A STUDY ON ADDITIVE ASSOCIATION OF LIPOPROTEIN (A) AND HOMOCYSTEINE IN ACUTE ST SEGMENT ELEVATION MYOCARDIAL INFARCTION

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ABSTRACT

BACKGROUND

Prevalence of Coronary Artery Disease (CAD) is increasing in India. Acute myocardial infarction has very high morbidity and mortality, hence economically a big burden. However, established risk factors alone do not explain this excess of coronary artery disease, emerging risk factors like Hyperhomocysteinaemia and Lipoprotein (a) have been recognized as independent risk factors for CAD in many retrospective case control studies. This study is done to analyse plasma homocysteine and lipoprotein(a) levels in patients admitted to the hospital with Coronary Heart Disease for its role and its relation to other known risk factors.

MATERIALS AND METHODS

This study was conducted in 50 patients of acute ST elevation myocardial infarction (STEMI). Patients visiting OPD and inpatients in the department of medicine, Meenakshi Medical College Hospital and Research Institute during the period of February 2018 to August 2018 were included in the study. This study also included 50 people as control group. Patients were screened to look for traditional risk factors, and the homocysteine and lipoprotein(a) levels were documented.

RESULTS

When the association of both elevated lipoprotein (a) and homocysteine were compared with case and control group, it was seen that there is a high statistical significance with p-value of <0.001 and the relative risk is 25.6

CONCLUSION

The association of elevated lipoprotein(a) and hyperhomocysteinaemia with STEMI were significant. Hyperhomocysteinaemia seems to have the stronger association with STEMI than elevated Lipoprotein(a). The relative risk of STEMI was more when both homocysteine and lipoprotein(a) were elevated than the individual relative risk added together.

KEYWORDS

Homocysteine; Lipoprotein (a); Acute ST Elevation Myocardial Infarction.

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BACKGROUND

Atherosclerosis is an inflammatory state, and also as a common denominator of multi-dimensional diseases like coronary artery disease, cerebrovascular accident, aortic diseases and peripheral artery disease. Coronary artery disease is an important ischemic cardiovascular event, which contributes more morbidity and mortality, when compared to other vascular events like peripheral artery disease. Risk factor stratification is the only effective tool to assess the magnitude of this problem in the community. Traditional risk factor for CVD (cardiovascular disease) includes obesity, dyslipidaemia, diabetes, smoking, hypertension, and sedentary lifestyle. Recently the emerging risk factors are getting more attention for the prevention of adverse cardiac events. These may be lipid risk factors like triglycerides, lipoprotein remnants, apolipoprotein, lipoprotein(a), small LDL articles. And non-lipid factors like homocysteine, thrombogenic factors, impaired fasting glucose.

In these risk factors, lipoprotein(a) levels are very strongly associates with increase CVD. Similarly, high homocysteine levels are associated with high prevalence of CVD. Hence the combination of high lipoprotein a and homocysteine levels,[1] additively increase the chances of the occurrence of delirious effects like acute myocardial infarction. But at the same time, the combination of these two, one lipid and another one non-lipid factor would have multiplicative outcome than merely simple additive effect.

Lipoprotein(a) is a molecule consisting of LDL particle with apo a glycoprotein through apo B 100.[2] Apo a and plasminogen have structure similarities. This feature allows lipoprotein a to compete with plasminogen and to promote

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thrombosis and inhibiting fibrinolysis in blood vessels[3,4] Homocysteine is a metabolite of methionine which causes arterial disease by various mechanisms, like proliferation of smooth muscle, activation of platelets, increased permeability of inflammatory mediators and endothelial malfunction.[5,6,7] Recent studies have shown that Homocysteine increase the affinity of binding of lipoprotein a to fibrin and there by altering fibrinolysis.[8]

We focused to determine the risk of CAD, when these both factors are there and to evaluate the association of these factors with other traditional risk factor.

MATERIALS AND METHODS

This study was conducted in 50 patients of acute ST elevation myocardial infarction (STEMI). They were selected as outpatient and inpatients in the department of medicine, Meenakshi medical college hospital and research institute during the period of February 2018 to August 2018. This study also included 50 people as control group. Patients were screened to look for traditional risk factors, and the homocysteine and lipoprotein(a) levels were documented.

