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Original Research Article

The Association of Serum Iron, Zinc, and Copper Levels with Preeclampsia

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Abstract

Preeclampsia is defined as hypertension associated with proteinuria arise for the first time after the 20th week of gestation in a previously normotensive woman & resolve completely by the 6th postpartum week. In the present study trace and ultratrace elements iron, zinc, and copper were estimated to know the role of them in pathogenesis of pre-eclampsia. Case control study included 120 women, sixty of them were patients diagnosed with preeclampsia in the third trimester and the other sixty were healthy pregnant women (controls) in the third trimester. Serum levels of copper was measured by using graphite furnace atomic absorption spectrophotometer technique. While total serum iron and zinc concentrations were measured by using spectrophotometric method. The results were expressed as mean ± standard error of mean. A P value of < 0.01 was considered to be statistically significant. The levels of serum zinc and copper were significantly lower in patients with pre-eclampsia compared to control groups. While the concentration of total serum iron was significantly higher in patients with pre-eclampsia compared to control group. As conclusion, alteration in the serum levels of trace and ultratrace elements could contribute to the pathogenesis of PE.

Key words: Pre-eclampsia, Iron, Zinc, Copper, Graphite Furnace Atomic Absorption Spstrophotometry.

الخلاصة

تسمم الحمل (ما قبل الأرتعاج) ارتفاع ضغط الدم المرتبط بارتفاع البروتين بالإدرار بعد الأسبوع العشرين من الحمل في الحامل ذات ضغط الدم الطبيعي سابقا و الذي ينتهي تماما قبل الأسبوع السادس بعد الولادة.في هذه الدراسة تم تقدير بعض العناصر النزرة و فوق النزرة (الحديد و الزنك و النحاس) لمعرفة تأثيرها كمسببات أمراضية لمرض ما قبل الأرتعاج .وقد اشتملت الدراسة على١٢٠ امرأة، ستين امرأة تم تشخيص إصابتهن بمرض ما قبل الأرتعاج في الثلث الثالث من فترة الحمل والستين الآخر من النساء الحوامل الطبيعيات في الثلث الثالث. تم قياس مستويات الحديد و الزنك في المصل باستخدام المطيافية اللونية. في حين تم قياس تركيز النحاس في المصل باستخدام تقنية فرن الجرافيت– الامتصاص الذري الطيفي.كان مستوى الحديد في المصل أعلى بكثير في المرضى الذين يعانون من مرض ما قبل الأرتعاج مقارنة بالحوامل الطبيعيات. بينما كانت مستويات الزنك و النحاس أقل من ذلك بكثير في المرضى الذين يعانون من تسمم الحمل مقارنة مع مجموعة الحوامل الطبيعيات. استنتجت الدراسة أن تغير مستويات العناصر النزرة و فوق النزرة فيالدم يساهم في أمراضية ما قبل الأرتعاج.

الكلمات المفتاحية :مرض ما قبل الأرتعاج ، الحديد ، الزنك ، النحاس ، مطيافية الأمتصاص الذري– فرن الجرافيت.

Introduction

Preeclampsia is defined as hypertension associated with proteinuria arising de novo after the 20th week of gestation in a previously normotensive woman and resolving completely by the 6th postpartum week[1]. It is a hypertensive disorder of pregnancy and a leading cause of fetal and maternal morbidity and mortality[2].In UK preeclampsia affects 3-5% of pregnancies[3]. Its aetiology remains incompletely understood, and many theories have been considered. Factors

those are likely to be involved in the aetiology of this disease:

- abnormal trophoblastic invasion of uterine vessels.
- Impaired immunological adaptation between maternal, placental and fetal tissues.
- Impaired maternal adaptation to cardiovascular or inflammatory changes of normal pregnancy.
- Genetic factors that include inherited predisposing genes as well as epigenetic influences.
- Nutritional factors[4].

Vasospasm and endothelial cell dysfunction, with subsequent platelet activation and micro-aggregate formation, account for many of the pathological features of pre-eclampsia seen in almost every major organ system, for example in cardiovascular, liver, renal, coagulation, and central nervous systems [5].

