cytotoxicity, whereas reactive nitrogen intermediates are employed by macrophages in tumor cell killing. J Immunol *150*: 3478-3486, 1993.
29 Haffner SM: Risk constellations in patients with the metabolic syndrome: epidemiology, diagnosis, and treatment patterns. Am J Med *119(5 Suppl 1)*: S3-S9, 2006.
30 Komura T, Sakai Y, Honda M, Takamura T, Matsushima K and Kaneko S: CD14+ monocytes are vulnerable and functionally impaired under endoplasmic reticulum stress in patients with type 2 diabetes. Diabetes *59(3)*: 634-643, 2010.
31 Alexandraki KI, Piperi C, Ziakas PD, Apostolopoulos NV, Makrilakis K, Syriou V, Diamanti-Kandarakis E, Kaltsas G, Kalofoutis A: Cytokine secretion in long-standing diabetes mellitus type 1 and 2: Associations with low-grade systemic inflammation. J Clin Immunol *28(4)*: 314-321, 2008.

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