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Original article

The effects of zinc supplementation on inflammatory parameters in pregnant women with impaired glucose tolerance: a randomized placebo controlled clinical trial

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Summary. Pregnancy is hyperglycemic cycle of life and usually associated with insulin resistance from mid-gestation. Previous studies indicate that abnormal production of some proteins secreted from adipocytes (adipokines) encloses in pathogenesis of insulin resistance and gestational diabetes mellitus (GDM). It is proven that maternal zinc deficiency affects glucose metabolism, but the interaction between zinc and adipokines secretion are not well understood. This study aims to evaluate the effect of zinc supplementation on Vaspin and IL-6 levels in pregnant women with impaired glucose tolerance (IGT). In this matched, placebo controlled double blind clinical trial, 46 pregnant women with impaired glucose tolerance were randomly distributed to zinc (n=23) and placebo (n=23) groups and received 30 mg/day zinc gluconate or placebo for eight regular weeks. The study was conducted in Shabestar district, North West of Iran. Serum Vaspin and IL-6 levels were assessed before and after intervention. There was a significant decrease in Vaspin and IL-6 levels in zinc group (p= 0.004, p= 0.034, respectively). Further, changes in fasting Vaspin levels had a positive correlation with change in fasting IL-6 levels in both zinc (r= + 0.820, p<0.001) and placebo (r= + 1.000, p<0.001) groups. According to enhancement of inflammatory cytokines in pregnant women with IGT, zinc may be considered as a complimentary supplement together with medical management in patients with IGT and GDM. However, further studies with greater sample size and extended periods of intervention are needed to make definite conclusion.

Key words: zinc, vaspin, interleukin-6, pregnancy, Gestational Diabetes Mellitus

Introduction

Gestational diabetes mellitus (GDM) is stated as carbohydrate intolerance with varying severity which can be started or recognized in the middle of pregnancy (1). The disease occurs in 7% to 8% of pregnancies (2). It is interrelated with increase risks of maternal and prenatal interference (3). The etiology of GDM is specified by both insulin secretion impairment and insulin resistance, which increases with gestational age (4).

Inflammation is linked with the extension of gestational diabetes mellitus (GDM) and it might have a pathophysiological connection between GDM and future type 2 diabetes mellitus (DM) (5). It was indicated that adipose tissue plays a vital role in the process of insulin resistance in both non-pregnant and pregnant women (6).

In pregnancy, adipokines seem to affect both maternal glucose metabolism and gestational insulin resistance (7). It is approved that adipose tissue has an

important role in insulin regulation sensitivity by secreting some cytokines (adipokines) which are involved in the pathogenesis of pregnancy-induced insulin resistance (8, 9). Previous researches approved that subjects with high risk of developing glucose intolerance have fat cell dysfunction that leads to the production of great amount of pro-inflammatory adipokines (5).

Visceral adipose tissue-derived serpin A12 (Vaspin) is a new and well-known adipokine which is recognized by its potential insulin- sensitizing features (10, 11). Vaspin is expressed in rat and human placenta. Vaspin expression is minimal in early months of the pregnancy and increases with expanding gestational age(12). However, Vaspin is known as an insulin-sensitizing adipokine, its function in GDM is still unknown (6).

Interleukin-6 (IL-6) which produced by adipose tissue is able to intensify insulin resistance condition. The cytokine is reported to reduce glucose uptake in adipocytes (13). However, its role on hepatic glucose production is still unclear (14). Furthermore, plasmatic levels of IL-6 are increased in those subjects with type 2 diabetes (15). Researchers have indicated that elevated levels of IL-6 may worsen insulin resistance in pregnancy and lead to pathogenesis of GDM (16).

Zinc is one of the most abundant elements that are essential for a broad range of physiological processes (17). It's vital role in insulin's function has been established previously(18). The production and signaling of numerous inflammatory cytokines such as tumor necrosis factor-α (TNF- α), IL-6 and IL-1β is influenced by mild to moderate zinc deficiency in humans (17). Pioneering studies supported a relationship between plasmatic zinc concentration and the level of inflammatory cytokines in insulin resistance. The effect of zinc on nuclear transcription factor kappa B (NF-ϰB) activity and nitric oxide signaling pathway are potential mechanisms for supporting this protective effect of zinc (19).

To the best of authors' knowledge, there are no published reports related to the effect of zinc on mentioned inflammatory parameters in pregnant subjects with impaired glucose tolerance up to now. So, for the first time, the effect of zinc supplementation on serum levels of Vaspin and IL-6 in pregnant women with impaired glucose tolerance test was investigated in this study.

