In the name of God

Shiraz E-Medical Journal
Vol. 12, No. 1, January 2011

http://semj.sums.ac.ir/vol12/jan2011/89007.htm

Effect of Zinc Supplementation on Inflammatory Markers in Women with Polycystic Ovary Syndrome.

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Received for Publication: May 24, 2010, Accepted for Publication: September 11, 2010.

Abstract:

Background: Polycystic ovary syndrome, as a low-grade chronic inflammatory state, may stimulate the immune response, increasing inflammatory factors such as C-reactive protein (CRP) and Interleukin 6 (IL-6) on one hand and zinc, another effective anti-inflammatory agent, on the other. The aim of this study is to evaluate the effect of zinc on inflammatory markers in women with polycystic ovary syndrome.

Patients and Methods: In a randomized, double-blind, and placebo controlled clinical trial, sixty patients with polycystic ovary syndrome were randomly divided into two groups, each group received one of the following daily supplement for eight weeks; group Zn: 50 mg elemental zinc (n = 30), and group P: Placebo (n =30). Fasting serum zinc levels, inflammatory markers, systolic-and diastolic blood pressure, anthropometric indices, and nutritional intake were measured at the baseline and the end of 8th week.

Results: Results indicate that after eight weeks of supplementation, mean value of nutritional intake, anthropometric indices, and systolic-and diastolic blood pressure did not change significantly in the two groups. Zinc supplementation had significant effects in increasing serum Zn ($p < 0.05$) and in decreasing serum hs-CRP and IL-6 levels ($p < 0.05$). There were no significant changes in the levels of these parameters in the placebo group.

Conclusions: The findings of the present study indicate that zinc supplementation may be considered as an inexpensive adjunct to treatments in patients with polycystic ovary syndrome in the hope of reducing cardiovascular disease risk factors, particularly inflammation.

Keywords: Polycystic ovary syndrome, Zinc, inflammation.

Introduction:

Polycystic ovary syndrome (PCOS) is a heterogeneous disease that affects about 5-10% of reproductive-age female population, which is predominantly characterized by chronic anovulation and hyperandrogenism. It also shares components of the metabolic syndrome (MS) manifested by abdominal obesity, insulin resistance, dyslipidemia, and endothelial dysfunction.[1, 2] Central adiposity appears to play an important role in the metabolic phenotype through the production of various adipocyte-derived cytokines and proteins known as adipokines.[3] Furthermore, PCOS has been described as a state of chronic low-grade inflammation mainly characterized by a modest rise in serum C-reactive protein (CRP) compared to the weight matched controls.[4-6] Substantial experimental evidence and more recent cross sectional data suggest that interleukin 6 (IL-6) and CRP, two sensitive physiological markers of subclinical systemic inflammation, are associated with hyperglycemia, insulin resistance, and overt type 2 diabetes mellitus (DM). Both of these inflammatory biomarkers are known to predict the development of cardiovascular disease in diabetic and also healthy populations.[7] IL-6, a major proinflammatory cytokine, is produced in a variety of tissues including activated leukocytes, adipocytes and endothelial cells. CRP is the principal downstream mediator of the acute phase response and is primarily derived via IL-6–dependent hepatic biosynthesis. In rodent models of glucose metabolism, the in vivo infusion of human recombinant IL-6 has been shown to induce gluconeogenesis, subsequent hyperglycemia and compensatory hyperinsulinemia. Similar metabolic responses have been observed in human subjects after administration of subcutaneous recombinant IL-6.[8] Several studies have demonstrated elevated levels of IL-6 and CRP among individuals both with features of the insulin resistance syndrome and clinically overt type 2 DM.[7, 8]

Due to the proven relationship between inflammation and many of the chronic diseases that PCOS patients often develop [9], interventional planning that emphasizes anti-inflammatory nutrients to reduce inflammatory markers in this population is of great importance. Among micronutrients, zinc is one of the most important trace elements required as a catalytic, structural, and regulatory ion for the activities of more than 300 enzymes, proteins, and transcriptional factors. Therefore, zinc is a key element in many homeostatic responses of the body, including oxidative stress and in many biological functions, including immune efficiency.[10, 11] Multiple roles of zinc as a modulator of the inflammatory mechanisms in cell cultures and animal models have been observed [12]; However, the use of zinc in the management of inflammatory biomarkers such as IL-6 and hs-CRP in patients with PCOS has not been reported. So, this study is aimed at investigating the effect of zinc supplementation on biomarkers of inflammation in patients with polycystic ovary syndrome.

