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VALIDITY OF MATERNAL SERUM FERRITIN LEVELS AS DIAGNOSTIC TEST FOR PRETERM LABOUR

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ABSTRACT

Background

Preterm birth is the leading cause of neonatal morbidity and mortality worldwide. There are various causes of preterm birth including multifetal pregnancy, intrauterine infections, hydramnios, etc. There have been various biochemical markers used to predict preterm labor such as CRH, ILs, CRP, fFN. In this study an attempt has been made to assess serum ferritin as marker of preterm labor, as subclinical maternal infection is responsible for elevated serum ferritin levels.

Aims and objectives

1. To assess the validity of maternal serum ferritin levels as diagnostic marker for preterm labor.
2. To compare the levels of serum ferritin in term and preterm gestation.

Methods

This study is conducted in the department of Obstetrics and Gynaecology, in Karwar Institute of Medical Sciences for a period of 18 months from August 2022 to January 2024. This was a prospective study. 208 patients were included in the study after satisfying inclusion and exclusion criteria, 104 patients in preterm group and 104 patients in term group. Serum ferritin level of 25ng/ml was taken as cut off. Statistical analysis done and results presented as tables and graphs.

Results

There was a statistically significant association between gestational age and serum ferritin levels at cut off of 25.64ng/ml with a sensitivity, specificity, PPV, NPV and accuracy of 86.5%, 82.7%, 83.3%, 86% and 84.6%.

Conclusion

From our study it can be concluded that serum ferritin levels were significantly higher in preterm group than in term group. There was an increased incidence of preterm labor in those with elevated maternal serum ferritin levels. Hence, serum ferritin can be used as a diagnostic test for predicting preterm labor.

Key words: Preterm Labor, Serum Ferritin, PPROM, Acute Phase Reactants, Subclinical Infections.

INTRODUCTION

Preterm birth (PTB) is defined as birth between 28 weeks and 37 completed weeks of gestation.[1] It is the leading cause of neonatal morbidity and mortality worldwide and accounts for 75% of neonatal deaths and 50% of long-term morbidity, including respiratory disease and neurodevelopment impairment.[2] There are various causes of preterm birth such as multifetal pregnancy, intrauterine infection, hydramnios, PPROM, iatrogenic preterm birth, etc. Irrespective of the cause there are profound neonatal morbidity and mortality associated with preterm birth including, respiratory distress, necrotising enterocolitis, intraventricular hemorrhage, immune deficiency, hyperbilirubinemia. In the background of these complications, it is essential to diagnose and prevent preterm labor at the earliest. Since a long time, there have been various biochemical markers used to predict preterm labor, such as CRP, CRH, progesterone, ILs, prostaglandins, fetal fibronectin. Several newer markers have been studied to diagnose preterm labor. In this study an attempt has been made to assess serum ferritin as marker of preterm labor.

AIMS AND OBJECTIVES

- To assess the validity of maternal serum Ferritin levels as diagnostic marker for preterm labor.
- To compare the levels of Serum Ferritin in term and preterm gestation.

MATERIALS AND METHODS

Source of the Data

This study is conducted in the department of Obstetrics and Gynaecology, in Karwar Institute of Medical Sciences for a period of 18 months from August 2022 to January 2024.

Method of Collection of Data

All pregnant patients attending OBG OPD from 28 weeks onwards, along with pregnant patients admitted in maternity ward with threatened preterm after 28 weeks were recruited for the study.

Inclusion Criteria

All pregnant women attending OBG OPD after 37 weeks and and all the patients who are admitted in maternity ward with labour pains after 28 weeks, after informed consent will be recruited for the study.

Exclusion Criteria

All pregnant women after 28 weeks with:

1. Anemia (Hb< 10g/dl)
2. Multiple gestation
3. Chronic infectious disease
4. Gestational Diabetes mellitus
5. Gestational hypertension
6. Known malignancy

Sample Size

Based on study by Tayebeh Jahedbozorgan et al[3]

$$n = \frac{Z^2 * sens(1-sens)}{d^2 * p}$$

Where, **n** is sample size;
Z is 1.96 at 5% level of significance;
Sens is sensitivity
p is prevelance
d is error = 8%
Based on this, the final sample size is calculated to be 208.