Subjects and methods

Study design and participants

In this matched, randomized controlled double blind clinical trial (allocation ratio 1:1) 46 pregnant women with IGT were voluntarily recruited. The sample size was calculated using the previous study comparing the effect of zinc supplementation on IL-6 levels (20). Figure 1 demonstrates design and protocol of study. Those pregnant women who attended Rohzendeh health centre in Shabestar city, North West of Iran during December 2012 –April 2013 have been chosen as our participants. The participants received 50 g glucose for oral glucose challenge test (OGCT) in their 24-28 weeks of pregnancy. The participants, who were given the specified 50 g glucose, were asked to attend the measurement after an hour. If the blood sugar results were ≥130mg/dL, oral glucose tolerance test (OGTT) is used to distinguish those whom had GDM. The diagnostic criteria are as follows:

- FBS≥92 mg/dL
- 1 h ≥180 mg/dL following a 75 g oral glucose load
- 2 h ≥153 mg/dL following a 75 g oral glucose load (20).

The exclusion criteria consists the past history of diabetes or chronic disease and specific infections, drinking alcohol and smoking in the registration. The

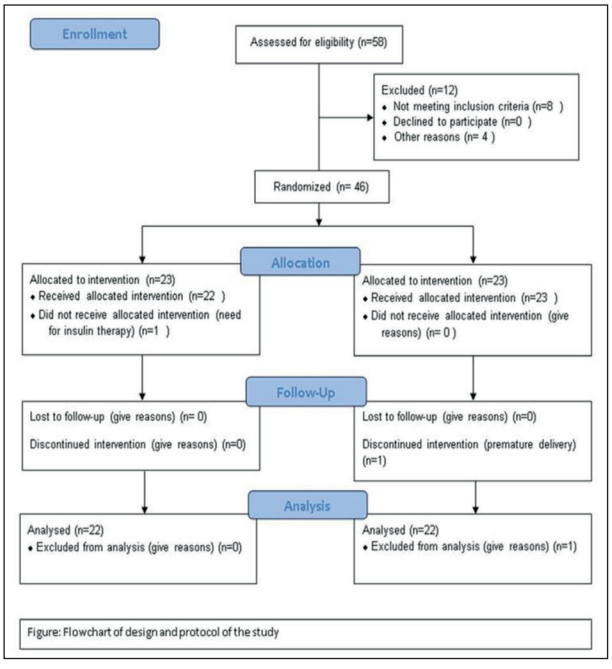

Figure 1. Consort diagram of the study

informed written consent was obtained from all participants, with ethical clearance for the study obtained from the ethics committee of Tabriz University of Medical Sciences. This study is registered at the Iranian Registry of Clinical Trials (IRCT registration number: IRCT 201212265670N6) and trial protocol can be accessed in IRCT website.

These 46 pregnant women with IGT were selected by strict following of inclusion and exclusion criteria and were randomly assigned to 2 intervention groups: zinc group (n=23), and placebo group (n=23).

The supplementation protocol followed a randomized, double-blind, placebo controlled design. The method of random permuted blocks was used to random allocate women to either the placebo.

All researchers and participants and staff of patient's recruitment center were blinded to the treatment assignment. A researcher prescribed the zinc group with 30 mg of zinc gluconate (Nature Med, USA) daily between meals and the participants were informed not to use any kind of vitamin or mineral supplements totally. The placebo group used a placebo tablet made of starch with the same method. Furthermore, participants received a dietary plan based on their gestational condition by an expert dietitian. One of the health staff was asked to be in touch with all subjects to ensure full compliance of the tablets one a week regularly. The doctor of the center observed all the participants closely once a month during the trail. The subjects' body mass index (BMI) was figure out as weight in kilograms divided by height in meters squared based on pre-pregnancy weight. The gestational age of pregnant women was specified using 1st trimester ultrasound.

To measure levels of serum Vaspin, IL-6, Fasting blood glucose (FBS) and Insulin, 5 millilitre fasting blood sample was taken before and after of the intervention. Vaspin and IL-6 were measured by human ELISA kit (BioVendor,Germany). Fasting blood glucose was measured enzymatically by an auto-analyzer (Hitachi, Tokyo, Japan). Chemiluminescent immunoassay method was used to calculate participants' serum fasting insulin (DiaSorin, Liaison, Italy). Insulin resistances were calculated via the following formula based on the Homeostatic model assessment of insulin resistance (HOMA-IR) method; Fasting Glucose (mg/dL) × fasting insulin (mU/L)/450.

Statistical analysis

To analyze distribution of the data a Kolmogorov-Smirnov goodness of fit test was used. Results are presented as median and upper and lower quartiles for non- normal data. To compare the results, paired t-test was used. Percentage changes was calculated by {using the formula: ((after intervention values– baseline values)/baseline values) × 100) between groups. The independent sample *t*-test was used for comparisons between two groups. Correlations were assessed by (Pearson) and (Spearman) correlations respectively for normal and non-normal data. An analysis of covariance test (ANCOVA) was used to adjust the influence of confounding factors. The changes in laboratory markers before and after intervention within the zinc and placebo group were compared. P value <0.05 was considered statistically significant. The statistical software SPSS version 21 (SPSS Inc. IL, Chicago, USA) was utilized for data entry and analysis.

