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The Relation between Serum Homocystiene Level and Recurrent Abortion in Egyptian Women

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ABSTRACT

Background: Early pregnancy loss is defined as the termination of pregnancy before 20 weeks' gestation or with a fetal weight of <500 g. Of those that are recognized, 15-*20%* result in spontaneous abortions (SABs) or ectopic pregnancies. Approximately 5% of couples trying to conceive have two consecutive miscarriages, and approximately 1% of couples have three or more consecutive early pregnancy loss. Increased levels of homocysteine may be due to inadequate dietary intake of folate and vitamin B12 and inherited defects within the methionine-hmocysteine pathway such as MTHFR c677T gene polymorphism. Hyperhomocysteinemia is associated with the syndromes of repeated miscarriage.

Objective: The aim of the current study is to estimate the serum levels of Homocystine and vitamin B12 and their relation to prevalence of recurrent spontaneous abortion in pregnancies with history of recurrent miscarriage. **Subjects and Methods:** In this study **80** pregnant women classified into two groups: GroupI:**60** consecutive pregnant women who had suffered from two or more miscarriages, and Group II: **20** pregnant women with no history of abortion .The following estimations were done (for each woman in the study): serum homocysteine and vitaminB12 were estimated using ELISA technique. **Results**: Results showed a significant increase in serum level of homocysteine in the first group compared to control group($p<0.0001$) and a low serum level of vitamin B12 which was significantly decreased in the study group (group 1) compared to control group($p<0.0001$). Moreover, Our results showed a negative correlation between homocysteine and vitamin B12. **Conclusion:** The elevated serum homocysteine levels during pregnancy were significantly associated with recurrent pregnancy losses. Moreover, reduced serum vitamin B12 level was considered a risk for recurrent miscarriage. Homocysteine is most sensitive and specific parameters in the recurrent pregnancy losses.

Keywords: Recurrent pregnancy losses, Homocysteine, Vitamin B12.

INTRODUCTION

Recurrent miscarriage is classically defined as the loss of three or more consecutive pregnancies before the 20th gestational weeks**.** Some experts consider two consecutive pregnancy losses is sufficient for the diagnosis of RM because the recurrence rate and risk factors are similar to that after three losses [(1)]**.** Spontaneous pregnancy loss is a surprisingly common occurrence, approximately 15% of all clinically recognized pregnancies result in pregnancy failure, there are many pregnancies that fail prior to being clinically recognized [(2)].

The etiology of recurrent pregnancy loss (RPL) include uterine abnormalities, chromosomal abnormalities, endocrine disease, alloimmune abnormalities, autoimmune disease, thrombophilia and infection [(3)]**.**

Elevated homocysteine concentrations may be associated with some fetal abnormalities and with potential blood vessel problems in the placenta, causing abruption [(4)]**.** Hyperhomocysteinemia is therefore a possible risk factor for coronary artery disease. Coronary artery disease occurs when an atherosclerotic plaque blocks blood flow to the coronary arteries[(5)]**.**

Hyperhomocysteinemia has been correlated with the occurrence of blood clots, heart attacks, strokes and has also been associated with early pregnancy loss and with neural tube defects[(5)]**.**

The placentas with hyperhomocysteinemia were found to have infarction, retroplacent alhaematoma, and uteroplacental vascular thrombosis [(6)]**.** Plasma homocysteine levels are elevated in vitamin B-12 and, vitamin B-6 and in folic acid deficiency[(7)]. Vitamin B12 deficiency can affect the pregnancy outcome for both mother and the offspring. For women who want to get pregnant, a vitamin B12 deficiency is considered an

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increased risk factor of developing pre-eclampsia, intra-uterine growth retardation, preterm labor[8].

Recent studies have also found an association between low vitamin B12 status in mothers and neural tube defect. This suggests an increased risk for birth defects when starting pregnancy with a deficient or inadequate vitamin B12 status[8]. The aim of the present study was to estimate the serum levels of Homocystine and vitamine B12 and their relation to prevalence of recurrent spontaneous abortion in pregnancies with history of recurrent miscarriage.

SUBJECTS AND METHODS

The study samples were patients who referred to Al-Gamaa Tanta hospital outpatient Obstetrics and Gynecology clinic in the period from January 2014 till December 2014and their ages ranged between 18-35 years.

