

The Impact of Third Trimester Maternal Serum Vitamin B12 and Folate Status on Fetal Birth Weight. Is Maternal Serum Homocysteine a Predictor of Low Birth Weight Infants?

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Abstract

Objective: The aim of this study is to evaluate vitamin B12, folate, and homocysteine status in pregnant women in the third trimester of pregnancy and their relationship to fetal birth weight and their correlation to corresponding neonatal cord blood levels, and in addition, to evaluate the possibility of maternal serum homocysteine level as a predictor of low birth weight infants.

Subjects and Methods: In this cross-sectional study, a total of two hundred pregnant women in third trimester (≥ 28 weeks) were recruited. After a detailed obstetrical and medical history, and clinical assessment, participants were subdivided into two groups: Group (A)—pregnant women who delivered average birth weight (ABW) infants and Group (B) for those who delivered low birth weight (LBW) infants between completed 37 and 42 weeks.

Results: Vitamin B12 deficiency was observed in 24.1% of the total cohort. The mean vitamin B12 level was significantly lower in group (B) compared to group (A) (195.2 ± 38.9 vs. 225.9 ± 66.59 respectively $P = 0.008$). The mean level of homocysteine for women in group (B) was significantly higher than those determined from women in group (A) (9.10 ± 5.9 vs. 7.6 ± 3.83 respectively, $P = 0.042$). On the other hand, the mean folate levels showed statistically insignificant differences between both groups. The mean cord vitamin B12 level was significantly lower in LBW infants in comparison to ABW infants (277 ± 61.93 vs. 312.03 ± 81.87 respectively, $P = 0.015$), while the mean level of cord homocysteine for LBW infants was significantly higher than those levels determined from ABW infants (7.9 ± 3.79 vs. 6.6 ± 2.09 respec-

tively $P = 0.0049$). **Conclusion:** Maternal micronutrients particularly cobalamin deficiency could be significant risk for LBW infants. Hyperhomocysteinemia has been shown to be a predictor for adverse pregnancy outcomes particularly LBW.

Keywords

Low Birth Weight, Vitamin B12, Homocysteine

1. Introduction

Pregnancy is a period of fetal growth and development which necessitates increase in the nutrients [1]. A pregnant woman requires extra amounts of nearly all essential nutrients, including iron, vitamin B12 and folic acid [2]. These maternal micronutrients are involved in the one-carbon metabolism (1-C) required for methylation capacity which can cause adverse metabolic processes.

Vitamin B12 (cobalamin) is a micronutrient coenzyme synthesized in the liver and called as extrinsic factor essential for DNA, and RNA synthesis, erythropoiesis, and proteins, and lipids synthesis in the cytoplasm [3]. A progressive reduction in vitamin B12 levels has been documented to occur during pregnancy despite the maintenance of normal dietary intakes. Guerra-Shinohara *et al.* [4] reported 37% decrease in vitamin B12 levels in pregnancy which considered owing to physiological changes in hormonal status and increase in plasma volume, and returned to pre-pregnancy levels within few weeks after delivery [5]. Severe cobalamin deficiency leads to insufficient DNA synthesis which results in pernicious anemia and neuropsychiatric symptoms which cannot coexist with pregnancy [6]. In a recent systemic review, cobalamin insufficiency reported to be common among pregnant women in all trimesters by 20% - 30% [7].

Animal proteins are the main dietary sources of cobalamin, and its absorption requires binding to intrinsic factor secreted by gastric cells forming a complex in the terminal ileum then released into the portal circulation and binding to transcobalamin II [8]. It is a water-soluble vitamin and can cross the placenta. Low vitamin B12 and folate in pregnant women have an impact on fetal birth weight by influencing placental development [9]. Previous studies explored that there is risk for low birth weight (LBW) infants born to vitamin B12 and folic acid deficient mothers [10] [11] [12].

Vitamin B12 maintains normal folate metabolism and its deficiency results in secondary folate deficiency which is crucial for cell multiplication, specifically in the rapidly dividing placental and fetal tissues [11]. Folate in pregnancy plays an essential role in embryonic formation particularly at the time of neural tube closure [13], in addition to prevention of adverse fetal outcomes such as other birth defects and growth retardation [11]. Maternal folate deficiency has been linked to increased risk of stillbirth, spontaneous abortion, abruptio placentae, preterm delivery, and LBW [14]. Relton *et al.* [15] in their large study stated that early pregnancy red cell folate level was an important determinant of infant birth

weight. Therefore, the maternal micronutrients status during pregnancy has a direct impact on birth weight, and the adequate supply of these micronutrients is known to be very crucial during pregnancy, and has been shown to increase birth weight by approximately 100 g [2] [16].