Results

Forty-six pregnant women with IGT were invited to participate in the study. In the zinc group, one subject did not receive allocated intervention due to need for insulin therapy. In the placebo group, one subject discontinued intervention due to premature delivery. In figure 1 flowchart of the design and protocol of the study was illustrated.Thus 44 subject (zinc group =22; placebo group=22) finished the study with the mean age of 29.45± 4.21 years in the zinc group and 29.82± 5.41 years in the placebo group (gestational age was 24-28 weeks in both group).

As it is clear in Table 1, none of the variables presented any statistically significant variety between the two groups on the baseline features. Table 2 includes serum levels of Vaspin and IL-6 before and after the supplementation. After adjusting for confounding variables (age, BMI, baseline values and energy intake), Analysis of Covariance test, ANCOVA, showed a significant reduce in Vaspin and IL-6 levels in zinc group (Table 2). The percentage changes of Vaspin and IL-6 levels between zinc and placebo supplemented groups were shown in Fig 2.

In the subgroups, correlation analysis showed that change (after intervention values– baseline values) in

fasting Vaspin levels had a positive correlation with change in fasting IL-6 levels in zinc group (r=+ 0.820, p<0.001) and placebo group (r=+ 1.000, p<0.001). Serum IL-6 was correlated with age, change in FBS and vaspin levels in the whole study group (Table 3).

Discussion

The current study demonstrated that zinc supplementation could remarkably reduce Vaspin and IL-6 levels in pregnant women with IGT. In addition, a strong positive correlation was found between the two adipocytokines.

Pathogenesis of both IGT and GDM involves very complex metabolic pathways. Despite many progresses, till know, no clear evidences are available on underlying mechanisms in which some factors were taken into part in the pathophysiology of GDM, thus this subject remains as an interesting research topic for further studies. More recently, notable progresses have been made in respect of involvement of adipose tissue derived hormones in the pathophysiology of GDM (21).

Table 1: Baseline characteristics of subjects (mean ± SD) in two groups

Variables	zinc (n=22)	placebo (n=22)	p*
Age (year)	29.45± 4.21	29.82± 5.41	0.805
Weight (Kg)	70.05± 11.23	68.43± 11.33	0.638
Height (m)	1.58± 0.05	1.60± 0.03	0.057
BMI (Kg/m^2)	28.34± 4.17	26.82± 3.73	0.210

*Abbreviations: SD: standard deviation, BMI: Body Mass Index, * Independent Sample t-test*

The important role of oxidative stress in the pathogenesis of diabetes mellitus has been repeatedly approved in many studies. The antioxidant feature of the zinc protects insulin and pancreatic cells against free radicals, interestingly (22). This element also came out to be effective for insulin synthesis, storage and its secretion (23). In particularly, it improves insulin function via stimulating of insulin tyrosine kinase receptors and increases the phosphorylation of tyrosine kinase (24).

Till now, there have never been published reports including the effect of zinc supplementation on metabolic and inflammatory indices in either diabetic pregnant women or among those with IGT. Pregnant women with IGT, are expecting high risk of perinatal complications regardless of lacking clinical diagnosis

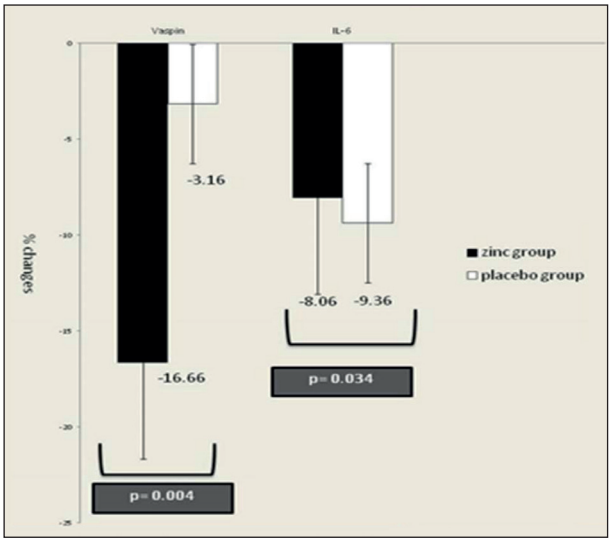

Figure 1. Percentage changes of adipocytokines. Comparison of percentage changes in inflammatory parameters between the two study groups. * P< 0.05, based on ANCOVA.