Inclusion Criteria

Age-15 years and above.
Typical ECG pattern of STEMI.
a. ST segment elevation of ≥0.1 mV in at least 2 consecutive limb leads or ≥0.2 mV in at least 2 consecutive chest leads for ST elevation MI.
b. New onset LBBB
Elevated cardiac enzyme levels (CKMB or Troponin T).

Exclusion Criteria

Patients with stable or unstable angina, non-STEMI.

Study Population

Cases

A total of 50 patients were recruited for the study based on clinical, biochemical and ECG evidence. An informed consent was taken from the patient. They included 38 males and 12 females. Patients with confirmed diagnosis of AMI and satisfying the inclusion and exclusion criteria with no previous ischemic heart disease were included in the study group.

Controls

50 normal individuals symptomatically and electrocardiographically were selected as control.

Data Collection

All patients (cases and controls) underwent a standard clinical examination by nurses and physicians, which included anthropometry (height, weight, waist-hip ratio), blood pressure, and a blood draw for a lipid profile. Patients also received dietary and smoking counselling when necessary. Individuals also completed a questionnaire that incorporated numerous risk related issues, including a history of hypertension, family history, cholesterol medication use, and diabetes (ever treated or diagnosed by a physician), and, in women, menopausal status and use of hormones. Patients were classified as either never- or ever-smokers.

Biochemical Analysis

Following investigations were done in all patients in the study, and the control groups.

1. Early morning fasting samples of blood were collected and sent for lipid profile, lipoprotein(a)
2. Homocysteine level
3. Complete blood count
4. Random blood sugar, urea, creatinine
5. CKMB
6. TROPONIN T
7. ECG
8. X-ray chest PA view
9. Echocardiography

Fasting total cholesterol, HDL-C, LDL-C, and triglyceride concentrations in the blood sample drawn at the same time as the sample for measurement of tHcy and Lp(a) were measured in all subjects after six weeks of acute coronary event.

Statistics

t-test for equality of means is used to assess the significance of the comparable factors. Also, mean and standard deviation were calculated. Pearson chi-square test is used to know the statistical significance. Odds ratio is used to assess the relative risk. p value of < 0.05 indicates strong significance.

RESULTS

This study group consisted of 100 patients of those 50 were cases and 50 were controls. The case group consist of patients with age ranging from 35 to 85, and a mean age of 55.9. In the control group age ranged from 35 to 70, with a mean age of 51.5. The cut-off value of homocysteine in this study was 17 micromoles/L. In case group 86 percent of patients were showed raised homocysteine, and in control group 24 percent of patients were showed raised homocysteine. Chi-square test was used to verify the statistical significance. And here the p value is <0.001 with the relative risk of 19.4. It shows raised homocysteine is statistically significant. Lipoprotein (a) level of more than 30 mg/dl is considered as increased. In case group 38 percent of patients were had elevated lipoprotein (a) and in control group these were 18 percent of patients. p value here is 0.026. It shows increased lipoprotein (a) is statistically significant with the relative risk of 2.7.

Age, life style, Family history of premature CAD, and parameters like BMI and waist to hip ratio were not statistically associated with levels of lipoprotein (a) and homocysteine. It was observed that patients with elevated levels of both lipoprotein (a) and homocysteine were showed high LDL-C and this association is statistically significant (p-value of 0.002). This association was not seen with other parameters of lipid profile.

When the association of both elevated lipoprotein (a) and homocysteine were compared with case and control group, it shows high statistical significance with p-value of <0.001. Lipoprotein (a) and homocysteine both are higher than the taken cut off value in 20 patients in study group, where 16 in case group and 4 in control group which has sensitivity of 76% and specificity of 89% with odds ratio of 25.6 Patients with elevated levels of both lipoprotein (a) and homocysteine were showed statistical association with hypertension, diabetes, history of smoking and alcoholism. Hypertension is present in 71% of patients with elevation of both lipoprotein (a) and homocysteine, which is highly significant with p value of less than .000, but diabetes association of this category of patients is less, even though statistically significant p value of .023