Study Design
Longitudinal study

Sample Collection
Blood sample will be collected from the pregnant patients with threatened preterm after 28 weeks and term antenatal patients. About 5ml of blood will be collected from large peripheral vein under aseptic conditions in plain vial for biochemical evaluation.Serum Ferritin levels will be measured by the Latex Enhanced nephelometry end point methods using reagent kits from Agappe diagnostics with MISPA-i_2 machine .The principle for the measurement of serum ferritin is, latex particles coated with anti-ferritin antibody (rabbit) are agglutinated when mixed with samples containing. The agglutination is directly proportional to the concentration of ferritin in the sample. Serum Ferritin levels above 25ng/ml will be taken as cut-off.
Data was collected using a proforma meeting the objectives of the study by consecutive sampling method.
The demographic data of the patients, along with the presenting complaints and examination findings were noted, and patients with preterm labor were classified into test group and patients with term gestation into control group. The routine investigations of patients (hemoglobin, total counts, platelet count, urine routine and microscopy, 2nd hour OGTT values) were noted, to satisfy exclusion criteria. Serum Ferritin levels were analysed in term and preterm groups.

Statistical Analysis
Continuous variables were presented as mean +/- SD and T test was used to test for the significant difference between the means. Correlation coefficient (r) was calculated to examine the correlation between quantitative variables to compare strength and direction of association between the two variables. Categorical variables were described as frequencies and percentages and association was found using Chi-square test. Validity of the biomarker was assessed using ROC in terms of sensitivity, specificity, NPV, PPV, accuracy and AUC. A P value of <0.05 was considered to be significant.

RESULTS AND DISCUSSION

Maternal Age (in Years)	**Gestational age**			**p-value**
	Pre Term (104) n (%)	**Full Term (104) n (%)**	**Total (208) n (%)**	
< 20	1 (0.9)	3 (2.9)	4 (1.9)	0.366
20 - 25	20 (19.2)	30 (28.8)	50 (24)	
25 - 30	37 (35.6)	35 (33.7)	72 (34.6)	
30 - 35	34 (32.7)	26 (25.0)	60 (28.8)	
> 35	12 (11.5)	10 (9.6)	22 (10.6)	
Table 1: Correlation between maternal age and gestational age at delivery				

Table 1 shows that there was 1 patient (0.9%) aged less than 20 years, 20 patients (19.2%) in age group 20 to 25 years, 37 patients (35.6%) in age group 25 to 30 years, 34 patients (32.7%) in age group 30 to 35 years and 12 patients (11.5%) aged more than 35 years, who had preterm birth. There were 3 patients (2.9%) aged less than 20 years, 30 patients (28.8%) in age group 20 to 25 years, 35 patients (33.7%) in age group 25 to 30 years, 26 patients (25%) in age group 30 to 35 years and 10 patients (9.6%) aged more than 35 years, who had full term birth. Majority of the patients in the study, in both the term and preterm group were between age group of 25 to 30 years. However, there is no statistical significance between maternal age and gestational age at delivery.

Study Variables		Frequency (n-208)	Percent (%)
Gestational age (in Weeks)	28 - 32	9	4.3
	32 - 34	14	6.7
	34 - 37	81	38.9
	> 37	104	50.0
Table 2: Distribution of gestational age at birth			

Table 2 show that there were 9 patients (4.3%) who delivered between 28 to 32 weeks, 14 patients (6.7%) who delivered between 32 to 34 weeks, 81 patients (38.9%) between 34 to 37 weeks, and 104 patients (50%) who delivered at >37 weeks of gestational age. 81 patients (38.9%) of the preterm deliveries were in the gestational age group of 34 to 37 weeks.