Plasma homocysteine concentration during normal pregnancy is lower than non-pregnant state with the nadir at second trimester and then relatively increases at 32 weeks of gestation until term [17]. It is a non-protein forming amino acid, and is not taken from diet, and it is synthesized from methionine. Most homocysteine is catabolised to cystathionine by cystathionine-h-synthase. Another pathway to be regenerated into methionine by methionine synthase where vitamin B12 and folate are necessary cofactors for the conversion of homocysteine to methionine, required for the synthesis of phospholipids and neurotransmitters [7].

Elevated plasma homocysteine concentration can arise from inadequate folate or vitamin B12 status, but also from physiological, genetic, as well as pathological causes such as neural tube defects [18], placental abruption [19], and pre-eclampsia [20]. Hyperhomocysteinaemia has been associated with high risk for renal, cardiovascular, and neurodevelopmental diseases [21].

The aim of this study is to evaluate vitamin B12, folate, and homocysteine status in pregnant women in the third trimester of pregnancy and their relationship to fetal birth weight, and in addition, to investigate the correlation between maternal micronutrients to their corresponding neonatal cord blood levels, and the possibility of maternal serum homocysteine level to be a predictor of LBW.

2. Subjects and Methods

2.1. Study Design

This cross sectional study was conducted at Obstetrics and Gynecology Department of IBN Sina College Hospital, Saudi Arabia, from September 2016 to May 2107. A total of two hundred pregnant women in the third trimester (≥ 28 weeks) attending the department for antenatal care were recruited for this study. Participants were subdivided into two groups, group (A) pregnant women who delivered average birth weight (ABW) infants ≥ 2.5 Kg, and group (B) for those who delivered low birth weight (LBW) infants < 2.5 Kg. This study was approved by the Hospital Research Ethics Committee and has been performed in accordance with the ethical standards as in Declaration of Helsinki (1964) and its latter amendments, and a written informed consent was obtained from each participant prior to the study. All deliveries occurred within the gestational age between completed 37 and 42 weeks.

2.2. Exclusion Criteria

Exclusion criteria include pregnant women with multiple pregnancies, or with chronic diseases such as diabetes mellitus, hypertensive disorders, heart disease, thyroid disease, metabolic disease, hepatic disorder, malnutrition and preterm deliveries.

2.3. All Participants Were Subjected to

- Thorough history taking (obstetric history, family history, socioeconomic status, educational level, and anti-anemic and multivitamins supplements).
- Clinical assessment regarding anthropometric measurements (height, weight, and body mass index (BMI)), and vital signs.
- Gestational age was calculated in weeks from the first day of the last menstrual period confirmed by subsequent ultrasonography.
- Maternal blood samples were obtained from the antecubital vein under complete aseptic conditions from all subjects.

The blood is collected in three tubes namely Sodium Fluoride tube (for Blood Glucose), EDTA tube (for CBC), and Plain Tube (Red Top). The plain tube was left to clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1000× g at room temperature. Freshly prepared serum was used for estimation of liver function tests, renal function tests, vitamin B12, folic acid, and homocysteine levels.

2.4. Serum Vitamins Measurements

Serum vitamin B12, folic acid, and homocysteine assay were done by architect i system from abbot diagnostics using chemiluminescent microparticle immunoassay (cmia) for quantitative determination of each of them. The reference range for serum B12 was 200 - 662 pg/ml, and for serum folate was 3.1 - 20.5 ng/ml. Deficiency of the two micronutrients was defined as <200 pg/ml, and <3.1 ng/ml, respectively [22]. The reference range for serum homocysteine for females was 4.44 - 13.56 µmol/l [23].