This research was carried on two different groups:

Group (I): 60 pregnant women who had suffered from two or more miscarriages. Inclusion criteria were a history of multiple miscarriages (two or more). Patients who had no history of miscarriage or had any medical disorders were excluded from the study. Group (II): 20 pregnant women who had no abortion history with at least one successful pregnancy considered as control.

All women of the two groups were subjected to the following:

1-Complete history taking and general examination including weight, blood pressure and pulse.

2-Laboratory investigation: CBC, PTT, PT, CT BT,VB12,HCYand random sugar.

Sample collection

5 ml of venous blood sample was obtained from both women groups and divided into portions:

1- The first portion (2.5 ml)of fresh blood were immediately citrated for the determination of CBC, PTT, PT and random sugar levels .

2- The remaining(2.5ml) portion was left to clot at room temperature 10-20 min, then serum was separated by centrifugation for 20min at the speed of 2000-3000 r.p.m and the resulting serum was divided into two portions each of them was kept into a clean epindorf and stored at minus 20 C until used for the determination of the following:

-Estimation of serum homocysteine(HCY): (by ELISA kits by spectrophotometer) (This Kit was supplied by Shanghai Sunred Biological Technology Co., No:201-12-8014, Size: 96 de terminations ,Shanghai).

-Estimation of serum vitaminB12 (VB12): (by ELISA kits by spectrophotometer) (This Kit is supplied by Shanghai Sunred Biological Technology Co., No:201-12-1545 ,Size: 96 de terminations, Shanghai).

The study was approved by the Ethics Board of Ain Shams University.

Statistical analysis

Statistical Package of Social Science (SPSS) version 16 was used for the analysis of data. Data were summarized and presented as mean and SE. T-test was used for analysis of quantitative data. Pearson's correlation was also done. r was consider weak if < 0.25, mild ≥ 0.25 - < 0.5, moderate ≥ 0.5 - < 0.75 and strong if > 0.75. P-value is significant if < 0.05.

RESULTS

The demographic data of the study and control groups are shown in the following table:

Table (1): Demographic data of the studied groups:

		Control N=20	RPL N=60	p-value
Age (Years)	(min-max)	19-35	18-35	0.535
	(M±SE)	26.75±1.061	26.03±0.525	
BMI (Kg/m2)	(min-max)	23.88-36.44	23.52-36.5	0.1293
	(M±SE)	30.38±0.613	29.45±0.361	

The mean of age and BMI were lower in RPL compared to control groups with no significant differences between RPL patients and control.

Table (2): Hematological parameters of RPL and control groups

		Control N=20	RPL N=60	p-value
WBCs (K/ul)	(min-max)	4.4-9.6	4.3-10.5	0.1933
	(M±SE)	7.480±0.3934	7.955±0.280	
Haematocrit%	(min-max)	37-41	21-35.5	**0.0001*** **
	(M±SE)	38.39±0.238	28.53±0.477	
RBCs (M/uI)	(min-max)	4.23-4.8	2.8-4.6	**<0.0001*****
	(M±SE)	4.511±0.038	3.5±0.057	
Hemoglobin (g/dL)	(min-max)	12.50-13.40	7.100-13.10	**<0.0001*****
	(M±SE)	12.77±0.049	9.450±0.18	
Platelets (K/UI)	(min-max)	152-401	84-400	0.0709
	(M±SE)	257.25±17.59	223.9±9.487	

*** Highly significant difference

The mean value of white blood cell(WBCs) was higher in RPL compared to control groups (p=.01933) . While the mean of haematocrite , Red blood cell(RBCs)and hemoglobin were significantly lower in RPL than control groups (p=0.0001,p<0.0001 and p<0.0001)respectively. Also, the mean of platletes count was lower in RPL and control groups(p=0.0709).