Pregnancy is a period of rapid growth and cell differentiation for both the mother and fetus. Consequently, it is a period during which both are vulnerable to changes in dietary supply, especially of those micronutrients that are marginal under normal circumstances. Essential trace elements are involved in various biochemical pathways [6].

Their specific and the most important functions are the catalytic role in chemical reactions and in structural function in large molecules such as enzymes and hormones [7]. Alterations in concentrations and homeostasis of each of these micronutrients in body are well-known contributors in pathophysiology of various disorders and disease [6]. Trace elements such as zinc (Zn), selenium (Se) and copper (Cu) have antioxidant activity, while others like calcium (Ca) and magnesium (Mg) are essential micronutrients[8]. Despite several studies had conducted on these elements in pre-eclampsia, its aetiology has not yet been fully elucidated. Some have shown that changes in the levels of serum trace elements in pre-eclamptic patients may have a role in its pathogenesis [9] while others have failed to show an association of serum levels of trace elements and pre-eclampsia [10].

It has reported an increased incidence of pre-eclampsia in zinc-deficient regions and it was later found that zinc supplementation reduced the high incidence of the disease. Furthermore, decreased levels of zinc, selenium and copper have been observed in patients with pre-eclampsia. Ugwuja and et al. reported that only copper was found to be statistically significant. The previous studies on serum calcium and magnesium levels in pregnant women showed that there is significant difference between patients with pre-eclampsia and normal control group [11].

In pre-eclampsia, when tissues become ischemic, reactive oxygen species (ROS) such as superoxide and hydrogen peroxide are produced, but these ROS may not be able to initiate any cellular damage directly. The transition of metal ions such as iron, arising from ischemic placenta by destruction of red blood cells from thrombotic, necrotic and hemorrhagic areas can generate highly reactive hydroxyl radical by Fenton reaction. This radical can initiate lipid peroxidation, which if uncontrolled, results in endothelial cell destruction.

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$ (Fenton reaction)

$Fe^{3+} + O_2^{\cdot -} \rightarrow Fe^{2+} + O_2$

$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + OH^{\cdot} + OH^-$ (HABER–WEISS REACTION)[12].

Alteration of zinc (Zn) may cause pathogenesis of several diseases [13]. Normal homeostasis of Zn is regulated by the actions of Zn transporters like zinc-related protein. These transporters control the level of Zn inside and outside the cell **[**14, 15**].** It has been shown that zinc performs a considerable role for optimal function of more than 300 different enzymes [16]. Some studies have indicated that decreased zinc concentration is associated with fetal malformations, fetal growth restriction, preterm delivery, pre-eclampsia, and

bleeding after delivery [17, 18]. Serum or placental Zn levels have been shown to be lowor without change in pre-eclamptic women[19, 20]. It has been revealed that concentrations of zinc and copper reduced in pre-eclamptic patients [21]. While study of Ugwuja et al showed that only copper was statistically different[22]. There are also contradictory studies on the association of serum trace element levels with the event of pre-eclampsia [23]. Studies have indicated the possible association of trace elements with fetal growth and development and its relationship to newborn body weight, neonatal morbidity and mortality [24].

The aim of this Study is to estimate the trace elements (iron, zinc, and copper) and assess the role of them in pathogenesis of pre-eclampsia.

Materials and Methods

This study was carried out on patients collected from Babylon Teaching Hospital for Gynecology & Pediatrics, in Babylon Province, Hilla City. All samples were collected from November 2014 till February 2015.This is a case control study included 120 women, sixty of them were patients diagnosed with preeclampsia in the third trimester and the other sixty were healthy pregnant women (controls) in the third trimester.

Thorough assessment of these women which included history, physical examination and investigations in the form of complete blood count, liver function test, renal function test, coagulation profile and ultrasound and sometimes Doppler study of umbilical artery.Exclusion criteria included women with age over 40, BMI > 30, previous history of pre-eclampsia family history of pre-eclampsia, multiple pregnancy &hydropsfetalis, pre-existing hypertension or renal disease, pre-existing vascular disease, antiphospholipid syndrome, and smoking.

The age ,gestational age, and BMI of patients group were matched to age, gestational age, and BMI of control group, where statistical analysis showed non-significant differences in the age, gestational age, and BMI between patient and control groups (P> 0.05).