After delivery of the fetus, umbilical vein samples were taken immediately following clamping of the cord before separation of the placenta, then serum was separated and serum vitamin B12, folic acid, and homocysteine levels were measured. Furthermore, anthropometric measurements (birth weight, length, head circumference), and Apgar score were taken from newborns. Classification of birth weights was evaluated according to intrauterine growth curves and maturity by Hadlock [24]. Small for gestational age (SGA) were those babies with weights below 10th percentile for sex and gestational age, those between 10th and 90th percentile were considered to be appropriate for gestational age (AGA), and those over 90th percentile were considered to be large for gestational age (LGA).

3. Statistical Analysis

The data was collected and entered into the personal computer. Statistical analysis was done using Statistical Package for Social Sciences (SPSS/version 21) software. Arithmetic mean, standard deviation was calculated for numerical data, while number and percent was calculated for categorized data. To compare the categorized parameters Chi-square test was used while for numerical data t-test was used to compare two groups. The level of significant was 0.05.

Sample Size Calculation

Sample size calculation was conducted using Epi-save software to conduct study on micronutrient deficiency during pregnancy. The sample was estimated to be 200 subjects to detect the incidence of low birth weight among pregnant with micronutrient deficiency. The estimated sample size is made at assumption of 95% confidence level and 80% power of study.

4. Results

Two hundred consented women participated in the study, 187 of them (93.5%) delivered in our hospital between completed 37 and 42 weeks of gestation with a known pregnancy outcome. The demographic characteristics of the study participants are illustrated in **Table 1**. There were no statistically significant differences between the two groups for maternal age, gestational age, parity, and BMI. When micronutrients were analyzed according to the gravidity, 55 (29.4%) participants were primigravida. Smoking habit was observed in 14 (7.8%) participants, and showed statistically insignificant differences between both groups.

Approximately 58% (108/187) of the participants used anti-anemic preparations during pregnancy, 20.3% (38/187) used anti-anemic and multivitamins preparations, 9.6% (18/187) used multivitamins only, and 12.2% (23/187) did not use any preparations at all. Moreover, 64% of participants had a post-high school educational degree, while 36% were educated at different levels up to high school.

For the whole cohort, the mean serum vitamin B12 and folate levels at the third trimester of pregnancy was 221.0 ± 63.9 , 9.50 ± 3.50 respectively. When all participants were classified according to birth weight, the mean vitamin B12 level was significantly lower in group (B) compared to group (A) (195.2 ± 38.9 vs. 225.9 ± 66.59 respectively $P = 0.008$). Vitamin B12 deficiency (lower than 200 pg/ml) was observed in 24.1% of the total cohort and in 31% of pregnant women in group (B) compared to only 22% in group (A). Furthermore, hyperhomocysteinaemia was detected in 11.7% (22/187) of the participants, and the mean level of homocysteine for women in group (B) was significantly higher than that determined from women in group (A) (9.10 ± 5.9 vs. 7.6 ± 3.83 respectively, $P = 0.042$). On the other hand, the mean folate level showed statistically insignificant differences between group (A) and (B) (9.57 ± 3.63 vs. 9.1 ± 2.73 respectively, $P = 0.27$). Moreover, folate deficiency (lower than 3.1 ng/ml) was rare and observed only in 3.2% of the total cohort (**Table 2**).

Cord blood samples for vitamin B12, folate, and homocysteine levels were analyzed. Although, the mean cord B12 levels were significantly lower in LBW infants in comparison to ABW infants (277 ± 61.93 vs. 312.03 ± 81.87 respectively, $P = 0.015$), but the mean folate levels showed statistically insignificant differences between neonates in both groups (11.02 ± 3.17 vs. 10.03 ± 3.25 respectively, $P = 0.062$). Moreover, the mean levels of cord homocysteine for LBW infants were significantly higher than those levels determined from ABW infants (7.9 ± 3.79 vs. 6.6 ± 2.09 respectively $P = 0.0049$) (**Table 3**).

Table 1. Demographic characteristics of patients in group (A), and (B).