Patients and Methods:

This study is a double-blind, randomized, parallel-group clinical trial of zinc supplementation at a dose (50 mg/d), compared to placebo in women with PCOS

volunteers. The study is approved by the Institutional Review Board and Regional Ethical Committee at the Medical Science University of Tabriz, Iran (5/4/2484),registered at IRCT (IRCT138803212017N2),and informed consent was obtained from participants. The diagnosis of PCOS is based on the Rotterdam criteria [13] with women satisfying at least two of the following three criteria: 1) oligomenorrhea/oligo-ovulation; 2) clinical or biochemical hyperandrogenism; 3) polycystic ovaries on ultrasound examination. Inclusion criteria for all participants were: unchanged regular physical activity at moderate level, BMI ≥ 25, and age range of 20 to 45 years. Exclusion criteria were: chronic or acute illnesses, pregnancy, hypothyroidism, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, androgen-secreting neoplasms, and current or previous (within last 2 months) use of any medications known to affect inflammation (statins, thiazolidinediones, and corticosteroids), anti-diabetic and anti-obesity drugs, insulin or vitamin and mineral supplements. None of the patients were affected by neoplastic, metabolic, or cardiovascular disorder or other concurrent medical illness (including diabetes, hypertension or kidney, liver, thyroid, autoimmune, cerebrovascular, and ischemic heart disease). All subjects were non-smokers, had normal physical activity and none of them drank alcoholic beverages. Patients were permitted to continue taking current medications, including estrogen-progestron compounds, but no new medication within 60 days and no change in dosage for the last 30 days.

In the present study, according to inclusion and exclusion criteria and medical records, 200 patients that attended the infertility and general clinics of Alzahra Hospital in Tabriz city,Iran, were chosen. After face to face interview and explanation of the objectives of the trial, 65 patients consented to participate in the study.

Patients were randomized to receive 50 mg of zinc in the form of zinc-sulphate and placebo for 8 weeks (60 days). Patients took one capsule per day in the morning for the duration of the trial. Placebo capsules contained corn sturch and they were identical to the treatment capsules in size, shape and color. At study entry and at the end of week 8, 10 cc venous blood sample was taken from each patient for complete hormonal and biochemical assays. All blood samples were obtained in the morning between 08:00 and 09:00 a.m. after an overnight fast and resting in the bed during the early follicular phase (d 2–5) of a spontaneous or P-induced menstrual cycle. During the same visits, all subjects underwent anthropometric measurements, including body mass index (BMI) and waist to hip ratio (WHR) ,and nutritional intakes for three days (two workdays and one holiday) by use of 24-hour Food Record questionnaire and analyzed by Nutrition III software. Blood samples were centrifuged immediately and sera were stored at −70°C until assayed. Serum zinc (Zn) was analyzed using an atomic absorption spectrophotometer (Model CTA-2000, Chem Tech, USA) in the laboratory of Nutrition Research Center (Faculty of Health & Nutrition). Hs-CRP concentration was determined by immuno-

turbidometric methods using autoanalyzer (model Alcyon 300 Abbott, USA and Germany). IL-6 concentration was also determined by ELISA methods (Bio-Source Europe S.A.). The systolic and diastolic blood pressure (SBP and DBP) (10 minutes seated rest, mean of two readings) was measured manually with a sphygmomanometer at baseline and after two months' supplementation. The subjects were asked not to alter their usual diets and physical activity throughout the study, and any changes in their medication were avoided whenever possible. Statistical analyses were performed with SPSS software (version 13.0). Only data from patients who had baseline and final data and did not violate the protocol, were used in the calculations. All values are expressed as mean ± SD (standard deviation). Means before and at the end of week 8 were analyzed by paired t-test. Normal distribution was tested by the Kolmogorov–Smirnov test while differences in normally distributed continuous variables were analyzed with the help of independent 't' test. The level of significance was defined at $P<0.05$.

Results:

Sixty-five patients were randomly assigned into Zn group (50 mg/d, n =35), and placebo group (n =30) and were followed up for 8 weeks. Five patients were excluded after three weeks because of intervention intolerance, and sixty patients (n =30, in the Zn group, n =30 in the placebo group) completed the study (Figure1). The baseline characteristics of the subjects allocated to zinc or placebo are presented in Table 1. There were no significant differences in age, body mass index (BMI) and nutritional intake (Table 2). At the beginning of the study, the groups were similar based upon serum levels of Zn. During the study, the Zn group showed a significantly greater increase in serum zinc compared to the placebo group ($p < 0.05$; Table 3).

