Effect of metabolic control on oxidative stress, subclinical atherosclerosis and peripheral artery disease in diabetic patients

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Abstract

Introduction: By rising diabetes mellitus prevalence, the prevalence of its most complication; cardiovascular disease (CVD) is also increasing. Moreover, oxidative stress has important role in pathogenesis of diabetes and its complications. We investigated relationship between total antioxidant status (TAS) and surrogate measures of subclinical atherosclerosis (SA) with glycemic status in diabetics.

Methods & materials: In a cross-sectional study, we recorded height, weight, waist circumference (WC) and blood pressure of 267 subjects. Blood samples were collected to measure fasting blood sugar (FBS), glycated hemoglobin (HbA1c), lipid profiles and TAS. The surrogate measures of SA were Carotid Intima Media Thickness (CIMT), and Ankle Brachial Index (ABI).

Results: We found significantly lower TAS levels and ABI values and higher CIMT in diabetic patients especially in poor glycemic group. There was a nonsignificant, weak correlation between TAS, ABI and CIMT with glycemic status ($r = -0.10$, $-0.16$, and $+0.09$, respectively). Multivariate regression analysis showed a significant influence of increasing age and diabetes duration on worsening CIMT in poor glycemic group.

Conclusions: Our study showed poor glycemic control leads to worse CIMT by increasing age and duration of diabetes. However we did not find a significant correlation between glycemic status and TAS levels. We suggest CIMT measurement along with other SA markers in poor glycemic diabetics, especially in older patients with longer duration of diabetes, to identify high risk CVD patients.

Keywords: Diabetes, Oxidative stress, Subclinical atherosclerosis, Peripheral artery disease
As the prevalence of DM is rising, it will be a major contributory risk factor of increased CVD events. Like other developing countries we are confronting an escalating trend of CVD in Iran [7]. So measurement of surrogate markers of atherosclerosis is of utmost importance for circumventing CVD end-points, especially in diabetics subjects. A reliable measure of metabolic control in diabetics is glycosylated hemoglobin (HbA1c) which reflects recent mean glucose concentration over the past 3 months and provides a relatively substantial base for prediction of CVD events [8, 9]. It is expected that improving glycemic control with HbA1c would delay the onset and ameliorate the severity of diabetic complications [6].

It is possible to evaluate plasma markers of oxidative stress. One of oxidative stress indicators is total antioxidant status (TAS) which represents the plasma level of cumulative antioxidant reserve of the body and enables the evaluation of the average antioxidant potential [10]. Conflicting reports on serum concentration of TAS in patients with CVD have been reported in epidemiological studies [11]. However, given its feasibility, measurement of TAS can be used as a valuable tool for investigating the association between dietary antioxidant status and CVD [12].

Atherosclerosis is the most common type of CVD. There are several non-invasive methods to assess subclinical atherosclerosis (SA) including Carotid Intima Media Thickness (CIMT) [13], and Ankle Brachial Index (ABI) [14]. CIMT is a valid surrogate marker of CVD that can detect intimal atherosclerotic process and medial hypertrophy in carotid arteries. Increased CIMT is an early phenomenon in development of atherosclerosis even in asymptomatic individuals [13].

It is established both asymptomatic and symptomatic PAD can increase the risk of CVD. Ankle brachial index (ABI) is a simple, inexpensive, and noninvasive measure and also sensitive and cost-effective screening tool for peripheral artery disease (PAD) [15]. ABI can also provide useful information about cardiovascular health both in general population and in clinical settings [15].

There are various reports about the effect of glycemic control on CVD risk reduction in type 2 diabetes mellitus (T2DM) [16, 17]. However, we didn’t find any study to assess the association between glycemic status with oxidative stress and surrogate measures of CVD in T2DM. This cross-sectional study was designed to determine the effect of metabolic control on plasma status of TAS and surrogate measures of atherosclerosis in T2DM.

