ORGINAL ARTICLE

The Effect of Selenium on Micro-Albuminuria in Diabetic Patients: A Randomized Clinical Trial

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Abstract

Objective: Oxidative stress plays an important role in the onset and progression of diabetic nephropathy. So antioxidant agents may be one of the key treatment strategies for prevention of diabetic nephropathy progression .The aim of this study was to determine the effect of selenium (Se) on micro-albuminuria in type 2 diabetic (T2DM) patients.

Materials and Methods: This study was a clinical study on 60 T2DM patients with micro-albuminuria who were referred to Yazd diabetic research center from March 2016 till April 2017. Patients were randomized in to two groups: Se (200 microgram Se daily) and control. The control group did not receive placebo. The intervention duration was 12 weeks. Micro-albuminuria, fasting blood sugar (FBS), cholesterol, triglycerides, HDL-C, LDL-C, urea, creatinine, HbA1c, plasma Se concentration were measured at the baseline and after 12 weeks.

Results: Micro-albuminuria decreased after 12 weeks in both studied groups but it was not statistically significant. Cholesterol and LDL levels improved statistically in both studied groups at the end of study (*P*-value: 0.034, 0.023 respectively). Plasma Se level increased in intervention group (*P*-value< 0.001). There were clinically improvement in other studied variables after 12 weeks in two studied groups but not statistically significant

Conclusion: Our study demonstrated that Se supplementation for 12 weeks among patients with micro-albuminuria had no beneficial effects on micro albuminuria.

Keywords: Selenium, Diabetic Nephropathy, Oxidative stress, Albuminuria, Diabetes Mellitus, Type II

Introduction

Type 2 diabetes mellitus (T2DM) is a growing health problem all over the world. T2DM is a public health problem affecting life quality, and leading to high management costs (1). The main clinical characteristic of T2DM is hyperglycemia caused by impaired insulin secretion and abnormal glucose metabolism (2). Micro and

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macro vascular complications are as the consequences of choronic hyperglycemia (2). T2DM is one of the most prevalant choronic diseases in Yazd-Iran. The prevalence of diabetes was 16.3% in Yazd-2014 (3). Low level of physical activity and unhealthy lifestyle are the risk factors of diabetes (4).

Diabetic nephropathy is the microvascular complication of diabetes. Inflammation, oxidative stress, metabolic and hemodynamic changes are the key pathology of diabetic nephropathy (5). Micro-albuminuria is the accurate risk predictor of developing diabetic nephropathy (6). Micro-albuminuria is an indicator of endothelial cell dysfunction due to inflammation, insulin resistance and oxidative stress (7). If micro-albuminuria did not timely diagnose, it will lead to severe kidney failure. The end point of diabetic nephropathy is end stage renal disease (ESRD). So, control of renal function is important in diabetic patients (7).

Selenium (Se) is an essential trace element. It has antioxidant and anti-inflammatory function. (8). Se due to its antioxidant function may reduce diabetes complications (9). In animal studies, Se supplementation reduced proteinuria (10,11) and glomerular sclerosis (11). A recent trial showed that Se supplementation in diabetic nephropathy reduced parameters of glucose homeostasis (HOMA-IR) without any effect on fasting plasma glucose (FBS) and lipid profiles (12).

In animal studies, Se supplementation increased low density lipoprotein (LDL)-receptor activity and mRNA expression (13) and decreased β-hydroxy-β-methylglutaryl coenzyme A reductase mRNA expression. Also Se decreased total cholesterol, LDL and apolipoprotein B (apo B) (13). As a consequence, it was proposed that Se supplementation may beneficially affect the lipid profile (13-14).

Oxidative stress plays an important role as an etiologic cause of the onset and progression of diabetic nephropathy. So antioxidant agents are considered as the prevention strategies of diabetic nephropathy progression. A meta-analysis of randomized controlled trials (15) concluded that larger studies are needed to investigate the effects of Se supplementation on T2DM prevention among various populations (15). The latest meta-analysis (1) also concluded that it remains unclear about the effect of Se on the risk for T2DM and complications that may vary by duration of Se exposure and further studies on the effects of Se and T2DM are needed (1). We aimed to evaluate the effect of Se on micro-albuminuria in T2DM patients with nephropathy.