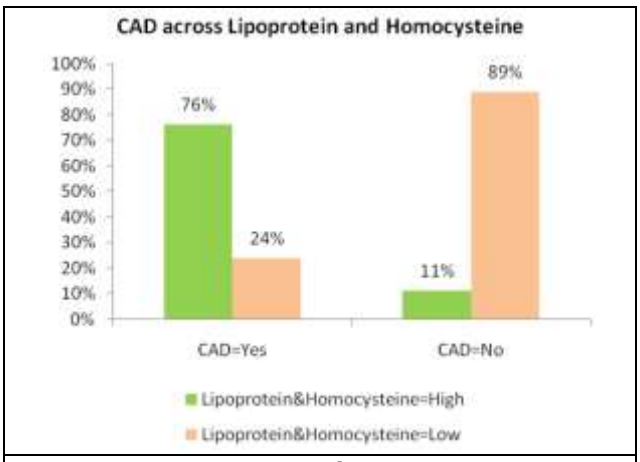
In total of 20 patients with smoking history 11 have elevated levels of both lipoprotein(a) and homocysteine, reminding did not have that elevation, and the association is significant with p value of .021 Total number of alcoholics in this study group was 18. In those 10 patients showed the elevation of both lipoprotein (a) and homocysteine, which is statistically significant with p value of .028 On comparing the established risk factor like LDL-C with emerging risk factors lipoprotein (a) and homocysteine, the association is very significant with p value of .002

These results provide evidence of important differences in the joint effect of Lp(a) and tHcy on CAD risk, and add insights into the role of these two risk factors in the pathogenesis of atherosclerosis. These data provide an interesting hypothesis-generating finding regarding the differential interactive effects of two emerging cardiovascular risk factors and may have important implications for the prevention and treatment of CAD in select high-risk people.

1. Both Homocysteine and Lp(a) Levels in Case and Control Group

Group * Lipoprotein and Homocysteine Crosstabulation

			Lipoprotein and Homocysteine		
			High	Low	Total
Group	Case	Count	16	5	21
		% within Group	76.2%	23.8%	100.0%
		% within Lipoprotein and Homocysteine	80.0%	13.5%	36.8%
	Control	Count	4	32	36
		% within Group	11.1%	88.9%	100.0%
		% within Lipoprotein and Homocysteine	20.0%	86.5%	63.2%
Total		Count	20	37	57
		% within Group	35.1%	64.9%	100.0%
		% within Lipoprotein and Homocysteine	100.0%	100.0%	100.0%



Graph 1

Sensitivity = 16/21 = 0.76 = 76%
Specificity = 32/36 = 0.89 = 89%
Odds Ratio = (16 x 32)/(4 x 5) = 25.6

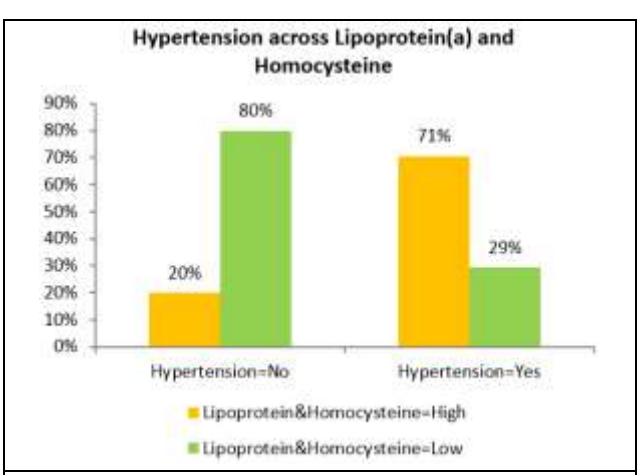
Chi-Square Tests

	Value	df	P-value
Pearson Chi-Square	24.663	1	.000
N of Valid Cases	57		

2. Both Homocysteine and Lipoprotein(a) Levels with Hypertension

Hypertension * Lipoprotein and Homocysteine Crosstabulation

			Lipoprotein and Homocysteine		
			High	Low	Total
Hypertension	NO	Count	8	32	40
		% within Hypertension	20.0%	80.0%	100.0%
		% within Lipoprotein and Homocysteine	40.0%	86.5%	70.2%
	YES	Count	12	5	17
		% within Hypertension	70.6%	29.4%	100.0%
		% within Lipoprotein and Homocysteine	60.0%	13.5%	29.8%
Total		Count	20	37	57
		% within Hypertension	35.1%	64.9%	100.0%
		% within Lipoprotein and Homocysteine	100.0%	100.0%	100.0%