Gestational age (in weeks)	Birth Weight (in Kgs)				p-value
	VLBW (< 1.5) n (%)	LBW (1.5 - 2.5) n (%)	Normal (> 2.5) n (%)	Total n (%)	
28 - 32	5(55.6)	4(44.4)	0	9(100)	< 0.001*
32 - 34	1(7.1)	11(78.6)	2(14.2)	14(100)	
34 - 37	1(1.2)	51(63)	29(35.8)	81(100)	
> 37	0	9(8.7)	95(91.3)	104(100)	
Total	7(3.36)	75(36.05)	126(60.5)	208	
Table 3: Comparison of birth weight with gestational age at delivery					
*signifies statistically significant					

According to table 3, on comparison between birth weight with gestational age at delivery, we found out that 126 patients (60.5%) had normal birthweight (>2.5kg) babies, 75 patients (36.05%) had LBW (1.5 to 2.5 kg) babies, and 7 patients (3.36%) had VLBW (<1.5kg) babies. The lowest birthweight noted in our study was 1.2kg. In those who delivered at term, 95 (91.3%) had a normal birth weight baby, 9 patients (8.7%) had a LBW baby. Among patients who delivered preterm, 66 babies (63.5%) were LBW babies, 31 patients (29.8%) were normal birth weight babies, and 7 babies (6.7%) were VLBW. The P value was calculated to be< 0.001, which shows a statistically significant association between gestational age and birthweight. As gestational age increases, birth, weight increases. Gestation age is directly proportional to birthweight.

This finding of our study was comparable with that of a study conducted by Topcu et al [4], in the year 2014, in which he concluded that the birth weight increases in a nonlinear pattern as the gestational age increases, which he represented in percentile charts.

Group	Serum ferritin <25ng/ml n (%)	Serum ferritin >25ng/ml n (%)	Total (208)	p value
Preterm	13(12.5)	91(87.5)	104	0.004*
Term	84(80.7)	20(19.3)	104	
Table 4: Comparison of Serum ferritin levels in term and preterm group				

Gestational age (in weeks)	Serum ferritin <25ng/ml n (%)	Serum ferritin >25ng/ml n (%)	Total (208)	p value
28 - 32	0	9 (100)	9	0.004*
32 - 34	0	14 (100)	14	
34 - 37	13 (16.1)	68 (83.9)	81	
> 37	84 (80.7)	20 (19.3)	104	
Table 5: Correlation between gestational age and maternal serum ferritin levels				
* signifies statistically significant				

Serum ferritin levels were noted and analysed in patients in each gestational age group, which is presented in table 4 and 5. An arbitrary cut-off of serum ferritin level at 25 ng/ml was taken and results noted. According to the table, among the 104 patients in preterm group, 91 patients (87.5%) had a serum ferritin level of >25ng/ml, and 13 patients (12.5%) had a serum ferritin level of <25ng/ml. Out of the 104 patients in the term group, 84 patients (80.7%) had a serum ferritin level of <25ng/ml and 20 patients had a serum ferritin level of >25ng/ml. Among patients who were in the preterm group, 68 (83.9%) patients had a serum ferritin level of more than 25 ng/mL and 13 (16.1%) patients had a serum ferritin level less than 25ng/ml. The P value was calculated to be 0.004, which was statistically significant. The maternal serum ferritin levels are inversely proportional to the gestational age. Hence, as gestational age increases maternal serum, ferritin levels decreases.

In a study by Anupam Goel et al,[5] which included 140 women at risk of preterm labor, such as history of preterm labor, low birth weight babies in previous pregnancies, history of threatened abortion in present pregnancy, serial venous blood samples were drawn at 26, 30, 34 weeks of gestation and various parameters including serum ferritin was estimated. They were then compared between subjects who delivered before and after 37 weeks. In preterm group, serum ferritin was increased in patients with preterm labor which was statistically significant. A serum ferritin level of 40 microgram/dl gave a sensitivity and specificity of 75% and 67.39% respectively for prediction of preterm delivery.

Such similar results were obtained in a cross sectional study with 300 patients in labor in Tehran by Tayebeh Jahedbozorgan et al.[3] The mean ferritin level in all preterm groups was significantly higher than in the term group. A ferritin level of 37.5 ng/mL was identified as the best cut-off for preterm delivery, as compared with term delivery, with a sensitivity, specificity, and diagnostic accuracy of 78.7%, 68.7%, and 73.6%, respectively.

Elnasr et al [6] showed that the mean of serum ferritin concentrations in the term patients was 89.04 ± 9.7 (ng/ml) and the range was 69–119 (ng/ml), while in the preterm group patients the mean was 116.27 ±4.9 (ng/ml) and the range was 109–125 (ng/ml), which was statistically significant. He also showed that the area under the ROC curve (AUC) for serum ferritin is 0.97, the cutoff value 110.5 ng/ml with sensitivity 86.7%, Specificity 94.1% and 95% CI 0.94-1.0. The values of his study was higher compared to our study, although the conclusion of his study was similar to ours.