Ethical Issues: included**:**

a- Approval of scientific committee of the Clinical Biochemistry Department in Babylon Medical College/ University of Babylon/ Iraq.

b- Approval of Babylon Health Directorate/Ministry of Health &Information Center for Research & Development of Babylon Province.

c- The objectives and methodology were explained to all participants in the current study and their verbal consent was gained.

Samples collection

Venous blood samples were drawn from patient and control subjects. Five (ml) of blood were obtained from each subjects by vein punctureand put in the gel containing tubes, then allowed to clot at room temperature for 10-15 minutes and centrifuged at (2000 × g) for approximately 2-5 minutes then the serum were obtained was stored at -20˚C until analysis (measuring of Total Iron, Zinc, and Copper concentrations).

Determination of Serum Iron Conc:

Serum iron was measured by colorimetric method.Iron reacts with chromazurol B (CAB) and cetyltrimethyl-ammonium bromide (CTMA) to form a colored ternary complex with an absorbance measured at 623 nm. The intensity of the color produced, is directly proportional to the concentration of iron in the sample[25, 26, 27, and 28].

Determination of Serum Zinc Concentration:

Serum zinc was estimated by spectrophotometric method. Zinc forms with 2-(5-Brom-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol a red chelate complex. The increase of absorbance can be measured and is proportional to the concentration of total zinc in the sample [29 and 30].

Determination of Serum Cu, Concentration:

Copper concentration was determined by using Furnace Graphite Atomic Absorption Spectrometer (FGAAS). PG Instruments Ltd (UK), Cu hollow cathode lamp from Varian were used in the procedure.

Results

Demographic characteristics in pre-eclamptic and healthy women.

Table 1 showed the characteristics of study sample:

Table 1: Demographic characteristics of pre-eclamptic and healthy women

NO.	Characteristics	Control	Patient	P. Value
1	Maternal Age (Years) Mean ± SEM	26.85 ± 0.53	27.72 ± 0.66	> 0.05
2	Gestational Age(Weeks) Mean ± SEM	37.28± 0.28	36.93± 0.46	> 0.05
3	BMI (Kg/m^2) Mean ± SEM	27.83 ± 0.24	28.31 ± 0.20	> 0.05
4	Diastolic BP(mmHg) Mean ± SEM	77.60 ± 0.69	106.42 ± 2.03	< 0.01
5	Systolic BP(mmHg) Mean ± SEM	115.62 ± 0.90	159.63 ± 2.63	< 0.01
6	Protein/Creatinine Ratio (mg/mmol) Mean ± SEM	27.94 ±0.19	38.82 ± 0.31	< 0.01
7	**Parity**			
	ParityCharacteristics	**Control**		**Patient**
		No.	**%**	**No.**	**%**
	0	4	6.666	31	51.666
	1-3	54	90	22	36.666
	≥ 4	2	3.333	7	11.666
8	**Number of samples**	60		60	

Age distribution in pre-eclamptic and healthy women:

There were no statistically significant difference between the mean age of pre-eclamptic women (patients) (27.72 ± 0.66 years) and the mean age of healthy women (control) (26.85 ± 0.53 years), (P. value > 0.05) as shown in Table 1.

Distribution of patients and control by maternal gestational age:

Table 1 showed the distribution of maternal gestational age in patients and control. There were no statistically significant difference between means of gestational ages for pre-eclamptic women (patients) (36.93± 0.46 weeks) and healthy women (control) (37.28± 0.28 weeks), (P. value > 0.05).

Distribution of patients and control by body mass index:

There were no statistically significant difference between the means of body mass index of pre-eclamptic women (patients) (28.30 ± 0.20 Kg/m^2) and the mean of body mass index of healthy women (control) (27.83 ± 0.24Kg/m^2), (P. value > 0.05) as shown in Table 1.

Distribution of patients and control by blood pressure:

There were statistically significant differences between the mean of diastolic blood pressure of pre-eclamptic women

(patients) (106.42 ± 2.03 mmHg) and the mean of diastolic blood pressure of healthy women (control) (77.60 ± 0.69 mmHg), (P. value < 0.01) as shown in Table 1.