Methods
Study population
We conducted a cross-sectional study in 267 participants; 150 non-diabetics and 117 T2DM patients during a period of 12 months between January 1st and December 31st 2012 in diabetes clinic of Dr. Shariati Hospital, Tehran/ Iran. T2DM was diagnosed according to American Diabetes Association criteria [9]. Inclusion criteria were T2DM or non-diabetics aged between 35–75 years (mean ± SD 51.5 ± 8.1 years). Subjects who ingested antioxidant supplements and drugs in last 3 months ago, suffering from acute or chronic inflammatory/ infectious conditions, past medical record of CVD or acute myocardial infarction, and chronic diseases such as chronic renal failure, or cirrhosis were excluded from the study. After obtaining the written consent of participants, T2DM patients were divided into two groups of good control (HbA1c ≤ 7.5 %) and poor control (HbA1c > 7.5 %) according to their glycemic status.

A personal and demographic questionnaire was filled for all of the patients. Height, weight, waist circumference (WC) and blood pressure were measured with standard tools and recorded. Body mass index (BMI) was calculated by dividing weight (kg) to height squared (m)². WC was measured on broadest area between the edge of lower ribs and the iliac crest in standing position. Systolic and diastolic blood pressures were measured after 5–10 min rest, on the right arm in sitting position. Hypertension was defined as systemic blood pressure/ diastolic blood pressure ≥ 140/90 mmHg or under treatment with antihypertensive medications.

Laboratory measures
Blood samples were collected in fasting state and were analyzed for fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) by autoanalyser, Pars Azmoon Kit (Iran). The low density lipoprotein cholesterol (LDL-C) was derived by Fredrickson-Friedwald’s formula [LDL-C = (TC− HDL-C− TG)/5], if TG level was < 400 mg/dl [18]. Dyslipidemia was defined as TC ≥ 200, TG ≥ 150 mg/dl, LDL-C > 100 mg/dl, HDL-C < 40 mg/dl in men and < 50 mg/dl in women based on definition of dyslipidemia in NCEP ATP III [19] or taking anti-hyperlipidemic medications. HbA1c was measured by DSS chromatography; Drew Scientific Limited Company (UK). TAS levels were measured by ELISA kits [Cayman (US)].

Subclinical atherosclerosis measures
We measured CIMT and ABI for detection of subclinical atherosclerosis. Ultrasonographic analysis of the carotid arteries was performed with a high-resolution ultrasound scanner, equipped with a linear array 13 MHz transducer (MyLab 70 X vision, biosound esaote USA). For detection of CIMT special software (vascular tools 5, Medical Imaging Applications LLC, USA) was employed.
The means of the three maximum right and three maximum left far wall measurements of the proximal part, mid part and the bulb were calculated for each common carotid artery. In our study, all the six right and left wall values were measured and the average values noted. The cut off point for SA definition by CIMT was considered an extreme increase of common carotid IMT ≥ 0.8 mm [20]. ABI was measured as the ratio of the average systolic blood pressure at the ankle of each leg divided by the average systolic blood pressure in the arm. Then the highest blood pressure of limbs considered. The ABI ≤ 0.9 was considered diagnostic for PAD [15].

This study was approved by the Ethics Committee of Tehran University of Medical Sciences of Tehran/ Iran.

Statistical analysis
The normal distribution of data was verified by Kolmogrov-Smirnov analytic test. All variables had normal distribution except TG, TAS, and CIMT. Paired T-Test was applied to variables with normal distribution, and Wilcoxon and Mann–Whitney non-parametric tests for analysis of following parameters: TG, TAS, and CIMT. Also, we used univariate and multivariate logistic regression models according to HbA1c categorized status (<7.5/ ≥7.5 %) adjusted for sex, age, duration of diabetes, BMI, WC, hypertension, and hyperlipidemia. Outcome variables included plasma levels of TAS, and surrogate measures of SA, that were CIMT and ABI. All independent variables which had \( p < 0.2 \) in the univariate model were selected for the multivariate analysis. The \( p \) value ≤ 0.05 was considered as statistically significant.

Results
The baseline characteristics of all participants are shown in Table 1.

Of our diabetic patients, 7 patients (6 %) were on dietary restriction alone, while 14 (12 %) and 21 (17.9 %) were taking metformin or glibenclamide, respectively. 62 patients (53 %) were under treatment with combination of metformin and glibenclamide, and 13 subjects (11.1 %) were on combination therapy with metformin and insulin. Mean ± Standard deviation (SD) of diabetes duration was 8.9 ± 6.6 years. The number of smokers, hypertensive and hyperlipidemic subjects in diabetes group were 10 (8.5 %), 60 (51.3 %) and 108 numbers (92.3 %), respectively.