Graph 2

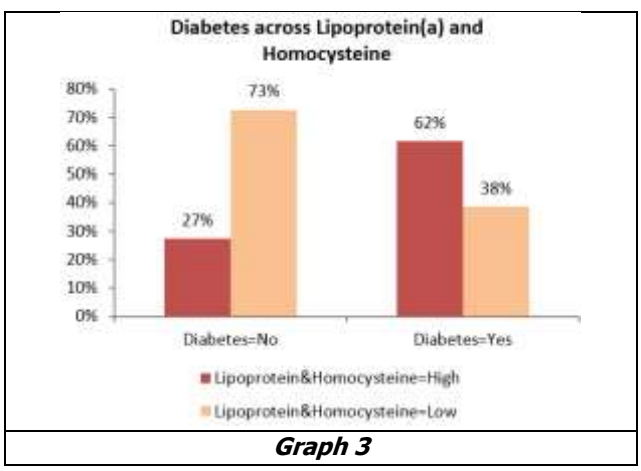
Chi-Square Tests

	Value	df	P-value
Pearson Chi-Square	13.405	1	.000
N of Valid Cases	57		

3. Both Homocysteine and Lipoprotein(a) Levels with Diabetes

Diabetes * Lipoprotein and Homocysteine Crosstabulation

			Lipoprotein and Homocysteine		
			High	Low	Total
Diabetes	NO	Count	12	32	44
		% within Diabetes	27.3%	72.7%	100.0%
		% within Lipoprotein and Homocysteine	60.0%	86.5%	77.2%
	YES	Count	8	5	13
		% within Diabetes	61.5%	38.5%	100.0%
		% within Lipoprotein and Homocysteine	40.0%	13.5%	22.8%
Total		Count	20	37	57
		% within Diabetes	35.1%	64.9%	100.0%
		% within Lipoprotein and Homocysteine	100.0%	100.0%	100.0%