Table-1 demonstrated that there was statistically significant differences between the mean of systolic blood pressure of pre-eclamptic women (patients) and the mean of systolic blood pressure of healthy women (control) (159.63 ± 2.63mmHg, 115.62 ± 0.90mmHg) respectively, (P. value < 0.01).

Distribution of patients and control by urinary total proteins/creatinine ratio:

Table 1 showed the distribution of maternal gestational age in both groups. There were statistically significant differences between mean of urinary total proteins/creatinine ratio for pre-eclamptic women (patients) (38.82 ± 0.31 mg/mmol) and healthy women (control) (27.94 ± 0.19 mg/mmol), (P. value <0.01).

Distribution of patients and control by parity:

Table 1 revealed the deference in parity between patients and control. (51%) of patients were primigravida while (6.666 %) were primigravida in the control group this result can be explained by the fact that first pregnancy is regarded as a risk factor for pre-eclampsia.

Trace and Ultra Trace Elements:

Iron (Fe) Concentrations

Figure 1 revealed that the levels of serum iron concentrations were statistically higher in pre-eclamptic women than in healthy pregnant women (186.498± 5.514 µg/dl vs.94.392 ± 9.962 µg/dl), (P. value < 0.01).

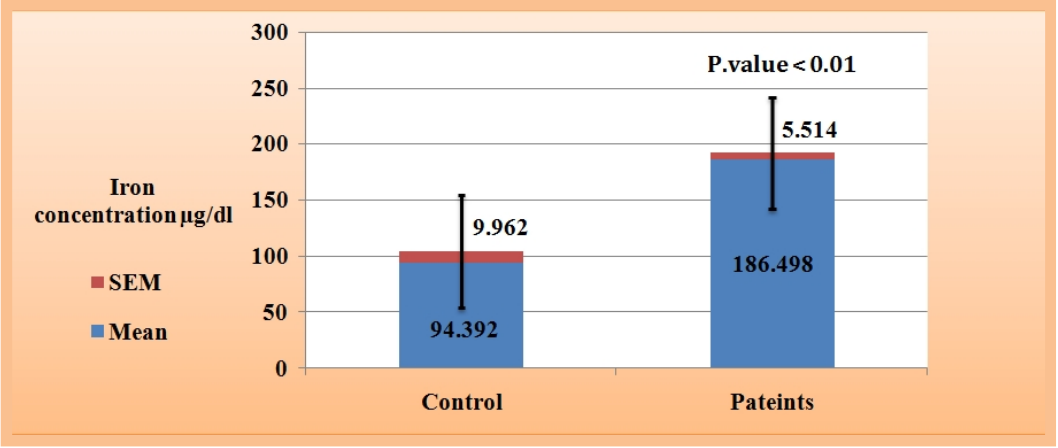

Figure 1 : Serum iron concentration (µg/dl) in pre-eclamptic and normal pregnant women (mean ±SEM)

Zinc (Zn) Concentrations

Figure 2illustrated that the levels of serum zinc concentrations were statistically lower in pre-eclamptic women than in healthy pregnant women(57.283 ± 1.740 µg/dl vs. 87.535 ± 3.710 µg/dl), (P. value < 0.01).

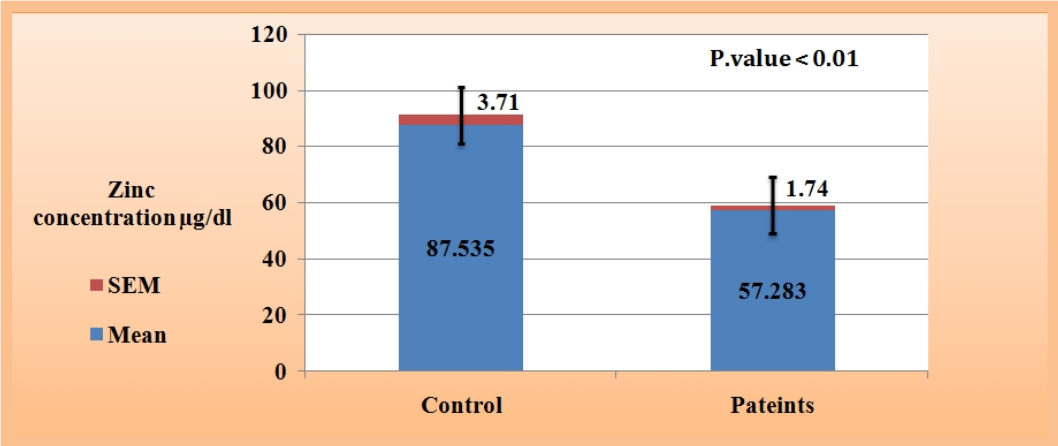

Figure 2 : Serum zinc concentration (µg/dl) in pre-eclamptic and normal pregnant women (mean ±SEM)