Effects of zinc on inflammatory biomarkers were evaluated by comparison of mean serum levels of hs-CRP and IL-6 after 60 days of treatment. At baseline hs-CRP and IL-6 concentrations were not significantly different among the groups. Table 3 documents the significant declines in the mean serum levels of hs-CRP and IL-6 in Zn group after 8 weeks ($P< 0.05$).

Table1. Baseline characteristics of study participants

Variable	Zinc group (n= 30)	Placebo group (n=30)
Age (y)	27.17±4.58	26.93±4.77
Marriage(n)		
Single	12	10
Married	18	20
Occupation (n)		
Unemployed	10	7
Student	3	4
Housekeeper	14	18
Employee	3	1
Education (n)		
Illiterate	1	2
Elementary	6	5
Incomplete secondary	8	9
Diploma	12	10
University	3	4
Habitation (n)		
Rural	16	13
Urban	14	17
Use of medications(n)		
Type of medications		
Provera	8	7
LD	20	20
Yasmin	2	3

n = number of subjects
Data are means ± SD and were analyzed by the Student's t-test.

Table2. Comparison of clinical data of patients with PCOS in zinc and placebo groups at the baseline and at the end of 8 weeks.

Variables	All subjects (n = 60)		Zinc group (n =30)		Placebo group (n = 30)	
	At baseline	After 8 wk	At baseline	After 8wk	At baseline	After 8 wk
Weight (kg)	75.87±4.41	75.88±4.44	76.13±4.39	76.03±4.36	75.61±4.49	75.73±4.60
BMI (kg/m2)	29,39 ±1,65	29.40±1.70	29.50±1.68	29.47±1.69	29.28±1.64	29.33±1.72
WC (cm)	97.24±6.42	97.10±6.51	97.28±5.9	97.05±6.21	97.20±6.96	97.15±6.90
Hip (cm)	109.34±8.31	109.09±8.31	109.58±6.31	109.15±6.3	109.11±10.02	109.02±10.0
	0.89±0.03	0.89±0.04	0.88±0.04	0.88±0.04	0.89±0.03	2
WHR	131.69±4.95	131.96±5.04	131.08±4.86	130.65±4.7	132.31±5.04	0.89±0.03
SBP (mmHg)	87.39±2.24	87.84±1.89	87.39±2.38	87.51±2.01	87.40±2.12	133.27±5.06
DBP (mmHg)						88.17±1.72
Nutritional intake						
Energy (kcal)	1733.87±71.59	1738.86±68.67	1737.84±78.89	1742.26±76.79	1729.91±64.58	
Carbohydrate(g)	212.44±11.41	214.27±11.90	214.42±12.79	216.27±13.30	210.45±9.66	1735.47±60.
Protein (g)	62.08±4.19	62.07±4.20	62.05±4.31	61.47±3.95	62.11±4.14	61
Fat (g)	70.64±4.92	70.38±5.06	70.21±4.41	70.14±4.94	71.07±5.43	212.27±10.1
Zinc (mg)	5.45±0.80	5.59±0.81	5.36±0.83	5.57±0.91	5.54±0.78	5
						62.66±4.42
						70.63±5.26
						5.62±0.71

Note: Data are expressed as mean ± SD. Means before and at the end of week 8 were analyzed by paired t-test, and t-test was used to compare mean differences among groups. Baseline differences between groups were not significant (t-test). P values for differences in characteristics between placebo and zinc groups were all statistically insignificant (P >0.05)
SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Table 3 .Effects) change from baseline) of zinc supplementation on serum (hs-CRP, IL-6 and Zn) levels

Outcome variable	Placebo (n = 30)	Zinc (n = 30)	P versus placebo
Zinc (µg/dl)			
Baseline	78.25±4.78	76.11±5.85	_____
Final	79.30±4.70	108.18±9.70	_____
Change	1.05± 3.87	32.06±9.68	0.0001
P	0.14	0.0001	
Hs-CRP (mg/l)			
Baseline	3.47±1.60	3.62±1.47	_____
Final	3.54±1.60	1.54 ±0.80	_____
Change	0.7±0.25	-2.07±1.0	0.0001
P	0.13	0.0001	
IL-6 (pg/ml)			
Baseline			
Final	25.57±5.87	25.89±5.75	_____
Change	25.71±5.77	15.44±1.98	_____
P	0.14±0.8	-10.45±4.27	0.0001
	0.32	0.0001	

Data are means ± SD and were analysed by Student's t-test.
Baseline differences between groups were insignificant.(t-test).
*P < 0·05 for changes compared to baseline.