Variables	Group (A) (n = 158)	Group (B) (n = 29)	Total (n = 187)	P
Age (Year)				
Mean $\pm$ S.D	26.9 $\pm$ 6.05	25.4 $\pm$ 5.56	26.7 $\pm$ 5.95	0.11 ^a
Gestational age (Weeks)				
Mean $\pm$ S.D	38.9 $\pm$ 1.46	38.9 $\pm$ 1.35	38.9 $\pm$ 1.43	0.453 ^a
Gravidity				
Mean $\pm$ S.D	2.88 $\pm$ 1.46	2.69 $\pm$ 1.14	2.73 $\pm$ 1.43	0.251 ^a
PG/MG	46/112	9/20	55/187	0.398 ^b
Parity				
Mean $\pm$ S.D	1.88 $\pm$ 1.45	1.69 $\pm$ 1.14	1.76 $\pm$ 1.2	0.260 ^a
BMI (kg/m²)				
Mean $\pm$ S.D	25.12 $\pm$ 3.03	24.69 $\pm$ 2.20	25.05 $\pm$ 2.91	0.234 ^a
Smoking	12/158 (7.5%)	2/29 (6.8%)	14/187 (7.5%)	0.399 ^b

Data presented as mean $\pm$ SD; *Significant ($P < 0.05$); ^aBased on t-test; ^bBased on Chi square test; PG: primigravida; MG multigravida; BMI (body mass index).

Table 2. Maternal serum vitamin B12, folate, and homocystiene levels in relation to birth weight.

Variables	Group (A) (n = 158)	Group (B) (n = 29)	Total (n = 187)	P
Vitamin B 12 (pg/ml)				
Mean $\pm$ S.D.	225.9 $\pm$ 66.59	195.2 $\pm$ 38.9	221.0 $\pm$ 63.9	0.008 ^{a*}
<200 pg/ml	(36/158 = 22%)	(9/29 = 31%)	(45/187 = 24.1%)	0.013 ^{b*}
Folic acid(ng/mL)				
Mean $\pm$ S.D	9.57 $\pm$ 3.63	9.1 $\pm$ 2.73	9.50 $\pm$ 3.50	0.27 ^a
<3.1 ng/mL	(5/158 = 3.1%)	(1/29 = 3.4%)	6/187 = 3.2%)	0.109 ^b
Homocystiene (μmol/L)				
Mean $\pm$ S.D	7.6 $\pm$ 3.83	9.10 $\pm$ 5.9	7.86 $\pm$ 4.22	0.042 ^{a*}
>15 μmol/L	(15/158 = 9.5%)	(7/29 = 24.1%)	22/187 = 11.7%	0.048 ^{b*}

Data presented as mean $\pm$ SD; *Significant ($P < 0.05$); ^aBased on t-test; ^bBased on Chi square test.

Table 3. Neonatal serum vitamin B12, folate, and homocystiene in relation to birth weight.

Variables	Group (A) (n = 158)	Group (B) (n = 29)	Total	P
Vitamin B 12 (pg/ml)				
Mean $\pm$ S.D.	312.03 $\pm$ 81.87	277 $\pm$ 61.93	306.6 $\pm$ 80.1	0.015 ^{a*}
Folic acid (ng/mL)				
Mean $\pm$ S.D	11.02 $\pm$ 3.17	10.03 $\pm$ 3.25	10.86 $\pm$ 3.19	0.062 ^a
Homocystiene (μmol/L)				
Mean $\pm$ S.D	6.6 $\pm$ 2.09	7.9 $\pm$ 3.79	6.85 $\pm$ 2.46	0.0049 ^{a*}

Data presented as mean $\pm$ SD; *Significant ($P < 0.05$); ^aBased on t-test.

The clinical characteristics of the newborns are described in **Table 4**. There were no statistically significant differences between the neonates in the two groups regarding sex, head circumference, and Apgar score. On the contrary, the

Table 4. Anthropometric measurements of neonates in both groups.

Variables	Group (A) (n = 158)	Group (B) (n = 29)	P
Weight (Kg)	3.4 ± 0.33	2.1 ± 0.18	0.022 ^{a*}
Length (cm)	46.0 ± 2.11	43.6 ± 1.04	0.031 ^{a*}
Head Circumference (cm)	36.0 ± 2.01	33.66 ± 1.56	0.082 ^a
Sex	64 (40.5%)	11 (37.9%)	0.365 ^b
Male Female	94 (59.5%)	18 (62.1%)	
APGAR Score	7.0 ± 0.25	6.4 ± 0.23	0.105 ^a
1 min 5 min	8.0 ± 0.36	7.1 ± 0.75	0.241 ^a