The baseline characteristics of diabetic patients according to glycemic status are shown in Table 2. The poor control group were dominantly women, had higher levels of FBS, TC, TG, LDL-C, HbA1c, CIMT, WC, BMI and longer duration of diabetes with lower TAS levels and ABI compared to the good control group (Table 2).

| Variable                        | Control group | Diabetes group | \( P \) value |
|---------------------------------|---------------|----------------|--------------|
| Age (year)                      | 49.8 ± 7.5    | 53.8 ± 8.3     | <0.001*      |
| Female (n (%))                  | 87 (58)       | 61 (52.1)      | 0.38         |
| Diabetes duration (year)        |               |                |              |
| SBP (mmHg)                      | 126 ± 17      | 134 ± 18       | <0.001*      |
| DBP (mmHg)                      | 78 ± 11       | 78 ± 10        | 0.93         |
| BMI (kg/m\(^2\))               | 28.4 ± 4.3    | 28 ± 4.3       | 0.41         |
| WC (cm)                         | 92 ± 10.7     | 94.8 ± 10.8    | 0.04*        |
| TC (mg/dl)                      | 200 ± 34      | 176 ± 42       | <0.001*      |
| TG (mg/dl)*                     | 161 ± 80      | 193 ± 109      | 0.002*       |
| LDL-C (mg/dl)                   | 114 ± 23      | 95 ± 26        | <0.001*      |
| HDL-C (mg/dl)                   | 46 ± 10       | 42 ± 9         | <0.001*      |
| FBS (mg/dl)                     | 95 ± 12       | 166 ± 63       | <0.001*      |
| HbA1c (%)                       | 5.3 ± 0.6     | 8 ± 1.8        | <0.001*      |
| TAS (mmol/l)*                   | 3.2 ± 0.8     | 3.1 ± 0.6      | 0.02*        |
| Max. CIMT (mm)*                 | 1 ± 0.5       | 1.3 ± 1        | <0.001*      |
| ABI                             | 1.17 ± 0.11   | 1.13 ± 0.11    | 0.003*       |

\( SBP \) systolic blood pressure, \( DBP \) diastolic blood pressure, \( BMI \) body mass index, \( WC \) waist circumference, \( TC \) total cholesterol, \( TG \) triglycerides, \( LDL-C \) low density lipoprotein cholesterol, \( HDL-C \) high density lipoprotein cholesterol, \( FBS \) fasting blood sugar, \( HbA1c \) glycated hemoglobin A1c, \( TAS \) total antioxidant status, \( Max. \) CIMT maximum carotid intima media thickness, \( ABI \) ankle brachial index.

\*\( P \leq 0.05 \) was considered as statistically significant

\*\( For \) variables with abnormal distribution nonparametric analytic tests were used.

In diabetes group, TAS was similar in males and females (mean 3.14 mmol/l in men vs. 3.11 mmol/l in women. Inter-quartile range of TAS that determines difference between quartiles 75 and 25 was also similar in men [3.23–2.86 mmol/l] and women [3.16–2.80 mmol/l]; \( p = 0.79 \). In addition TAS levels decreased with age in men (3.17 vs. 3.13) and increased in females (3.10 vs 3.12) but without significant differences \( p = 0.82 \), and \( p = 0.93 \), respectively.

The correlation between indices of glycemic status and TAS was a weak negative correlation \( (r = -0.10) \) with nonsignificant statistical difference \( p = 0.30 \). This correlation for CIMT, and ABI was \( r = 0.09 \), and \( r = -0.16 \), respectively. The difference in all of these correlations was not statistically significant, \( p = 0.30 \), and \( p = 0.09 \), respectively. Coefficients of univariate and multivariate regression models between TAS, CIMT, and ABI as outcomes with demographic characteristics, BMI, WC, hypertension, and hyperlipidemia as covariates according to glycemic status \( (\leq 7.5/> 7.5) \) are shown in Table 3.