However, among the 20 patients in term group, who had a serum ferritin level of more than 25 ng/ml there were inflammatory conditions and infections seen such as urinary tract infection, tinea corporis, psoriasis, acute febrile illness, raised total counts, bronchial asthma. These conditions are known to increase acute phase reactants such as serum ferritin. This could be the reason for increased serum ferritin levels in patients in term group. Similarly, 13 patients in preterm group in whom serum ferritin level of <25ng/ml were noted belonged to the late preterm (34 to 37 weeks), with average birthweight of the newborn being 2.4 kg. This could be due to wrong dates or fetus already having attained maturity and triggering labor in such patients.

		N	**Mean**	**Std. Deviation**	**p-value**
Serum Ferritin (ng/ml)	Pre Term	104	78.40	212.24	0.004*
	Full Term	104	17.59	16.93	
Table 6: Comparison of mean Serum ferritin levels in Term and Preterm group					
*signifies statistically significant					

Table 6 shows that, the mean of serum ferritin levels in preterm group was found to be 78.4ng/ml with a standard deviation of 212.24ng/ml. The serum ferritin level of patients in term group was found to be <25ng/ml. The mean of serum ferritin levels in patients of term group was 17.59ng/ml with a standard deviation of 16.93ng/ml.

P value was found to be 0.004, which shows that mean serum ferritin levels of women with preterm labor was significantly greater compared to women in term group.

El-Shahawy et al[7] included in their study group 30 patients with established PTL between 30 to 34 weeks, Control group included 30 patients with uncomplicated pregnancies between 30 to 34 weeks.

They found that the median serum ferritin concentration in preterm labor group and control group was 150 (100-150) ng/ml and 20 (15- 25)ng/ml respectively. There was statistically significant difference between both groups in serum ferritin concentration as the p value was 0.0001.
Serum mean levels of hs CRP and ferritin were significantly higher in preterm labour cases when compared to controls, as studied by Nagaraj R S et al.[8] The difference was statistically highly significant ($p < 0.001$). Statistically significant elevation of mean serum ferritin level in patients with preterm labor (172.37±9.21 µg/L) when compared to that of control subjects (86.21±7.47µg/L). These results were similar to our study, except the serum ferritin values were much lower in our study as predictor for preterm labor.

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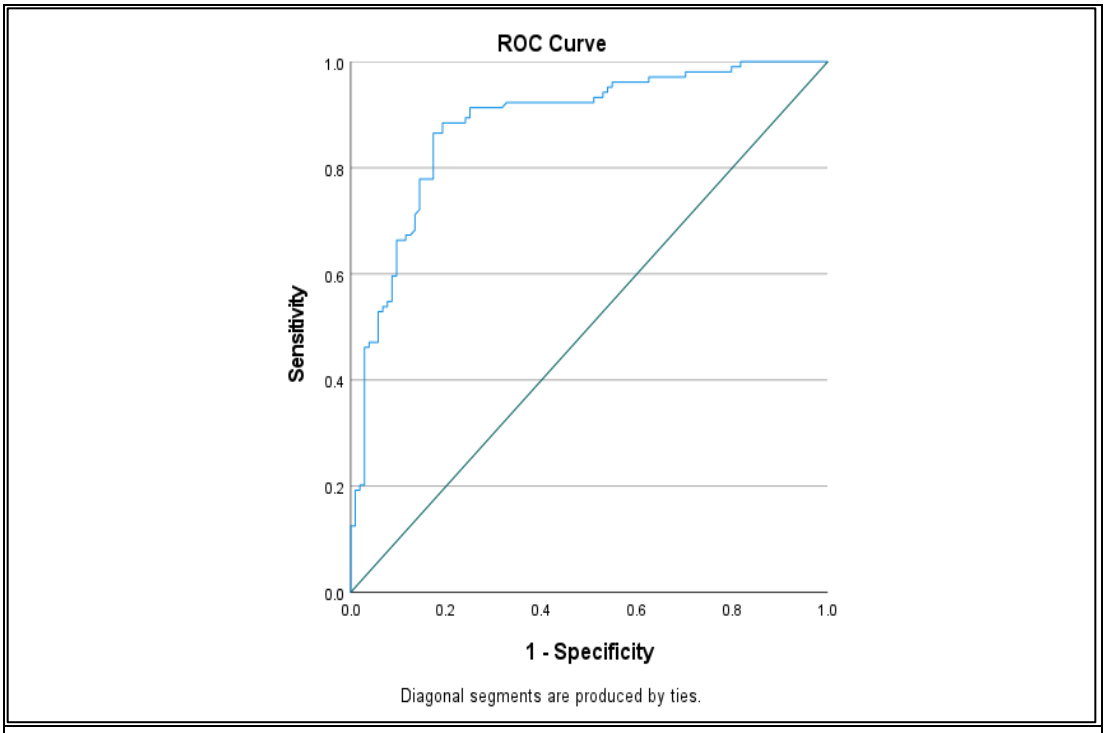
	Serum Ferritin Levels		**p value**
Deliveries	**>25.64ng/ml n (%)**	**<25.64ng/ml n (%)**	
Pre Term	90(83.3)	14(14)	0.001*
Full Term	18(16.7)	86(86)	
Total	108	100	
Table 7: Correlation of maternal serum ferritin levels (at cut off off 25.64 ng/ml) and incidence of preterm deliveries			
*signifies statistically significant			