Data presented as mean ± SD; *Significant ($P < 0.05$); ^aBased on t-test; ^bBased on Chi square test.

neonatal weight and length were significantly lower in group (B) compared to group (A). Correlations between maternal B12, folate, and homocysteine levels and neonatal clinical and biochemical variables are illustrated in **Table 5**. There was a strong significant positive correlation between maternal serum vitamin B12 and birth weight, we also observed a strong negative correlation between maternal serum homocysteine and birth weight. Ultimately, the risks of low vitamin B12 were significant for LBW infants (OR 8.22, 95% CI), and lower head circumference (OR 2.62, 95% CI). On the other hand, the risks of hyperhomocysteinemia were significant for LBW infants (OR 7.54, 95% CI), and lower head circumference (OR 2.01, 95% CI).

5. Discussion

In most developing countries, nutritional deficiencies of micronutrients are common in pregnant women [4]. Physiologically vitamin B12 decreases during pregnancy due to hemodilution, changes in binding proteins and active transport to the fetus [25] [26]. Allen *et al.* and Lima *et al.* do not believe that pregnant women have vitamin B12 deficiency since a balanced diet would maintain daily requirements [27] [28]. Deficiency of cobalamin and folate results in hyperhomocysteinemia which have been considered to be a sensitive marker of their concentrations [29], and subsequently causes intrauterine growth restriction [1]. The results of our study showed that one hundred and seventy eight participants completed the study till delivery, and the rates of vitamin B12 deficiency are 24.1% in the total population, 22% in women who delivered ABW infants group (A) and 31% for those who delivered LBW infants group (B). Worldwide the prevalence of maternal vitamin B12 insufficiency is different in many studies, 59%, in Venezuela [30], 46% in South Korea [31], 40% in UK [32], 36.5% in Turkey [33], and 8% in Sudan [34]. Recently Sukumar *et al.* [7] reported that the pooled estimate of maternal vitamin B12 insufficiency during pregnancy across all three trimesters were 21%, 19%, and 29% respectively.

The effects of maternal B12 level on birth weight is still controversial, our results showed that the mean maternal vitamin B12 level in mothers with LBW

Table 5. Correlation between maternal serum vitamin B12, folate, and homocystiene and different studied variables.

	Maternal Vitamin B12		Maternal Folic acid		Maternal Homocystiene	
	r	P	r	P	r	P
Neonatal Vitamin B12	0.652*	0.0001	0.112	0.127	-0.746*	0.0001
Neonatal Folic acid	0.068	0.354	0.072	0.326	-0.040	0.590
Neonatal Homocystiene	-0.537*	0.0001	-0.076	0.303	0.497*	0.0001
Weight	0.629*	0.0001	0.111	0.132	-0.598*	0.0001
Length	0.017	0.814	0.100	0.173	-0.094	0.201
Head Circumference	0.544*	0.001	-0.004	0.958	-0.547*	0.001
APGAR score 1min	0.043	0.560	0.022	0.760	-0.053	0.469
APGAR score 5 min	0.102	0.166	0.012	0.868	-0.114	0.121

r: Correlation; *: Significant correlation.

infants was significantly lower than that of mothers of ABW infants (195.2 ± 38.9 vs. 225.9 ± 66.59 respectively $P = 0.008$) which is in agreement with previous studies [12] [35] [36] [37]. Van Sande *et al.* [38] reviewed 29 articles and they concluded that although there is an association between low maternal vitamin B12 and intrauterine growth retardation, but no studies have definitively determined the cut off value for serum vitamin B12 level during pregnancy. Recently, a meta-analysis was done included eighteen studies observed no linear association between maternal B12 levels in pregnancy and birth weight, but B12 deficiency (<148 pmol/L) was associated with a higher risk of LBW in newborns (adjusted risk ratio = 1.15, 95% confidence interval (CI): 1.01, 1.31) [39].

However, several studies did not detect any association between maternal vitamin B12 levels and fetal growth restriction [3] [10] [33] [40] [41] [42]. Recently a systematic review and meta analysis done by Sukumar *et al.* [7] they reviewed 57 reviews and 23 articles, showed non significantly lower maternal vitamin B12 concentrations in LBW than in ABW infants but higher odds of LBW with lower vitamin B12 values (adjusted OR: 1.70; 95% CI: 1.16, 2.50), and concluded that there was no significant effect of low maternal vitamin B12 level and LBW. The authors concluded also that the lack of an association might have been due to an overall low vitamin B12 status or, it would be difficult to demonstrate a nutrient effect in these women who were vitamin B12 replete.