Discussion
We found significantly lower TAS levels and ABI values in diabetic patients. In addition CIMT was significantly
Table 2 Baseline characteristics of 117 diabetic patients according to glycemic status

| Variable                      | Good control diabetics | Poor control diabetics | P value |
|-------------------------------|------------------------|------------------------|---------|
| Age (year)                    | 54.3 ± 8               | 53 ± 8.5               | 0.50    |
| Female (n (%))                | 26 (42.6)              | 35 (57.4)              | 0.58    |
| Diabetes duration (year)      | 6.9 ± 5                | 10 ± 6.5               | 0.004** |
| SBP (mmHg)                    | 133 ± 19               | 135 ± 17               | 0.44    |
| DBP (mmHg)                    | 77 ± 11                | 79 ± 9                 | 0.20    |
| BMI (kg/m²)                   | 27.7 ± 4               | 28 ± 4                 | 0.58    |
| WC (cm)                       | 94.8 ± 9               | 94.7 ± 12              | 0.92    |
| TC (mg/dl)                    | 166 ± 32               | 184 ± 47               | 0.02*   |
| TG (mg/dl)                    | 174 ± 97               | 211 ± 115              | 0.02*   |
| LDL-C (mg/dl)                 | 89 ± 21                | 101 ± 28               | 0.01*   |
| HDL-C (mg/dl)                 | 42 ± 9                 | 41 ± 10                | 0.72    |
| FBS (mg/dl)                   | 126 ± 27               | 199 ± 65               | <0.001* |
| HbA1c (%)                     | 6.5 ± 0.7              | 9.3 ± 1.3              | <0.001* |
| TAS (mmol/l)                  | 3.23 ± 0.68            | 3.04 ± 0.52            | 0.30    |
| Max. CIMT (mm³)               | 1.25 ± 0.83            | 1.41 ± 1.08            | 0.30    |
| ABI                           | 1.16 ± 0.11            | 1.11 ± 0.11            | 0.03*   |

SBP systolic blood pressure, DBP diastolic blood pressure, BMI body mass index, WC waist circumference, TC total cholesterol, TG triglycerides, LDL-C low density lipoprotein cholesterol, HDL-C high density lipoprotein cholesterol, FBS fasting blood sugar, HbA1c glycated hemoglobin A1c, TAS total antioxidant status, CIMT carotid intima media thickness, ABI ankle brachial index.

*a For variables with abnormal distribution was used nonparametric analytic tests.

Higher in diabetics compared to the control group. After dividing diabetics into two subgroups according to the glycemic status, we did not find any significant influence of glycemic status on TAS and ABI. However, glycemic status showed a significant influence on the association between age and diabetes duration with CIMT. By increasing age and duration of diabetes, accrretion of CIMT was significantly more in poor glycemic group versus well controlled diabetics.

The excess risk for CVD mortality in diabetics can be justified to some extent by increased prevalence of traditional risk factors such as hypertension, obesity and hyperlipidemia in this group [21]. For example, the results of the United Kingdom Prospective Diabetes Study (UKPDS) showed a significant association between increased risk of CVD in diabetics with hyperlipidemia and increased HbA1c levels [22]. However, an important contributory factor for excess risk of CVD in diabetes would be oxidative stress which is associated with increased lipid peroxidation [2]. Hyperglycemia associated with hyperlipidemia is a causative factor for increased lipid peroxidation in diabetics [23]. This is supported by impaired antioxidant status and increased oxidative damage to lipids in type 2 diabetes. In this regard use of antioxidant nutrients is suggested as adjuvants in the prevention of CVD in T2DM [24]. All of the different parameters of hyperglycemia, acute or chronic, contribute to the vascular damage, likely via generation of reactive oxygen species. We observed significantly higher levels of dyslipidemia parameters in our poor glycemic group than those with good glycemic control. This is a substantial finding as the results of UKPDS have proven that good control of diabetes corrects lipid abnormalities in almost 65% of these subjects [25]. So, strict glycemic control should be the first objective to prevent CVD associated with oxidative damage to lipids in T2DM.

Of various markers for oxidative stress measurement in diabetes, total Antioxidant Status (TAS) considers the cumulative action of all the antioxidants present in the plasma.