In the data presented in table 7, we tried to find if there is a correlation between maternal serum ferritin levels and incidence of preterm and deliveries. Out of the 108 patients who had a serum ferritin level of more than 25.64 ng/mL 90 patients (83.3%) actually delivered preterm, while 18 patients (16.7%) delivered at term. Among the hundred patients who had a serum ferritin level of less than 25.64 ng/ml, 86 patients (86%) delivered at term, while 14 patients (14%) delivered preterm. Although there were few patients whose results did not correlate with the serum ferritin value (such as in chronic inflammatory condition, or in cases of late preterm), the P value was calculated to be 0.001. Hence there is a statistically significant association between maternal serum ferritin levels and incidence of preterm deliveries at the cut-off of serum ferritin level at 25.64ng/ml.
Saha et al[9] compared serum ferritin in preterm group, in patients with PPROM and in control group. Mean serum ferritin levels in patients with preterm labor and preterm premature rupture of membranes were 23.24±12.13 ng/ml and 29.44±28.41 ng/ml, respectively. The mean serum ferritin in control subjects was 8.69±3.7 ng/ml. The difference was found to be statistically significant. Thus he concluded that the serum ferritin level is significantly raised in pregnant women with preterm labor and preterm PROM.
A Y Weintraub et al,[10] concluded that second trimester maternal serum ferritin concentrations above 30 ng/ml can serve as a marker of preterm delivery in patients with preterm labor and intact membranes.

Serum Ferritin Cutoff	**Sensitivity**	**Specificity**	**Positive Predictive Value**	**Negative Predictive Value**	**Accuracy**
25.64	86.5%	82.7%	83.3%	86.0%	84.6%
Table 8: Validity of maternal serum ferritin levels as predictor for preterm labor at cutoff of 25.64 ng/ml					

The primary objective of our study is to assess the validity of maternal serum ferritin levels as diagnostic test for preterm labor, the results of which has shown us that serum ferritin level at the cut off of 25.64ng/ml has reasonably good performance in predicting preterm labor with high sensitivity (86.5%) and specificity (82.7%), as well as good positive (83.3%) and negative (86%) predictive values, with an accuracy of 84.6%.



Graph 1: Receiver Operating Characteristic (ROC) curve for Serum Ferritin

AUC (95% C.I) = 0.882 (0.836 – 0.929) with S.E = 0.024 & Significance, P < 0.001*

* signifies statistically significant

The ROC curve for serum ferritin at the cut-off of 25.64 ng/mL shows AUCS 0.882 with standard error of 0.024 and the P value of < 0.001, which is statistically significant. This indicates that at the confidence interval of 95% serum ferritin levels have good discriminatory power in predicting preterm labour.

This results are comparable with that of the study conducted by Dr. Aseel Adil Omran et al [11] in the year 2019 in 216 patients between age 18 to 40 years. Serum ferritin was significantly decreased in full term than preterm gestation (p < 0.001). He also showed that the level of serum ferritin declined with progression of gestational age in preterm labor.