Table (3): Biochemical parameter in RPL and control groups:

		Control N=20	RPL N=60	p-value
PTT /Sec	(min-max)	31.30-45.30	23.90-39	
	(M±SE)	34.3±0.799	31.07±0.428	**0.0003*****
PT /Sec	(min-max)	12-15.3	11.6-27.8	
	(M±SE)	13.35±0.19	14.82±0.493	0.3199
Random Sugar Mg/dl	(min-max)	62-94	63-98	0.0762
	(M±SE)	84±1.7	87±1.2	
CT /Min	(min-max)	5-7.12	5.1-7.2	
	(M±SE)	5.77±0.162	5.97±0.079	0.1287
BT /Min	(min-max)	2-2.9	2-2.55	
	(M±SE)	2.25±0.046	2.207±0.017	0.674

***Highly significant difference

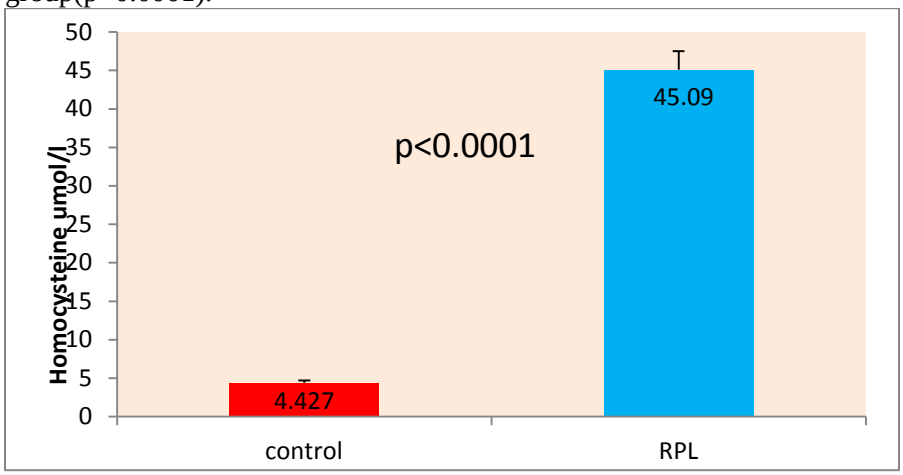
The mean of prothrombin time (PT), random sugar and clotting time(CT) were higher in RPL group than control groups(p=0.3199 and 0.1287) respectively.While mean of partial prothrombin time (PTT) was significantly lower in RPL than control groups(p=0.0003),also mean of Bleeding time(BT) was lower in RPL than control group(P=0.674).

Table (4): Homocysteine and VTB12 in RPL and control groups

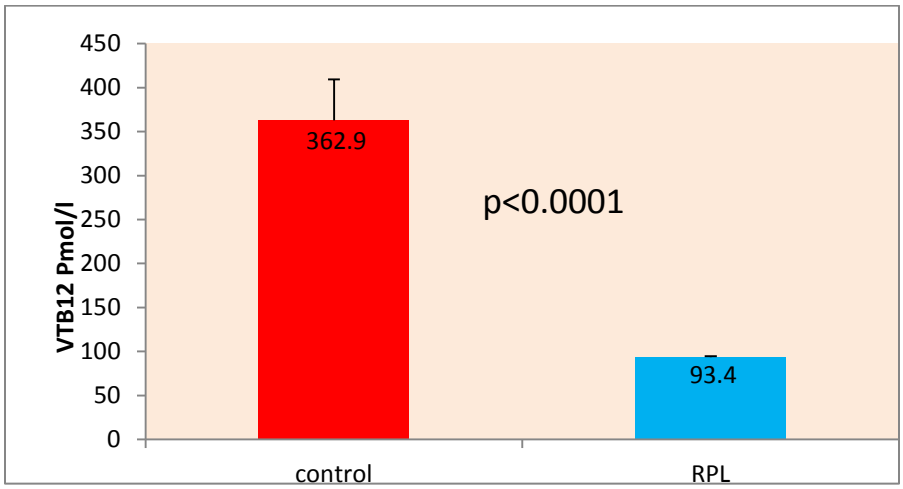
		Control N=20	RPL N=60	p-value
Homocysteine Umol/l	(min-max)	3.5-8.77	20.5-100.6	
	(M±SE)	4.427±0.2883	45.09±2.407	**<0.0001*****
VTB12 Pmol/l	(min-max)	200-794.7	50-110	
	(M±SE)	362.9±46.61	93.40±1.394	**<0.0001*****

***Highly significant difference

The mean value of Homocysteine (HCY)was highly significant in RPL in comparison with that of the control group (p<0.0001). while the mean of vitamin B12 (VTB12)was significantly lower in RPL than control group(p<0.0001).