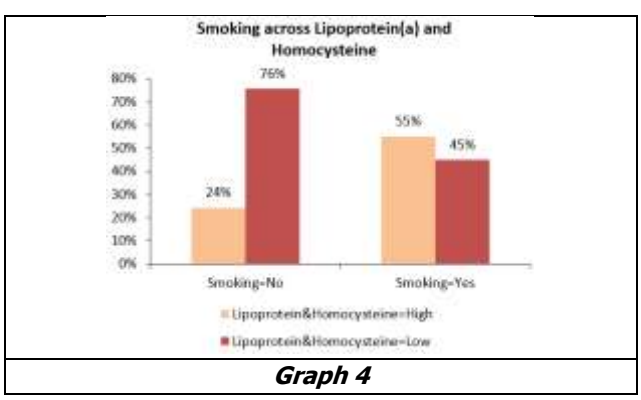


Graph 3

Chi-Square Tests

	Value	df	P-value
Pearson Chi-Square	5.173	1	.023
N of Valid Cases	57		

4. Both Homocysteine and Lipoprotein (a) Levels in Smokers and Non-Smokers



Graph 4

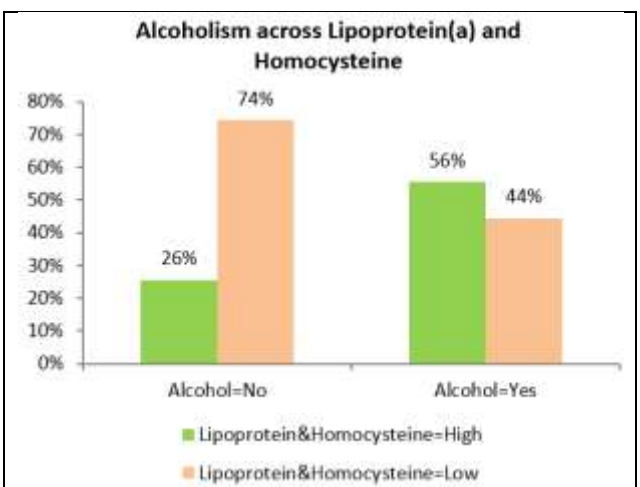
Chi-Square Tests

	Value	df	P-value
Pearson Chi-Square	5.364	1	.021
N of Valid Cases	57		

5. Both Homocysteine and Lipoprotein(a) Levels In Those Who Consumed Alcohol and In Those Who Did Not

Alcohol * Lipoprotein and Homocysteine Crosstabulation

			Lipoprotein and Homocysteine		
			High	Low	Total
Alcohol	NO	Count	10	29	39
		% within Alcohol	25.6%	74.4%	100.0%
		% within Lipoprotein and Homocysteine	50.0%	78.4%	68.4%
	YES	Count	10	8	18
		% within Alcohol	55.6%	44.4%	100.0%
		% within Lipoprotein and Homocysteine	50.0%	21.6%	31.6%
Total		Count	20	37	57
		% within Alcohol	35.1%	64.9%	100.0%
		% within Lipoprotein and Homocysteine	100.0%	100.0%	100.0%



Graph 5

Chi-Square Tests

	Value	df	P-value
Pearson Chi-Square	4.839	1	.028
N of Valid Cases	57		

6. Both Homocysteine and Lipoprotein(a) Levels with LDL-C

Group Statistics

	Lipoprotein and Homocysteine	N	Mean	Std. Deviation	Std. Error Mean
LDL	High	20	125.00	28.152	6.295
	Low	37	106.19	14.744	2.424

Independent Samples Test

	t-test for Equality of Means						
						95% Confidence Interval of the Difference	
	t	df	P-value	Mean Difference	Std. Error Difference	Lower	Upper
LDL	3.323	55	.002	18.81	5.661	7.466	30.156

DISCUSSION

Several clinical trials are underway to test whether homocysteine lowering will reduce CAD risk.[9] ATP III does not recommend routine measurement of homocysteine as part of risk assessment to modify LDL-cholesterol goals for primary prevention. This lack of recommendation is based on uncertainty about the strength of the relation between homocysteine and CAD, a lack of clinical trials showing that supplemental B vitamins will reduce risk for CAD. Recent recommendations state that lipoprotein(a) screening is not warranted for primary prevention and assessment of cardiovascular risk at present but that lipoprotein(a) measurements can be of use in patients with a strong family history of cardiovascular disease or if risk of cardiovascular disease is judged intermediate on the basis of conventional risk factors. Measurements of lipoprotein(a) levels might help to identify as yet unidentified high-risk individuals who could benefit from other aggressive, prophylactic measures, including statins directed at elevated cholesterol levels. Lipoprotein(a) may promote various pathophysiological pathways leading to the development of atherosclerosis and further propagation of thrombosis. Hyperhomocysteinaemia, when it presence increase the affinity of lipoprotein(a) to plasmin treated fibrin to twenty fold.[10]

Haemostatic function is influenced by both of these molecules either by altering the endothelial or platelet function and then by leading a thrombotic prone condition. The presence of both Homocysteine and Lipoprotein(a) is a situation that cannot be termed as additive or multiplicative. Generally, it increases the risk of the development of CVD. Homocysteine and Lipoprotein(a) interact to potentiate this risk more than tenfold when compared with fivefold increase when only the lipoprotein(a) is altered.

Harpel et al initially pointed that tHcy promoted binding of lipoprotein(a) to plasmin-modified fibrin. This would probably lead to more atherogenesis and atherothrombosis associated with elevations of both homocysteine and lipoprotein(a). It might be because of that lipoprotein(a) is composed of apo(a)-linked to an apoB- 100–LDL particle by a single disulfide bond. Thiols, such as homocysteine, are known to dissociate apo(a) from the lipoprotein(a) complex, leading to the exposure of an additional lysine binding site on apo(a).[11] This additional lysine-binding site may increase the affinity of apo(a) for plasmin-modified fibrin, thus inhibiting fibrinolysis.[12] This modification of Harpel's original hypothesis,[13] explains how the presence of homocysteine adds greater lipoprotein(a) fibrin binding. This theory is consistent with the suggestion that apo(a) is the atherogenic moiety of lipoprotein(a) as noted in transgenic mouse models. These findings support the hypothesis that homocysteine and lipoprotein(a) interact to increase the risk of CAD. In this case control study elevated levels of homocysteine and lipoprotein(a) are independently associated with CVD. And the significance of association is higher for homocysteine (odds ratio 19.45) than lipoprotein(a) (odds ratio 2.79). Some patients show elevated levels of both homocysteine and lipoprotein(a), the magnitude of this effect was in excess (odds ratio 25.6) of what would be expected if the risk factors were operating either additively or multiplicatively with regard to CAD risk. This clearly demonstrate that lipoprotein(a) and homocysteine interact to increase the risk of CAD.