Although in his study the validity of the test to diagnose preterm was assessed by using cutoff value of S. ferritin at 34.2 ng/L, and the AUC was 80% show that sensitivity was 91%, specificity 95%, NPV 94.8%, PPV 68% and accuracy of the test was 93%.

Movahedi et al[12] showed that, on 222 singleton pregnancies, 69 (31.1%) had PTD and 153 (68.9%) had term delivery). Women who delivered before 37 weeks had a higher mean serum ferritin concentration than those who delivered after 37 weeks of gestation (26.7 ±5.5 ng/ml vs. 19.8 ±3.6 ng/ml, (P < 0.001), which agreed with the results of our present study. Serum ferritin level of 22.5 ng/mL yielded the best combination with sensitivity of 78.3%, specificity of 83.0%, positive predictive value of 67.5%, and negative predictive value of 89.4% for prediction of preterm delivery.

Xiao et al,[13] in a case control study in Swedish Medical Centre, observed a twofold increased risk of preterm premature rupture of membranes among women with ferritin concentrations above 64.5ng/mL compared with women whose concentrations were <26.0ng/mL. He also noted that women with serum ferritin concentrations of >96ng/mL, compared with those whose concentrations were <26.0ng/mL, experienced a 2.7 fold increased risk of delivering at <34 weeks gestation.

Amina khambalia et al,[14] found that serum ferritin levels >75th percentile(≥43 µg/L) were associated with increased odds of sPTB (<37 weeks) and the sub-category of moderate-to-late sPTB (34-36 weeks). However, only the higher threshold (>90th percentile) for serum ferritin levels (≥68 µg/L) was significantly associated with early sPTB in the study.

Ramsey et al[15] in their study found that cervical ferritin levels were significantly higher in women who subsequently had spontaneous early preterm delivery (<32 weeks, mean ± SD, 37.7 ± 31.1 vs

21.5 ± 24.1 ng/mL, *P* =.002; and <35 weeks, 43.2 ± 62.7 vs 28.2 ± 36.7 ng/mL, *P* =.004) than in term controls. A cervical ferritin of >75th percentile in the controls (>35.5 ng/mL) was found in 52.9% (9/17) of the women delivered <29 weeks vs 17.7% (3/17) of the controls and in 43.5% (20/46) of the women delivered <32 weeks versus 10.9% (5/46) of the controls. Cervical ferritin levels had a weaker association with spontaneous preterm delivery <35 weeks and <37 weeks. Cervical ferritin levels correlated significantly with cervical lactoferrin, interleukin-6 (IL-6), and defensin levels. Elevated cervical ferritin levels at 22 to 24 weeks of gestation in asymptomatic women are associated with subsequent spontaneous preterm birth. The strong correlation of cervical ferritin with other inflammatory markers provides support for the hypothesis of infection as a mediator of preterm delivery.

In a study by Abdel Malek et al,[16] with serum ferritin cut off of 31ng/ml, the sensitivity, specificity, PPV, NPV and accuracy was found to be 92.8%, 99.4%, 97.5%, 98.4% and 98.3%.

LIMITATION

This study has certain limitations such as smaller sample size of cases and control. We compared serum ferritin levels of patients delivering at term and preterm at different gestational ages.

However, we did not compare the serum ferritin levels of patients delivering preterm and those at comparable gestational age who did not have preterm labor.

A serial estimation of serum ferritin level at different gestational ages in the same patients could have also pointed towards a rising or falling trend in serum ferritin levels. This aspect was not studied. Normally, there is a fall in serum ferritin level as gestational age increases.

CONCLUSION

In our study serum ferritin levels in preterm group was significantly higher than in the term group. Incidence of preterm labor was increased in patients with elevated serum ferritin levels. Measurement of serum ferritin levels is a cost effective, simple to perform and less time consuming test for diagnosing preterm labor. It is indicative of subclinical infections which can be one of the reasons for preterm labor. Serum ferritin at a cut off of >25.64ng/ml had a sensitivity, specificity, PPV, NPV and accuracy of 86.5%, 82.7%, 83.3%, 86% and 84.6%. Hence Serum ferritin can be used as a diagnostic test for predicting preterm labor.

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