Table 3 Association between diabetic control (HbA1c) with surrogated markers of subclinical atherosclerosis and total antioxidant score in Univariate and multivariable logistic regression models

| Independent variables | CIMT (≥0.8 / >0.8) | ABI (≥0.9 / >0.9) | TAS (≥ Median / > Median) |
|-----------------------|-------------------|-------------------|---------------------------|
|                       | Univariate odds   | Multivariate odds | Univariate odds           | Multivariate odds |
|                       | ratio CI 95 %      | ratio CI 95 %      | ratio CI 95 %              | ratio CI 95 %    |
| HbA1c %               | 1.12 (0.90 - 1.40) | 1.17 (0.87 - 1.56) | 1.18 (0.88 - 1.58)        | 1.16 (0.87 - 1.55) |
| Age (year)            | 1.09 (1.03 - 1.15) | 1.10 (1.03 - 1.18) | 0.98 (0.92 - 1.05)        | -               |
| Sex (F/M)             | 0.83 (0.37 - 1.84) | -                 | 0.42 (0.13 - 1.31)        | 0.51 (0.15 - 1.72) |
| Diabetes duration (year) | 1.06 (0.99 - 1.13) | 0.98 (0.91 - 1.07) | 0.99 (0.90 - 1.08)        | -               |
| BMI (kg/m²)           | 0.92 (0.83 - 1.02) | 1.00 (0.83 - 1.20) | 0.89 (0.77 - 1.03)        | 0.92 (0.80 - 1.07) |
| WC (cm)               | 0.94 (0.90 - 0.99) | 0.91 (0.83 - 0.99) | 0.98 (0.94 - 1.03)        | -               |
| Hypertension (Y/N)    | 2.87 (1.24 - 6.60) | 4.20 (1.41 - 12.51) | 1.53 (0.51 - 4.62)        | -               |
| High LDL-C (Y/N)      | 3.94 (1.19 - 13.01) | 3.77 (0.84 - 16.86) | 0.47 (0.06 - 3.86)        | -               |
|                       | 2.86 (0.79 - 10.30) | 3.26 (0.86 - 12.33) |                           |                 |

CIMT carotid intima media thickness, ABI ankle brachial index, TAS total antioxidant status, BMI body mass index, WC waist circumference, Hypertension high blood pressure ≥140/90 mmHg or history of hypertension, High LDL-C LDL-C ≥130 mg/dl

The variables with P < 0.2 in univariable models enter in multivariable models.
plasma and body fluids; and provides an integrated measure of oxidative stress rather than simply assessing sum antioxidants [26]. It is proven that measurement of TAS is of paramount importance in diagnosis of oxidative state [26]. In addition TAS is a useful marker to determine prognosis and guide antioxidant therapy [26].

The level of TAS in the poor glycemic group in our study, was lower than the well controlled glycemic ones; although with a non-significant statistical difference, which was in agreement with previous reports [27]. In a study by Willems et al. in type 1 diabetic patients, there was no difference in TAS levels between the 2 subgroups with and without subclinical complications; however both of these subgroups were glycemi- cally well controlled [28].

We suggest that the association of low TAS levels with poor glycemic status would justify prescription of antioxidant supplies as dietary supplements in the treatment of poor glycemic diabetics. Moreover, hypertension and hyperlipidemia which very often coexist with type 2 diabetes produce oxidative stress and contribute to low TAS levels in these patients [29, 30]. While antioxidant therapy is advantageous in secondary prevention of oxidative damage, it also assists in control of hypertension and dyslipidemia [31]. Antioxidant therapy also hinders progression of vascular damage in diabetics by helping optimal control of glucose in these patients [31].

An interesting finding of this study was an age dependent increase in TAS among women who were metabolically controlled; although this favourable effect was not observed in men.

Sundaram et al. have demonstrated an age-dependent reduction in total antioxidant capacity, and an age independent increase in lipid profile and oxidative damage in elderly diabetics [32]. In the elderly diabetics, age-related perturbations of TAS would contribute significantly to the development of cardiovascular complications. This idea is worth to be testified in complementary larger scale trials.

The association between increased CIMT and CVD is well established [33]. CIMT is increased in patients with T2DM [34]. In the Epidemiology of Diabetes Interventions and Complications (EDIC) study, CIMT was measured 18 months after the Diabetes Control and Complications Trial (DCCT) and the investigators did not find a significant correlation between mean levels of HbA1c with CIMT during the study period. However when this measurement was extended 6 years later, a significant positive association was observed in both sexes between mean HbA1c levels and CIMT [35]. After 18 years, this association was still consistent and also more robust in diabetic women. This may be related to stronger atherosclerotic effect of CVD risk factors in women compared to men.