A study of Homocysteine and Lipoprotein(a) Interact to Increase CAD Risk in Young Men and Women by JoAnne Micale Foody et al demonstrates that lipoprotein(a) and homocysteine interact to increase the risk of CAD in women. But this present study does not show any gender variation when both the levels of homocysteine and lipoprotein(a) were elevated.

In recent study the relationship of lipoprotein(a) and homocysteine with overt CAD in diabetics has been analysed an association of high lipoprotein(a) levels and apo(a)[14] phenotypes with CAD has been found in even with normal resting ECG.[15] In our study Neither isolated high homocysteine nor isolated high lipoprotein(a) was identified as an independent risk factor in diabetes. However, the conjoint presence of elevated homocysteine and elevated lipoprotein(a) indicated increased risk of CAD in diabetes. Similarly, neither isolated high homocysteine nor isolated high lipoprotein(a) was associated with LDL-C. But when both the levels were elevated the association with LDL-C is significant.

Another study on the relationship between hypertension and hyper homocystinaemia found that, in comparison with normotensives, hypertensive patients have higher plasma levels of homocysteine.[16] Similarly very highly significant association is observed from our study.

In the present study there is no positive correlation in between lipoprotein(a) and LDL, but it is there when both lipoprotein(a) and homocysteine were elevated. In addition, concomitant elevations of lipoprotein(a) and LDL cholesterol have been reported to have synergy in elevating risk in both men and women for CAD. Elevations of serum homocysteine are positively correlated with risk for CAD.[17]

As reported by earlier studies, smoking increases the level of serum total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol and decreases the levels of HDL cholesterol.[18,19,20,21,22] Smoking, in both active and passive forms, is a major risk factor for atherosclerosis and coronary heart disease.[23]

Elevated levels of both the homocysteine and lipid profiles amplifies the risk factors toward cardiovascular diseases; this is as same with the findings reported by O'Callaghan and colleagues.[24] A positive association is noted between elevated homocysteine and a number of atherogenic risk factors including, smoking as reported by Nygard et al.[25] The same result is obtained from this present study. Another recent study reports that Smokers with plasma homocysteine levels more 12 µmol./l had, a 12-fold

increase of cardiovascular risk when compared with the non-smokers. A recent evidence for an link between elevated homocysteine and increased oxidative stress was reported by Yamamoto et al.[26] Since it is now well proved that smoking is also associated with an increase in markers of oxidative stress. Similarly, in our study another lipid risk factor lipoprotein(a) is significantly elevated along with homocysteine in smoking population.

In this case control study elevated levels of homocysteine and lipoprotein(a) are independently associated with CAD. And the significance of association is higher for homocysteine (odds ratio 19.45) than lipoprotein(a) (odds ratio 2.79). Some patients show elevated levels of both homocysteine and lipoprotein(a), the magnitude of this effect was in excess (odds ratio 25.6) of what would be expected if the risk factors were operating either additively or multiplicatively with regard to CAD risk. Based on these observations, study of the interaction between homocysteine and lipoprotein(a), ultimately becomes a priority for better understanding into disease with a favourable impact on public health. We believe that these reports underline the significance of identifying patients with dual risk factor of elevated homocysteine and lipoprotein(a) to target on preventive measure that may decrease the risk of CAD. Hyperhomocysteinaemia may be treated with increased consumption of folate, vitamin B6 and vitamin B12. Elevated lipoprotein(a) can be treated by the use of nicotinic acid.

CONCLUSION

The association of elevated lipoprotein(a) and hyperhomocysteinaemia with CAD was significant. Hyperhomocysteinaemia seems to have the stronger association with CAD than elevated lipoprotein(a). The relative risk of CAD was more when both homocysteine and lipoprotein(a) were elevated than the individual relative risks added together.

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