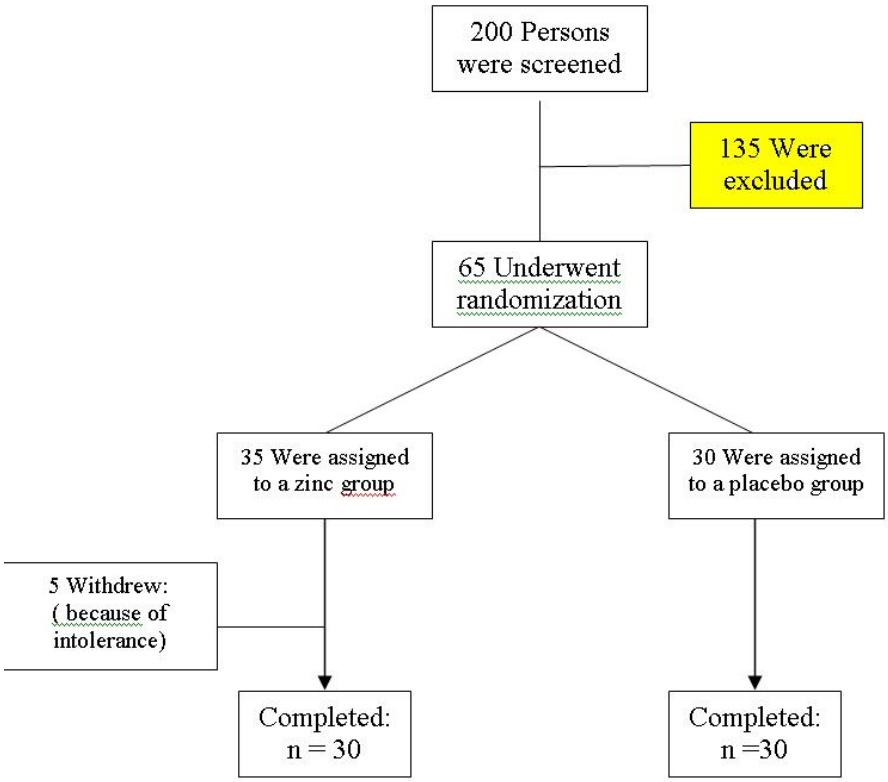

Figure 1

Discussion:

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder associated with long-term health risks, including diabetes mellitus and coronary artery disease.[14] There are a large number of evidences suggesting that patients with PCOS have increased cardiovascular risk compared with age matched controls. It has been estimated that myocardial infarction is seven times more likely in patients with PCOS.[15] Inflammation is now thought to play a key role in the pathophysiological mechanism of atherosclerosis and cardiovascular disease. Epidemiologic data have shown a relationship between elevation of CRP levels and cardiovascular risk in people with and without a history of heart disease; furthermore, it is a good predictor of vascular events.[16] Elevated CRP levels are a common feature of metabolic disease and also have been linked to insulin resistance, diabetes, the metabolic syndrome, hypertension, and other risk factors for cardiovascular diseases.[17] Hepatic production of CRP primarily is under the control of interleukin-6 stimulation.[18]

Recent evidences support an anti-inflammatory effect of zinc. Zinc is involved in fighting oxidative stress and inflammation.[12] Studies suggest that zinc nutrition can markedly modulate mechanisms of the pathology of inflammatory diseases such as atherosclerosis.[19] There are several mechanisms by which zinc might be capable of inhibiting atherogenesis. Zinc is an important component of biomembranes and an essential cofactor in a variety of enzymes.[20]

To our knowledge, this is the first prospective randomized double-blind placebo-controlled trial evaluating the effect of zinc supplementation on CRP and IL-6 levels in PCOS women. Tamakoshi et al [21] have suggested that metabolic syndrome may be related to a subclinical chronic inflammatory state. Thus, PCOS, as one of the diseases that is associated with metabolic syndrome, also may have changes in inflammation factors such as CRP and IL-6. Wu et al [22] found that the PCOS state, as a low-grade chronic inflammatory state, may stimulate the immune response, increasing inflammatory factors such as CRP and IL-6. We found that Zinc supplementation decrease CRP and IL-6 levels in PCOS women. In this study, nutritional intake and anthropometric indices is presumed as confounding factors. Therefore, neither nutritional intake change nor anthropometric indices, show the lack of effect of these variables on inflammatory markers. At the onset of our study, serum zinc, CRP and IL-6 levels were similar in two groups and there were no significant differences among them. After intervention, the zinc supplemented group presented a reduce in both CRP and IL-6 and an increase in zinc concentrations with a statistically significant difference in relation to the initial values ($p < 0.05$), as shown in Table 3. The placebo group did not present statistically significant differences.