Materials and Methods

This study was a quasi-experimental study to assess the effect of Se supplementation on micro-albuminuria in T2DM patients. In this study 60 T2DM patients with documented micro-albuminuria (before the onset of study) who had medical record in Yazd diabetic research center from March 2016 till April 2017 were included (Figure 1). The inclusion criteria were: T2DM, documented micro-albuminuria (refers to increased excretion rate of albumin in the urine between 30–299 mg/g creatinine (16), age between 30-60 years old, insulin treatment and angiotensin II receptor blockers drugs such as losartan or valsartan. Exclusion criteria were; infectious diseases, multivitamin and antioxidant supplementation during past 6 months, advanced heart failure, any known chronic diseases, history of hospital admission within 3 past months of trial and cognitive impairment (Patients who had history of dementia, Alzheimer's disease, Parkinson's disease and Huntington's disease history or medical history). It should be noticed that a neurologist visited all the patients. This exclusion criterion was done to increase the patient compliance. Also a patient who changed the medication two months before and during trial was excluded from study. Also subjects who taking multivitamin and antioxidant supplements before and during trial were excluded from study. The study design was parallel and allocation ratio was one (same in two groups).

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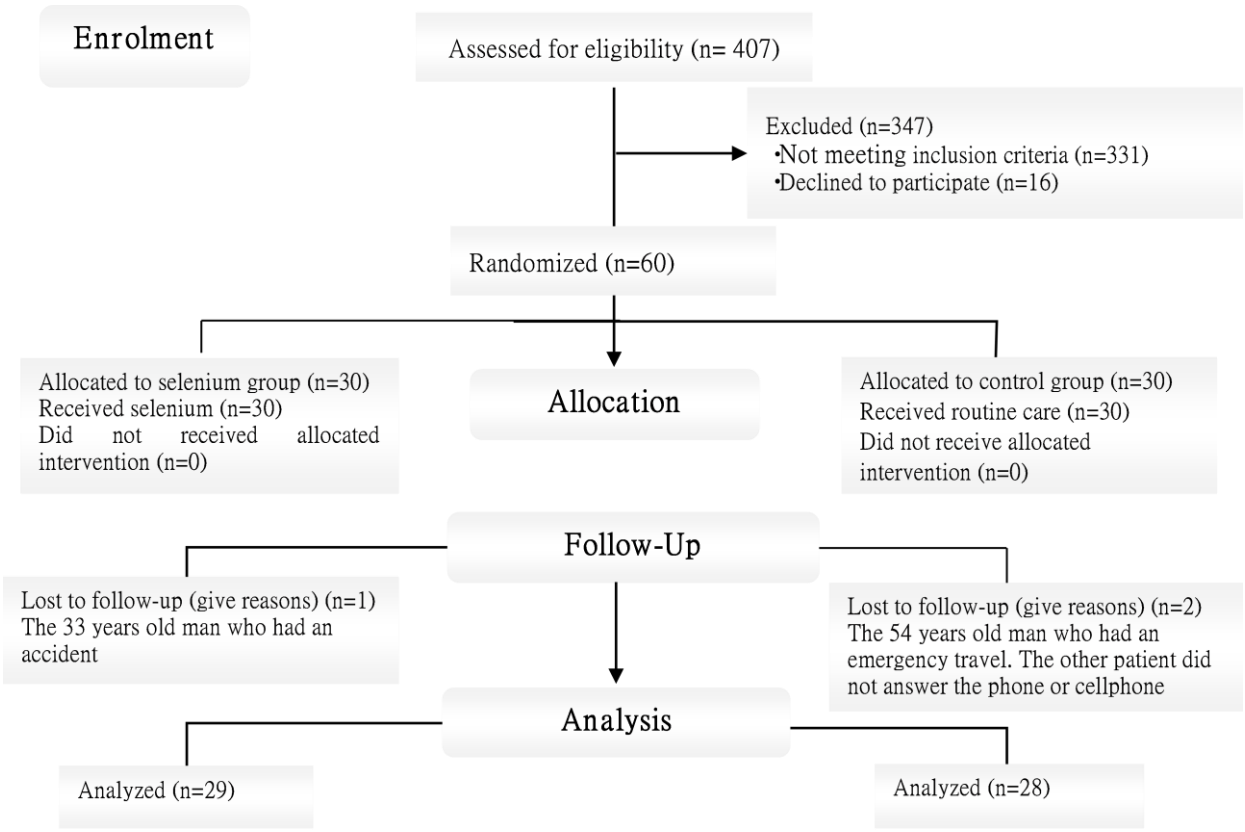