Regarding maternal folic acid, we observed only 3.2% folate deficiency in the total population, while Adaikalakoteswari *et al.* [32] reported 11% folate deficiency in their study on 91 pregnant women and their neonates. Moreover, maternal serum folic acid level showed insignificant differences between group A and B (9.57 ± 3.63 vs. 9.1 ± 2.73 respectively, $P = 0.27$), which contradicts other previous studies as [12] [36] [43]. A systematic review done by Fekete *et al.* [44] showed two-fold increase in folate intake in early pregnancy is associated with 2% increase in birth weight, which is a slight but significant increase. Therefore,

vitamin B12 and/or folic acid deficiency is likely to affect methylation pathway of homocysteine to methionine which represents a crucial part of the one-carbon metabolism for synthesis of DNA, and RNA. Hyperhomocysteinemia has been considered to be a sensitive functional marker of micronutrients concentrations [45].

Our results showed significantly high serum homocysteine level of mothers of LBW infants compared to mothers of ABW infants (9.10 ± 5.9 vs. 7.6 ± 3.83 $\mu\text{mol/L}$ respectively, $P = 0.042$) which is consistent with former studies which reported that higher total homocysteine was significantly associated with LBW but they suggested the cause of LBW is vitamin B12 deficiency rather than folate deficiency [3] [46] [47] [48]. On the other hand Guerra-Shinohara *et al.* [4] found no correlation was detected between birth weight and maternal or neonatal biochemical variables (B12, RBC and serum folate and total homocysteine). In our study we revealed a strong significant positive correlation between maternal antenatal serum vitamin B12 and birth weight which is supported by Ahmed *et al.* and Frery *et al.* [12] [49]. On the contrary, we also observed a strong negative correlation between maternal serum homocysteine and birth weight, and no correlation found between maternal serum folate and birth weight. Ultimately, maternal vitamin B12 and homocysteine levels found to be significant risks for LBW infants.

According to cord blood samples, neonatal folic acid showed insignificant differences between both groups. On the other side, cord vitamin B12 was significantly lower while homocysteine was significantly higher in LBW infants compared to ABW infants (277 ± 61.93 vs. 312.03 ± 81.87 , $P = 0.015$; 7.9 ± 3.79 vs. 6.6 ± 2.09 , $P = 0.0049$ respectively), which is in line with Muthayya *et al.* [37] who found that new born with birth weights (<2500 g and 2500 - 2999 g) had significantly lower mean cord serum vitamin B12 concentrations when compared to those who were ≥ 3000 g. We also observed a significant positive correlation between maternal and cord levels of vitamin B12 and homocysteine which is in agreement with other former studies [48] [49], in addition to significant inverse correlation between maternal B12 and neonatal homocysteine supported by Molloy *et al.* [11] in an Irish population documenting that low maternal B12 levels predicted hyperhomocysteinemia in both the newborns and the mothers. Therefore, neonatal total homocysteine levels were directly affected by maternal total homocysteine levels but inversely affected by neonatal vitamin B12 levels.

6. Study Limitation

Our study had some limitations particularly the costly screening of vitamin B12, folate, and homocysteine in both mothers and neonates. However, this study raises interesting issues requiring further investigations for better assessment of growth and neurodevelopment of infants.

7. Conclusion

The present study confirms that low maternal vitamin B12 concentration in the

third trimester of pregnancy is directly reflected on neonatal vitamin B12, and homocysteine status. Therefore, maternal micronutrients particularly cobalamin deficiency could be significant risk for LBW infants, and hyperhomocysteinemia has been shown to be a predictor for adverse pregnancy outcomes particularly LBW. Deficient pregnant women are not capable of supporting their fetuses with necessary micronutrients particularly in low socio-economic group and screening of micronutrients is unconventional, so we strongly support enriched diet and vitamin supplementation throughout pregnancy to decrease risk factors of LBW infants and other health hazards.

Declaration of Funding

This study was not funded.

Conflict of Interest

The authors declare that they have no conflict of interest.

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