Copper (Cu) Concentrations

Figure 2 showed that the levels of serum copper concentrations were statistically lower in pre-eclamptic women than in healthy pregnant women (143.153 ± 3.316 µg/dl vs. 209.657± 8.679 µg/dl), (P. value < 0.01).

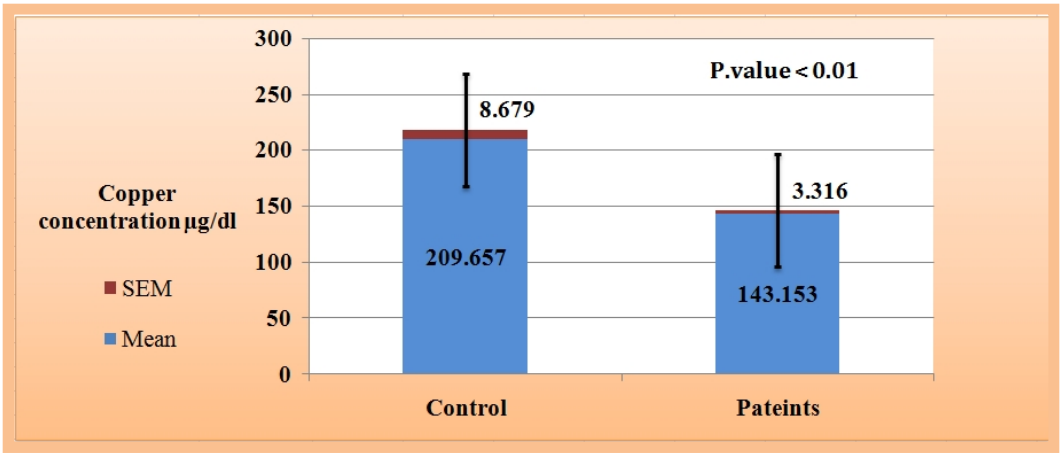

Figure 3 : Serum copper concentration (µg/dl) in pre-eclamptic and normal pregnant women (mean ±SEM)

Discussion

proteinuria

Testing for proteinuria is essential in differentiating pregnancies with pre-eclampsia from those with gestational or chronic hypertension and, thus, identify those pregnancies most prone to adverse outcome. measurement of significant proteinuria, traditionally 300 mg excretion in a 24-hour period, is prone to collection and measurement error. Because collection of 24-hour urine sample is not practical as a routine test, urine dipstick screening is employed as a first-line screening test with secondary tests employed to confirm positive dipstick test. Visual dipstick reading is not reliable but the use of automated dipstick readers significantly improves the accuracy of dipstick testing[31] and therefore is

recommended by The National Institute for Health and Care Excellence (NICE) for use in pregnancy.

NICE also recommends that quantification of proteinuria should follow diagnosis. There are two methods. The first is the 24-hour urine protein estimation and this needs that an assessment of sample completeness is undertaken, with measurement of creatinine excretion being the most common. NICE also supports the use of the protein/creatinine ratio test. This test is done on a 'spot' urine sample and is therefore much quicker. Numerous studies showed that this test to be comparable to the 24-hour urine protein estimation. Significant proteinuria by this test is> 30 mg protein/mmolcreatinine[2].