Figure 1. Shows the consort flow diagram of this trial

Simple randomization with stratification (age and sex) was done. One of the investigators (NN) who had no clinical involvement in the study did randomization by a random number table generated in Epi Info software. The other investigator (JA.S) enrolled and assigned the participants to intervention and control group.

We received written informed consent from all participants. Patients in intervention group treated with 200 microgram daily Seas Se-enriched yeast orally (Capsule, 21ST Century Company) for three months (12 weeks). No measurement was carried out to check the level of Se in the supplement. Other medication of two groups did not change two months before the start of study till the end. Patients of two groups were asked to maintain their routine diet and physical activity during the intervention. So no dietary record performed. Participants in the intervention group were given enough Se until three days after their next scheduled visit by researcher every 4 weeks and the remaining supplements were given until next visit. Patients were followed twice a week by phone call to take the capsule and presence of side effects during the three months period of study. One day after routine visit date if the patients did not come back, one of researchers' visited them in home. The patient's compliance was measured by capsule count and serum Se level at the end of study.

The control group did not receive placebo. Their routine care and visits were done. The control group was visited every 4 weeks. The control group also was followed twice a week by phone call about their routine medication. (The control group phone call follow up was done to reduce Hawthorne bias).

The study duration was 12 weeks since the minimum period of time to check the primary and secondary outcomes was recommended after 12 weeks.

A checklist consist of age, gender, height, weight, body mass index (BMI), blood pressure and history of hypertension and

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medication was completed through interview and physical examination of each patient was done at baseline. Micro-albuminuria was measured as primary outcome and fasting blood sugar (FBS), cholesterol, triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), urea, creatinine, hemoglobin A1c (HbA1c), plasma Se concentration were measured as secondary outcomes.

Blood sample was taken from both studied groups to measure FBS, cholesterol, TG, HDL-C, LDL-C, urea, creatinine, HbA1c and plasma Se concentration. Also morning urine checked for micro-albuminuria at baseline and 12 weeks later (Urine albumin-to-creatinine ratio).HbA1c was determined by TosohG8 HPLC Analyzer by high performance liquid chromatography method. FBS was measured by an enzymatic colorimetric method (glucose oxidize-peroxides) (Biosystem kit, Spain). Total cholesterol, TG, HDL-C and urea were measured by colorimetric enzymatic assays (Biosystem kit, Spain). LDL-C was calculated according to Friedwall's formula in subjects with triglyceride level less than 300 mg/dl [LDL-C = Total cholesterol - (HDL-C + TG / 5)]. Creatinine was measured by the Jaffe action using routine laboratory methods (Spinreact andLabomed Inc. equipment Spain).Urinary albumin was measured as the albumin-creatinine ratio(ACR) in a morning sample by immunoturbidometry assay (Biosystem kit, Spain) and urine creatinine was measured by an enzymatic colorimetric assay(Biosystem kit, Spain). The determination of selenium levels was performed by atomic absorption spectrometers (AGILENT). Samples were diluted with a solution containing nickel and nitric acid and measured by a standard additions method (17).

Statistical analysis

The sample size was calculated by two mean (micro-albuminuria) comparison formula. Considering, type one error 0.05, effect size (d: 0.55) and power 80%, the calculated sample size was 26 participants in each group. Also 15 % was added for attrition rate. Final sample size was 30 subjects in each group.