Prasad et al.[23] reported that in comparison to the younger adults, the elderly subjects had lower plasma zinc, increased generation of inflammatory cytokines, and increased oxidative markers. Following zinc supplementation to the elderly subjects, the plasma zinc increased, oxidative stress markers de-

creased and generation of inflammatory cytokine decreased in comparison to the placebo group. Zinc has several key roles relating to cell signaling, cell activation, gene expression, protein synthesis, and apoptosis. Zinc is crucial to the normal development of immune cells, and it plays an important role in maintaining the activity of a range of immune cells, including neutrophils, monocytes, macrophages, natural killer cells, and B and T cells.[24]

A few investigators [25, 26] have reported that inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-1β are generated by activated monocytes macrophages, and increases in these cytokines are associated with decreased zinc status in patients. In another study [27], zinc supplementation to healthy human subjects aged 20–50, reduced the concentrations of the oxidative stress–related byproducts malondialdehyde (MDA), 4-hydroxyalkenals (HAE), and 8-hydroxydeoxyguanine in the plasma; inhibited the ex vivo induction of TNF-α and IL-6 mRNA in mononuclear cells (MNCs); and provided protection against TNF- α –induced nuclear factor–kβ activation in isolated MNCs. Kahmann et al [28] showed significant reductions in basal cytokine levels after supplementation, on average, of 50mg pure zinc/d. As a main marker for total amount of inflammation, IL-6 is of special importance in their study and zinc treatment leads to a 96.5% decrease in basal IL-6 release. Kandhro et al [29] demonstrated that Zn supplementation was given 5 day/wk for 6 month (30 mg/day) to patients with goitrous diseases, improve the Zn level in biological samples and resulted in decreased concentration of CRP in both genders.

Conclusions:

This study demonstrated that zinc supplement in patients with PCOS receiving their regular therapy positively affected inflammatory biomarkers as measured by the serum hs-CRP and IL-6. Zinc supplementation may represent an effective adjunctive therapy for patients with PCOS to prevent cardiovascular disease. Results of this study warrant further evaluation of the effects of this supplement on other inflammatory parameters in PCOS subjects.

Acknowledgment:

This work was supported by grants from Nutrition Research Center and Research vice chancellor, Tabriz University of Medical Sciences. We are indebted to the patients for their cooperation.

References:

1. Ehrmann D: Polycystic ovary syndrome. N Engl J Med 2005;352:1223–36.

2. Lobo RA: What are the key features of importance in polycystic ovary syndrome? Fertil Steril 2003;80:259–61.

3. Pasquali R, Gambineri A, Pagotto U: The impact of obesity on reproduction in women with polycystic ovary syndrome. BJOG 2006;113:1148–1159.

4. Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Blumenfeld Z: Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. J Clin Endocrinol Metab 2004;89:2160–5.

5. Diamanti-Kandarakis E, Paterakis T, Alexandraki K, Piperi C,Aessopos A, Panidis D: Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. Hum Reprod 2006;21:1426–31.

6. Kelly C, Lyall H, Petrie JR, Gould GW, Conell JM, Sattar N: Low-grade chronic inflammation in women with polycystic ovarian

syndrome.J Clin Endocrinol Metab 2001;86:2453–5.

7. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker SPM: C-Reactive Protein, Interleukin6, and Risk of Developing Type 2 Diabetes Mellitus. JAMA 2001;286(3):327-334.

8. Verma S, Li S-H, Badiwala MV, Weisel RD, Fedak PWM, Li R-K,Dhillon B, Mickle DAG. Endothelin Antagonism and Interleukin-6 Inhibition attenuate the proatherogenic effects of C-reactive protein. Circulation 2002;105: 60-66.