Figure(1): M±SE of Homocysteine in studied groups.



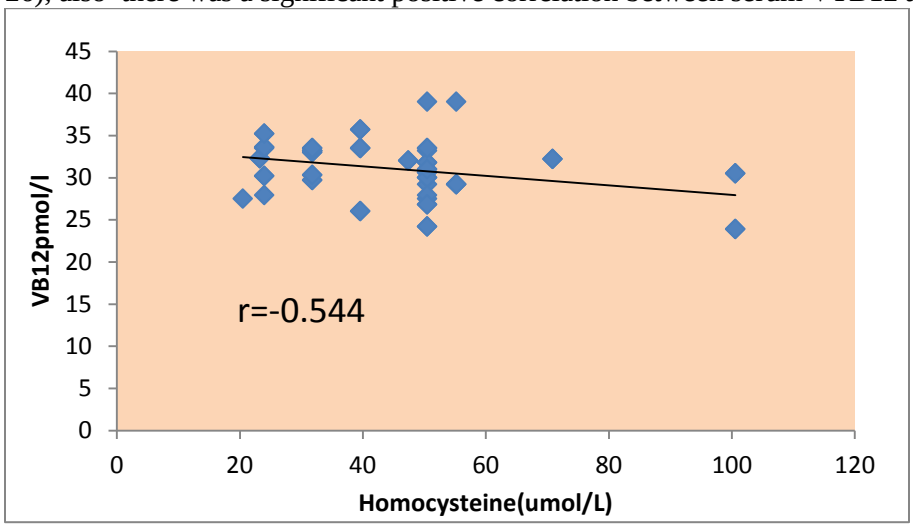
Figure(2): M±SE of VTB12 in studied groups.

Table (5): Correlation coefficient of Homocysteine and VTB12 with other variables in RPL group.

	Homocysteine		VTB12	
	R	P	R	P
Age	-0.056	0.559	0.48	0.671
BMI	-0.76	0.503	0.180	0.111
PTT	-0.464	0.001**	0.271	0.015*
PT	0.198	0.78	-0.142	0.210
CT	0.297	0.007**	-0.135	0.231
Hb	-0.553	0.001**	0.576	0.001**
RBCs	0.76	0.504	-0.49	0.664
Platelt	0.023	0.839	0.249	0.026*
W.B.Cs	0.144	0.203	-0.218	0.052
BT	-0.133	0.239	0.101	0.372
Homocysetine	-	-	-0.544	0.001**
VTB12	-0.544	0.001**		

* Significant correlation ** Moderate Significant correlation

There was a significant negative correlation between serum HCY and PTT, Hb and VTB12 (figure 25, 27 and 28 respectively) and there was a significant positive correlation between serum HCY and CT (figure 26), also there was a significant positive correlation between serum VTB12 and PTT , Platlet and Hb.