Data analysis was performed using Statistical Package for the Sciences, version 22.0, SPSS. All statistical analysis was performed according intention to treat method. Continuous variables were demonstrated as the mean ± standard deviation (SD). Independent and paired T-tests were done to compare the mean value of each parameter between the two groups and before after in each group. Chi-squared was used to evaluate the differences of retinopathy, smoking and cardiovascular diseases frequencies between two studied groups. *P*-value less than 0.05 were considered statistically significant. No additional analysis such as subgroup analyses and adjusted analyses were done.

Ethical considerations

The trial was registered at the Iranian registry of clinical trials (http://www.irct.ir) with the IRCT ID: IRCT2015112825266N1.

This study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences and approved by the internal medicine department. (IR.SSU.REC.1394.22).

The patients were informed about the objective and nature of the study, and each participant provided written consent prior to the study.

Results

The baseline characteristics of two groups are described in Table 1. During the study one participant of the Se group and 2 patients of the control group did not follow the study.

The patient who left the Se group was a 33 years old man who had an accident and did not want to follow the study. One of control group patients who did not follow the study was a 54 years old man who had an emergency travel during the study and the other patient did not answer the phone or cellphone. The entire drop out patients was male.

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At the end of study, 57 subjects (intervention group (n= 29) and control group (n =28) completed the trial. Figure 1 shows the consort flow diagram of the trial. All of studied patients had micro-albuminuria. There were no significant differences at the baseline characteristics between two groups (Table1). Comparison of clinical data in intervention and control groups after interventions showed that there were no significant differences between two groups after intervention except plasma Se level which was statistically significant differences between two groups which can show the compliance of patients (Table 2).

Table 3 revealed comparison of study variables before and after trial in intervention group. Cholesterol and LDL levels improved statistically in both studied groups after study. Also plasma Se level increased after trial (*P*-value< 0.001) but other parameters (FBS, HbA1c, TG, HDL, Cr and micro albuminuria) did not change after intervention. Micro-albuminuria clinically decreased after Se treatment but it was not statistically significant. It should be noticed there were

Table 1. The baseline characteristics of patients in intervention and control groups

Variables	Group	Mean ± SD (frequency %)	*P*-value
Age* (year)	Intervention	55.44 (±1.94)	0.71
	Control	56.51 (±2.10)	
Height *(cm)	Intervention1	63.06 (±1.78)	0.61
	Control	161.86 (±1.58)	
Weight* (kg)	Intervention	77.42 (±2.50)	0.71
	Control	78.65 (±2.20)	
Body mass index* (kg/m²)	Intervention	30.18 (± 4.58)	0.37
	Control	29.10 (± 4.55)	
Systolic blood pressure *(mmHg)	Intervention	138.10 (±3.46)	0.99
	Control	138.16 (±3.51)	
Diastolic blood pressure* (mmHg)	Intervention	80.17 (±2.28)	0.08
	Control	75.00 (±1.93)	
Duration of Diabetes* (year)	Intervention	10.00 (±1.29)	0.88
	Control	9.75 (±1.06)	
FBS* (mg/dL)	Intervention	154.51 (±10.47)	0.78
	Control	151.80 (±8.19)	
HbA1c* %	Intervention	8.55 (±0.31)	0.33
	Control	8.69 (±0.36)	
Triglycerides *(mg/dL)	Intervention	187.58 (±23.17)	0.65
	Control	175.60 (±13.15)	
Cholesterol (mg/dL)	Intervention	156.48 (±9.58)	0.76
	Control	152.70 (±8.51)	
HDL-C* (mg/dL)	Intervention	43.08 (±2.35)	0.57
	Control	41.32 (±2.08)	
LDL-C* (mg/mg/dL)	Intervention	86.62 (± 7.39)	0.81
	Control	84.22 (± 6.99)	
Selenium* (µ g/L)	Intervention	95.41 (± 9.18)	0.98
	Control	92.20 (± 9.90)	
Creatinine* (mg/dL)	Intervention	0.90 (± 0.03)	0.81
	Control	0.89 (± 0.04)	
Urea* (mg/dL)	Intervention	34.30 (± 2.46)	0.76
	Control	33.66 (± 2.57)	
Micro-albuminuria* (mg/gr creatinine)	Intervention	114.93 (± 12.92)	0.76
	Control	112.67 (± 11.23)	
GFR*	Intervention	99.66 (±6.45)	0.23
	Control	89.83 (± 5.02)	
Smoking(yes)**	Intervention	3 (±10.3%)	0.61
	Control	2 (±7.1%)	
Cardiovascular disease (yes)**	Intervention	1 (±3.4%)	0.05
	Control	5 (±17.8%)	
Retinopathy (yes)**	Intervention	2 (±6.9%)	0.62
	Control	3 (±10.7%)	