Table 2. The Median biochemical factors before and after intervention in both zinc and placebo groups[a]

Parameters		Zinc Group a (n= 22)	Placebo Group a (n= 22)	P-value
Vaspin (ng/mL)	Before	2.85(1.32-4.29)	1.89(0.84-3.62)	Pc =0.127
	After	1.17(0.86-2.80)	0.78(0.66-1.20)	Pd=0.004*
	P[b]	0.016	0.042	
IL-6 (pg/ml)	Before	793.71(496.52-1150.84)	794.96(678.40-1654.90)	
	After	469.96(425.59-633.40)	624.34(508.09-1236.84)	Pc=0.350
	P[b]	Pd=0.034*	0.041	0.059

[a]*Median(Q1-Q3)*
P[b], comparison within group by paired t test
P, comparison baseline values between groups by independent t test
P[d], comparison after intervention values between groups by ANCOVA

Table 3. Relationships of change in serum Vaspin level with biochemical and anthropometric parameters in the whole study groups and subgroups

	Total (n= 44)		Zinc Group (n= 22)		Placebo Group (n= 22)	
Parameter	**r**	**p**	**r**	**p**	**r**	**p**
BMI	-0.032	0.836	-0.029	0.899	0.019	0.934
Age	-0.209	0.173	-0.311	0. 158	-0.098	0.664
FBS change	-0.327	0.030*	-0.384	0.078	-0.290	0.190
Insulin change	0.241	0.115	-0.002	0.992	0.449	0.036*
IL-6 change	0.910	<0.001*	0.820	<0.001*	1.000	<0.001*

r, Pearson or Spearman correlations for normal and non-normal data
** Statistically significant*

of GDM (25, 26). Thus, looking for a solution to manage IGT and impede further development of GDM remains as an important measure to help improving healthy pregnancy outcomes. Thus, the current randomized controlled trial was performed to elucidate any possible effects of zinc supplementation in pregnant women with IGT.

Elevated levels of vaspin in inflammatory condition such gestational diabetes mellitus suggesting that it may exert pro-inflammatory effect, although the mechanism is largely unknown (27, 28). In our study, Vaspin concentration decreased significantly in both group, however, the percentage decrease was greater in the zinc group (-16.66% versus -3.16%). This finding is in line with previous studies reporting that the amount of serum Vaspin levels decrease from 2nd trimester to the 3rd trimester of pregnancy step by step (6, 29). Normally, in the early months of pregnancy there is a gentle rise in maternal fat stores and a decrease in free fatty acid (FFA) concentrations, which slowly reverses from mid pregnancy, leading in decreased maternal adipose tissue residue and increased postprandial FFA levels in late pregnancy (6, 7). This may justify gradual decrease in circulating Vaspin levels in our pregnant women in both groups. The more percentage decrease in the intervention group can be attributed to zinc supplementation.

Interlukine 6 is a pro-inflammatory cytokine which is involved in insulin resistance (30). It is secreted in response to infection, injury and pregnancy. It is known to inhibit insulin stimulated tyrosine phosphorylation at the insulin receptors and is found in trophoblast and endothelial cells of placenta (31). In agreement with previous reports (32-34), it was demonstrated that zinc supplementation reduced IL-6 levels. However, they are contradictory results that there is not any association between the mineral and IL-6 levels (19, 35). These discrepancies may be attributed to different amounts of zinc used in the studies, zinc exposure chemical combination of supplements as well as different population groups.

Interestingly, we did not find any correlation between Vaspin and BMI or age in none of the groups. Further, there was a negative correlation between FBS changes in whole study groups. The lack of association between BMI and Vapsin in this study was similar to results of previous studies (6, 29).

A positive correlation was observed between changes in Vaspin level and IL-6 concentrations among all subjects. In the other words, we can say that there is a strong correlation between decreasing Vaspin levels and decreased levels of circulating IL-6. Potential mechanisms of the influence of zinc supplementation on cytokines like IL-6 and vaspin may be for its interaction with a wide range of inflammatory factors, such as NF-κB and peroxisome proliferator-activated receptors (PPARs) signaling pathways (17). Nevertheless more studies are needed, particularly in the area of the cell culture.

To our knowledge, this is the first documentation to show the correlation between change in concentration of serum Vaspin and change in IL-6 level after 8 weeks of zinc supplementation. The current study had some limitations. These included relatively small sample size, short duration of the intervention and short follow-up of the patients.

Conclusion

We demonstrate that zinc supplementation decreased significantly Vaspin and IL-6 levels. These results support the beneficial effects of zinc supplementation in pregnant women with IGT. It is suggested that zinc may be considered as a complimentary supplement together with Metformin and Insulin treatment in patients with IGT and GDM.

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