Figure(3):Correlation between serum HCY and VTB12 in RPLgroup

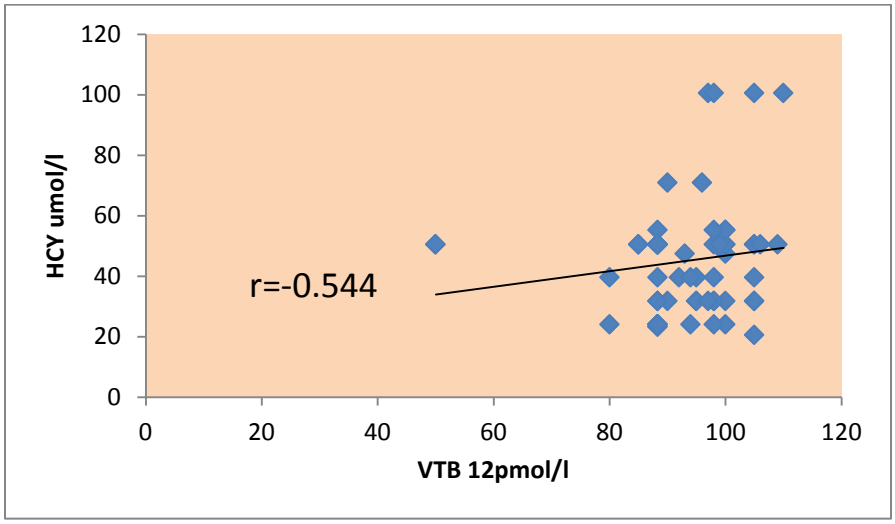


Figure (4): Correlation between serum VTB12 and HCY in RPL group

DISCUSSION

Recurrent pregnancy loss has been considered as a public health concern, defined as three or more spontaneous pregnancy losses before the 20th week of pregnancy. But in some studies, two or more losses were considered sufficient to diagnose RPL[3]. RPL is occurring in approximately 15–25% of pregnancies[9].Various disorders have been proposed as etiology of RPL including uterine abnormalities, chromosomal abnormalities, endocrine disease, alloimmune abnormalities, autoimmune disease, thrombophilia and infection[3].

In pregnancy, homocysteine levels tend to decrease. Elevated homocysteine concentrations may be associated with some fetal abnormalities and with potential blood vessel problems in the placenta, causing abruption[4].

The role of increased homocysteine as an independent risk factor for arterial and venous thrombosis has become increasingly appreciated. The proposed pathogenetic mechanisms are increasing in the levels of asymmetric dimethylarginine, and impaired methylation, oxidative damage of the endothelium through suppression of the vasodilator nitric oxide, promotion of platelet activation and aggregation, vascular smooth muscle proliferation and disruption

of the normal procoagulant-anticoagulant balance favoring thrombosis[10].

In this study we aimed at evaluating the association between serum homocystien as well as vitamin B12 concentrations and recurrent early pregnancy loss risk.

To achieve our aim, this study was conducted on 80 participants that were divided into two groups; case group consisted in 60 patients with RPL. In addition to, a control group consisted in 20 healthy volunteers.

The mean homocysteine level was higher in RPL group than control group (45.09±2.407 vs 4.427±0.2883, respectively; P<0.0001).similarly, **Nelen *et al*.**[11] found that elevated homocysteine level was a risk factor for recurrent early pregnancy losses[11]. Our findings concerning homocysteine level and RPL seem to be in agreement with those already reported[12;13;14] .

While, in contrast to our result **Sikora *et al*.**[15] found that the average homocysteine concentration in RPL group was not statistically higher (P>0.05) compared to the control group[15].

Also, **Meiyappan *et al*.**[16] reported that plasma homocysteine levels were decreased in pregnancy loss and ectopic pregnancy compared with normal pregnancy. But, they explained that their results were in contrast to the expected outcome of increased homocysteine levels, leading to increased oxidative stress in abortion. They hypothesized that, lower homocysteine levels resulted in ectopic pregnancy by increasing the activity of nitric oxide and thus causing tubal relaxation and retention of embryo in the fallopian tube[16].

In the county of Hordaland in Western Norway, a large population-based study was conducted on more than 18,000 men and women. In this large population, subjects with elevated homocysteine levels have increased risk of cardiovascular and noncardiovascular mortality, cardiovascular morbidity, and were more likely to suffer from elderly and depression. Among women, increased homocysteine levels were associated with an increased risk of having suffered from pregnancy complications and an adverse pregnancy outcome. In those mothers with elevated homocysteine levels, there was an increased incidence of malformations, particularly neural tube defects[17].

Homocysteine by itself can be embryo toxic[18]. The risk associated with maternal hyperhomocysteinemia could be due to ability of high homocysteine to activate factor V and to inactivate protein C, heparansulphate, thrombomodulin, tissue plasminogen activator binding to human endothelial cells or due to impaired cellular methylation that alters gene expression or due to uracil misincorporation in DNA resulting in DNA damage[19].

One of the plausible causes for RPL is the elevated levels of homocysteine in serum or plasma. Increased levels of homocysteine in blood may be related especially to nutritional influences, or to genetic factors such as mutation in the gene of methylene tetrahydrofolatereductase (MTHFR) enzyme. In the gene of MTHFR enzyme, cytosine 677 thymine (C677T) mutation has been identified that was found to be linked with recurrent miscarriage. The MTHFR gene mutation will decrease the activity of the enzyme and increase the homocysteine concentrations in blood[20] .