*- Independent T-test
**- Fisher's exact test

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clinically improvement in all studied variables after 12 weeks in two studied groups but they were not statistically significant.

In power analysis, the power of our study in micro-albuminuria differenced after intervention in two studied groups was 0.68.

Discussion

This randomized trial showed that Se supplementation had no beneficial effects on micro-albuminuria in T2DM nephropathy, although we found decrease of albuminuria in both groups. Plasma Se level showed statistically significant differences before and after intervention in intervention group and also between two groups at the end of study. Clinically improvement in all studied variables after 12 weeks in two studied groups may be explained by Hawthorne effect or trail effect. This is a type of reactivity that patients change their behavior and treatment compliance when they are observed. In our study, no side-effect

Table 2. Comparison of two groups' treatment parameters after interventions

Variables	Group	Mean ± SD*	*P*-value
FBS (mg/dL)	Intervention	138.66 (±7.73)	0.64
	Control	133.60 (±7.91)	
HbA1c (%)	Intervention	7.94 (±0.23)	0.09
	Control	7.53 (±0.27)	
Triglycerides (mg/dL)	Intervention	183.76 (±19.48)	0.29
	Control	159.68 (±11.28)	
Cholesterol (mg/dL)	Intervention	149.52 (±6.73)	0.10
	Control	134.47 (±6.07)	
HDL-C(mg/dL)	Intervention	42.33 (±1.80)	0.14
	Control	38.03 (±2.22)	
LDL-C(mg/dL)	Intervention	74.74 (±6.23)	0.81
	Control	68.04 (±7.90)	
Selenium(µ g/L)	Intervention	119.53 (±7.64)	0.02
	Control	80.20 (±10.71)	
Creatinine (mg/dL)	Intervention	0.90 (±0.05)	0.93
	Control	0.91 (±0.05)	
Urea (mg/dL)	Intervention	36.68 (±5.03)	0.72
	Control	38.67 (±2.28)	
Micro-albuminuria(mg/gr creatinine)	Intervention	98.01 (±14.61)	0.16
	Control	109.74 (±11.32)	
GFR	Intervention	97.06 (± 6.19)	0.53
	Control	90.89 (± 7.73)	

***- Independent T-test**

Table 3. The comparison of study parameters before and after trial in two studied groups*

Variables		Intervention	*P*-value	Control	*P*-value
FBS (mg/dL)	Before	154.51 (±10.47)	0.11	150.80 (±8.19)	0.13
	After	138.66 (±7.73)		133.60 (±7.91)	
HbA1c (%)	Before	8.55 (±0.31)	0.12	8.69 (±0.36)	0.34
	After	7.94 (±0.23)		7.53 (±0.27)	
Triglycerides (mg/dL)	Before	187.58 (±23.17)	0.45	175.60 (±13.15)	0.06
	After	183.76 (±19.48)		159.68 (±11.28)	
Cholesterol (mg/dL)	Before	156.48 (±9.58)	0.03	152.70 (±8.51)	0.03
	After	149.52 (±6.73)		134.47 (±6.07)	
HDL-C (mg/dL)	Before	43.08 (±2.35)	0.14	41.32 (±2.08)	0.29
	After	42.33 (±1.80)		38.03 (±2.22)	
LDL-C (mg/dL)	Before	86.62 (±7.39)	0.02	84.22 (±6.99)	0.01
	After	74.74 (±6.23)		68.04 (±7.90)	
Selenium (µ g/L)	Before	95.41 (±9.18)	<0.001	84.20 (±13.90)	0.66
	After	119.53 (±7.64)		80.20 (±14.71)	
Creatinine (mg/dL)	Before	0.9 (±0.03)	0.99	0.91 (±0.04)	0.73
	After	0.9 (±0.05)		1.01 (±0.05)	
Urea (mg/dL)	Before	34.30 (±2.46)	0.48	33.66 (±2.57)	0.42
	After	37.68 (±5.03)		35.67 (±2.28)	
Micro albuminuria (mg/gr creatinine)	Before	114.93 (±12.92)	0.08	112.67 (±11.23)	0.48
	After	98.01 (±14.61)		109.74 (±11.32)	