Iron (Fe) Concentrations

An imbalance between pro-oxidants and anti-oxidants results in oxidative stress which increases the potential for the development of preeclampsia [32]. Free iron acts as pro-oxidant agent and it is released from ferritin by the reducing agents that convert Fe^{3+} into Fe^{2+}. Under stress or pathological conditions, it undergoes Fenton reaction and Haber – Weiss reaction to generate ROS, which in turn damage the biological macro molecules [33].The elevated serum iron levels are due to hemolysis caused by physical destruction of red blood cells (RBCs) as a result of vasospam or abnormal endothelial cell erythrocyte interactions. Excess iron is a causative factor of oxidative stress (i.e., in its radical form) involved in the pathogenesis of preeclampsia [34].The excess iron released from destruction of RBCs can react with free radicals produced from cell membrane (as it is rich in polyunsaturated fatty acids) and circulating lipoproteins initiates lipid peroxidation[35]. In addition to this the damaged placenta is a site for release of free radicals (FR) in pre-eclampsia. The elevation or excess iron can also react with these released FR of placenta and can initiate and propagate lipid peroxidation both in placenta and systemic vasculature. This is one of the significant etiologic factors in the endothelial cell damage of preeclampsia [36].The present study findings were agreed with Osman R, Hameed R. and etal [37,38].

Zinc (Zn) Concentrations

Metalloprotein (Zn) is an important trace element in many functions of the human body including metabolism, growth, development and reproduction. It is a co-factor for the synthesis of a number of enzymes. It has important roles in nucleic acid metabolism and protein synthesis, as well as membrane structure and function [39 and 40]. Previous studies have suggested that alterations in maternal serum or plasma Zn levels are found in preeclampsia [41]. In this study it has been found significantly lower levels of Zn in the preeclamptic women when compared to control ($P < 0.01$). Similar results were obtained by earlier reports [17, 20, 42, and 43].

Zn deficiency has been found to be associated with complications of pregnancy including pre-eclampsia. Zn is transferred passively from mother to fetus across the placenta and there is decreased Zn binding capacity of maternal blood during pregnancy, this facilitates efficient transfer of Zn from mother to fetus. During pregnancy, there is a decline in circulating Zn and this increases as the pregnancy progresses, this might be attributed to decrease in Zn binding and increased transfer of Zn from the mother to the fetus. Zn deficiency is also related to hemodilution and increased urinary excretion[44]. In pregnant women with preeclampsia, low serum zinc may be partly due to reduced concentrations of Zn-binding protein and estrogen caused by increased lipid peroxidation [45 ,46]. This leads us to hypothesize that zinc may play a role in pre-eclampsia through an increase of lipid peroxidation. The association between zinc and pre-eclampsia although attempts to modify the frequency of pre-eclampsia with zinc supplementation has not been successful.

Recently, the role of oxidative stress or excessive lipid peroxidation has been implicated in the pathogenesis of pre-eclampsia. There is an imbalance between antioxidant enzyme activities and pro-oxidant production.

Maternal zinc deficiency is related with serum cortisol level that increases during normal pregnancy and it is much higher in preeclampsia. Several investigators have noted that women with preeclampsia as compared with normotensive pregnant women had lower zinc concentrations [47]. Zinc deficiency in the placental tissue might lead to insufficiency of superoxide dismutase, an antioxidant enzyme. Furthermore, deficiency in placental zinc also plays a role in the biosynthesis of connective tissue, maintaining its integrity, which might have an impact on the structure of the spiral arteries [48].

Copper (Cu) Concentrations

Copper has been shown to be involved in the function of several cuproenzymes that are essential for life. It is known to be a cofactor of the antioxidant enzyme superoxide dismutase [49]. Gurer et al and Açikgoz et al reported increased malondialdehyde and decreased ceruloplasmin activity in the plasma of preeclamptic women [50 and 48]. Based on these observations, low levels of copper in preeclamptic women may be associated with impairment of the cell antioxidant capacity and oxidant/antioxidant balance. Ceruloplasmin containing copper which catalyses the conversion of ferric ion to its ferrous form and favors the absorption of iron from the gastro-intestinal tract. It also plays a role in the mobilization of iron to plasma from the tissue stores. Findings of present study indicated a decrease in the serum concentrations of copper in preeclamptic patients when compared to control subjects. Previous studies have shown that copper deficiency is related more to fetal complications like birth defect that to maternal complication [51].

Conclusion

Alteration in the levels of serum trace and ultratrace elements could contribute to the pathogenesis of PE. There was a statistical reduction in the levels of zinc and copper in pre-eclamptic patients compared to control pregnant women. On the other hand, iron level was significantly increased in patients when in comparison to control group.

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