9. Lakhani K, Prelevic GM, Seifalian AM, Atiomo WU, Hardiman P. Polycystic ovary syndrome, diabetes and cardiovascular disease: risks and risk factors. J Obstet Gynaecol 2004,24:613–621.

10. Prasad AS. Zinc: mechanisms of host defense. J Nutr 2007;137: 1–5.

11. Rink L, Kirchner H. Zinc-altered immune function and cytokine production.J Nutr 2000;130: 1407S–1411S.

12. Prasad AS: Clinical, immunological, anti-inflammatory and antioxidant roles of zinc Experi Geron 2008; 43: 370–377.

13. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41–7.

14. Giallauria F,Orio F, Palomba S, Lombardi G, Colao A,Vigorito C. Cardiovascular risk in women with polycystic ovary syndrome.(Review articles). J Cardio Med 2008; 9(10):987-992.

15. Dahlgren E, Janson PO, Johansson S, et al. Polycystic ovary syndrome and risk for myocardial infarction. Acta Obstet Gynecol Scand 1992;71:599–603.

16. Ridker PM. C-reactive protein, inflammation, and cardiovascular disease: clinical update. Tex Heart Inst J 2005;32 (3):384-386.

17. Blake GJ, Ridker PM. Inflammatory biomarkers and cardiovascular risk prediction. J Intern Med 2002; 252:283–94.

18. Bastard J-P, Maachi M, Lagathu C, Kim M, Caron M, Vidal H, Capeau J, Feve B: Recent advances in the relationship between obesity,inflammation, and insulin resistance. Eur Cytokine Netw 2006 ;17(1):4-12.

19. Shen H, Oesterling E, Stromberg A,Toborek M, MacDonald R and Hennig B: Zinc Deficiency Induces Vascular Pro-Inflammatory Parameters Associated with NF-kB and PPAR Signaling. J Am Coll Nut 2008; 27: 577-587.

20. Mariani E, Neri S, Cattini L, Mocchegiani E, Malavolta M, Dedoussis G-V,Kanoni S, Rink L, Jajte J, Facchini A: Effect of Zinc supplementation on plasma IL-6 and MCP-1 production and NK cell function in healthy elderly: Interactive influence of +647 MT1a and _174 IL-6 polymorphic alleles. Exper Geron 2008;43 :462–471.

21. Tamakoshi K, Yatsuya H, Kondo T, Hori Y, Ishikawa M, Zhang H, et al. The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. Int J Obes Relat Metab Disord 2003;27: 443–9.

22. Wu Y, Zhang J, Wen Yu, Wang H, Zhang M, and Cianflone K: Increased acylation-stimulating protein, C-reactive protein, and lipid levels in young women with polycystic ovary syndrome. Fertil Steril 2009;91:213–9.

23. Prasad AS, Beck FWJ, Bao B, Fitzgerald JT, Snell DC, Steinberg JD,Cardozo LJ: Zinc supplementation decreases incidence of infections in the elderly: effect of Zinc on generation of cytokines and oxidative stress. Am J Clin Nutr 2007;85:837–44.

24. Prasad AS. Zinc, infection and immune function. In: Calder PC, Field CJ,Gill HS, eds. Nutrition and immune function. Wallingford, United Kingdom: CABI Publishing,2002:193–207.

25. Ozaki Y, Ohashi T, Kume S. Potentiation of neutrophil function by recombinant DNA produced interleukin-1α. J Leukoc Biol 1987;42:621–7.

26. Berkow RL: Enhancement of neutrophil superoxide production by pre-incubation with recombinant human tumor necrosis factor. J Immunol 1987;139:3783–91.

27. Prasad A, Bao B, Beck FWJ, Kucuk O, Sarkar FH. Anti-oxidant effect of Zinc in humans. Free Rad Biol Med 2004; 37:1182–90.

28. Kahmann L, Uciechowski P, Warmuth S, Plümäkers B, Gressner AM, Malavolta M, Mocchegiani E, and Rink L: Zinc Supplementation in the Elderly Reduces Spontaneous Inflammatory Cytokine Release and Restores T Cell Functions. Rej Res 2008;11(1):227-237.

29. Kandhro GA, Kazi TG, Afridi HI, Kazi N, Baig JA, Arain MB, Sirajuddin, Shah AQ, Sarfraz RA, Jamali MK, Syed N: Effect of Zinc supplementation on the Zinc level in serum and urine and their relation to thyroid hormone profile in male and female goitrous patients. Clin Nutr 2009; 28:162–168.