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of Se was seen in the intervention group during follow up because the mean supplemental Se intake in our study was lower than 400µ g (18).

Rees et al, systematic review reported Se supplementation reduced total cholesterol but it did not reach statistical significance (19). Recent study in Iran on diabetic nephropathy revealed that Se had no effect on lipid profiles (12). In our study, although total cholesterol decreases statistically significant after Se supplementation, other lipid panel especially LDL-C that is problematic particle was not changed.

In the previous studies, plasma Se level was lower in T2DM patients with micro-albuminuria than patients without micro-albuminuria (20) and healthy subjects (20-21) and inversely correlated to the stage of diabetic nephropathy (20).

Studies have shown that several components such as advanced glycation end products (AGE), protein kinase C, reactive oxygen species (ROS) play essential role in the pathogenesis of diabetic nephropathy (22-23).

Se intake may inhibit production of ROS through involvement in selenoprotein and glutathione peroxidase structures that protect cells from the adverse effects of reactive oxygen species and free radicals (24-25).

Kähler evaluated efficacy of antioxidant supplementation in T2DM. Result showed that daily intake of 100 micrograms of Se diminished urinary albumin excretion (26). This study published in German language and also assessed the effect of other agents such as alpha lipoic acid and alpha-tocopherol. Subjects in mentioned study probably were diabetic with and without nephropathy. But, all of participants in our study had micro-albuminuria. Bahmani showed that Se supplementation had no significant effects on FBS and lipid profiles in comparison with the placebo in patients with diabetic nephropathy (12). Assessment of albuminuria in Bahmani study was not performed, although participants had proteinuria with or without elevation of serum creatinine level.

In another study by Bahmani showed that Se supplementation among patients with diabetic nephropathy had no effect on markers of inflammation such as CRP, transforming growth factor β (TGF-β), AGE, protein carbonyl and malondialdehyde (27). Evaluation of albuminuria in this trial was not performed although subjects had proteinuria. The result of this study is in agreement with our result because evaluated factors such as CRP, TGF-β , AGE have critical role in the pathogenesis of diabetic nephropathy (22-23).

A systematic review and meta-analysis with the aim of assessing potential benefits of anti-oxidant agents for delaying diabetic kidney disease progression was published in 2017. In this study, any antioxidant supplementation (including vitamin A, vitamin C, vitamin E, Se, zinc, methionine or ubiquinone) alone or in combination were key words in search (28). Although Se was one of the antioxidant agents for search in this systematic review and meta-analysis, but no study found for analysis that means no study have assessed the effects of Se supplementation on micro-albuminuria.

Our study assessed the effects of Se supplementation on micro albuminuria in patients with diabetic nephropathy. It must be taken in to account that there was decrease of albuminuria in our study although it was not statistically significant. Our study had some limitations. We could not prepare placebo. Also we should notice that our study power at the end of study by considering two groups micro-albuminuria after intervention was 68%. Also we should notice that no dietary records were done at the baseline and end of intervention to show the diets/macro and micronutrients were equivalent between studied groups during the intervention. Also we should notice that dietary intake may be a major confounder. Another limitation was the lost to follow up of 3 patients and the sensitivity analysis was not done.

Conclusions

In summary, our study showed that Se supplementation for 12 weeks among patients

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with diabetic nephropathy had no statistically effects on micro albuminuria.
Further research with large sample size and longer follow up periods is recommended. Also prospective, randomized, double-blinded, placebo-controlled clinical trial may be necessary.

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Conflict of Interest

The authors have no conflicts of interest.

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