There are various reports in the literature regarding the association of CIMT with risk factors of carotid atherosclerosis. Some authors [36, 37] were unable to find a statistically significant correlation between CIMT and CVD risk factors in diabetic patients. Other investigators nonetheless, have reported a significant association between CIMT and age, sex, smoking, blood pressure, body mass index [38–40] and the presence of diabetes or glucose intolerance [34, 41, 42]. In our study, like the findings of two other research groups [36, 37], we did not observe a significant correlation between CIMT and gender, hyperlipidemia, hypertension, obesity, or HbA1c. However, we found just a significant association between age and duration of diabetes in poor glycemic group. It is probably because diabetes itself is of crucial importance for the development of atherosclerosis because of clustering of multiple interrelated metabolic disturbances over-shadows the contribution of other risk factors.

Peripheral Artery Disease (PAD), a manifestation of atherosclerosis in the lower limbs occurs with more than four-folds increased risk in diabetics. With each 1% increase in HbA1c, the risk of PAD increases 28%. Currently the most widely accepted test to assess PAD is the ABI. Decreased ABI is a major risk factor of CVD mortality [43] and low ABI (≤0.9) is an indicator of PAD [15]. These observations in diabetics may simply reflect the severity of diabetes due to its long duration and poor glycemic control. In general, risk factors for PAD are similar to those of coronary artery disease [44, 45].

There are diverse findings regarding the effect of glycemic control on PAD in T2DM. Some studies show a significant association between fasting serum glucose and HbA1c with PAD; in contrast to others [46, 47].

Although, there is no conclusive evidence to suggest that optimal glycemic control lowers the risk of PAD, in the presence of an increased risk of cardiovascular events, it is logical that optimal glycemic control would be desirable in patients with PAD. In our patients, there was no significant difference in ABI between two groups after adjusted analysis for glycemic control. One reason of this finding may be the absence of ABI ≤0.9 in our patients (The average of ABI within both good and poor glycemic groups was 1.0).

One limitation of the study was that we did not assess postprandial hyperglycemia and individual antioxidant intake of the participants. In addition, due to the cross sectional design of the study and small sample size, we were not able to evaluate the influence of age, sex, diabetes duration, obesity, hypertension and hyperlipidemia on oxidative stress and surrogate measures of subclinical atherosclerosis comprehensively in these patients. Our
results may be confounded as well by anti-diabetic, anti-hypertensive and lipid lowering medications; almost all of them have potent antioxidant effects and may have an independent effect on TAS and surrogate SA markers [48–50]. Complementary studies are needed in order to confirm our findings.

Conclusions
Our observations are in favour of the hypothesis that persistent hyperglycemia leads to decreased TAS levels and worse CIMT which is more pronounced in diabetics with poor glycemic control.

Considering the conflicting outcomes of different studies it is suggested that further cohort studies with larger number of patients are required to find out the interrelationship and contribution of various risk factors of atherosclerosis in diabetic patients. As expected the blood pressure, lipid profile, weight, oxidative stress and vascular stiffness in our patients with poor glycemic control were worse than well controlled ones. Increased CIMT, impaired ABI, and decreased TAS levels are considered to be the initial steps in cardiovascular complications of diabetes, which may be reversible. Thus in diabetic patients we suggest assessment of TAS levels concomitant with CIMT measurement for early initiation of antioxidant therapy. In addition, it is necessary to initiate primary and secondary preventive measures such as including plenary lifestyle modification and use of lipid-lowering, antihypertensive and glucose-lowering drugs to mitigate the devastating consequences of diabetes leading to CVD.

Endnotes
1National Cholesterol Education Program-Adult Treatment Panel III

Competing interests
The authors declare that they have no conflict of interest.

Authors’ contributions
OT-M conceived of the study, wrote draft the manuscript and performed the statistical analysis. FS performed the statistical analysis. MM participated in the design of study. SM-A helped finalizing the manuscript. ZB collected the data. MAK helped in statistical analysis. BL was the principle investigator, participated in its design and coordination and edited the final manuscript. HF was the co-principle investigator of the study. All authors read and approved the final manuscript.

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