The mean vitamin B12 was significantly lower in RPL than control group (93.40±1.394 vs 362.9±46.61, respectively; P<0.0001). Our results are similar to **Sikora *et al*.**[15] who suggested that the average vitamin B12 level in RPL group was statistically lower (178.3 pg/ml) than control group (268.6 pg/ml) (p<0,001)[15]. Also, **Bennett**[21] suggested that vitamin B12 deficiency may lead to fetal loss due to raised homocysteine levels that caused in hypercoagulability[21]. Furthermore, **Hubner *et al*.**[22] showed that Vitamin B12 was significantly decreased in patients with RPL compared to controls (197 vs. 300 pg/mL, p=0.004)[22].In contrast to our results **Sutterlin *et al*.**[23] found that the serum levels of vitamin B12 were not significantly altered in women with unexplained recurrent abortions[23].

Vitamin B12 is involved in the methionine metabolism and its deficiency is associated with hyperhomocysteinemia and vice versa. Vitamin B12 has been identified to play a pivotal role in RPL. The implicated mechanisms of vitamin B12 deficiency leads to faulty and sporadic ovulation producing a faulty oocyte. Also, vitamin B12 deficiency leads to incomplete trophoblastic invasion of spiral arteries thereby leading to defective placentation[24].In the human body, vitamin B12 is a component of two important enzymes (Methionine Synthase and Methylmalonyl

Coenzyme (COA) Mutase). In vitamin B12 deficiency, the mutase activity is compromised, leading to high plasma or urine levels of methylmalonyic acid which is a degradation product of methylmalonyl CoA. In contrast to adult, mutase has a very vital role in embryonic life and in early development [(24)]. Vitamin B12 deficiency can also cause megaloblastoid uterine epithelium leading to early spontaneous abortion[(21)].

There was a significant negative correlation between serum homocysteine and APTT, Hb and vitamin B12 (r= -0.464, -0.553 and -0.544, respectively). But, there was a significant positive correlation between serum homocysteine and CT (r= 0.297). While, there was a significant positive correlation between serum vitamin B12 and APTT, Hb and platelets (r=0.271, 0.576 and 0.249, respectively). These findings indicated the efficacy of vitamin supplementation and/or anticoagulation in prevention of further miscarriage in women with elevated homocysteine. In the same manner, **Puri *et al*.[(25)]** found that there was a significant negative correlation (r=-0.35, P<0.0001) between serum homocysteine and vitamin B12 levels[(25)] .While, in contrary **Nadir *et al*.[(26)]** found that there was a weak negative correlation between homocysteine and vitamin B12 levels (r=-0.281, P=0.034)[(26).] which is in agreement agreement with our results, **Sirdah *et al*.[(27)]** studied the homocysteine and vitamin B 12 status in females with iron deficiency anemia. They found that there were significant negative correlations between homocysteine level and vitamin B12 and hemoglobin levels (r=-0.361 and r=-0.364 respectively; P<0.0001)[(27)].Also, in the study of **Yassin *et al*.[(28)]**, to assess the association between homocysteine and different hematological indices in hemodialysis patients, they found that homocysteine correlated inversely with hemoglobin (r=-0.733, P=0.0001) and APTT(r=-0.690, P=0.0001)[(28)].Moreover, **Narang *et al*.[(29)]** studied vitamin B12 level in patients with metabolic syndrome and they found that there was a positive correlation (r=0.346, P=0.002) between vitamin B12 and hemoglobin level[(29)].

CONCLUSION

This study proved that elevated homocysteine blood levels during pregnancy are significantly associated with recurrent pregnancy losses. Moreover, reduced serum vitamin B12 is a significant risk factor for recurrent miscarriage.

Because homocysteine and vitamin B12 have got the most probable association ship with miscarriage rate in women, they may represent a promising area in the biomarker discovery for recurrent pregnancy loss.Homocysteine is most sensitive and specific parameters in recurrent pregnancy losses.

This study also has shown that the defect of some co-agulation factors are associated with recurrent pregnancy losses and by the therapeutic corrrection,it will permit normal birth and there is clear decrease in numbers of early pregnancy loss. We recommended testing for homocysteine blood levels and pre-conceptional supplementation with vitamin B12. These steps might be beneficial to improve pregnancy outcome, although further studies on larger number of patients are necessary to prove this matter.

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