

LDL and Small Dense LDL in Normal Pregnancy and Post partum

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Abstract: Normal pregnancy is associated with significant hyperlipidemia. Hyperlipidemias are well known as a modifiable risk factor for ischemic heart disease, which is one of the leading causes of morbidity and mortality worldwide. Traditionally LDL is referred to as 'Bad Cholesterol'. It is now established that more than LDL as a whole, it is the small dense subtype of LDL in particular which is responsible for the atherogenicity of LDL. In this study we aimed to determine whether normal pregnancy is associated with a shift in distribution of LDL subtypes, and whether a shift to a more atherogenic lipid profile is an inevitable consequence of normal pregnancy. 100 women with normal, uncomplicated pregnancy were enrolled in the study. Blood samples were collected in the middle of each trimester and 3-4 months post partum, and LDL and small dense LDL levels were estimated. Repeated Measures Anova with post hoc correction was used to compare the mean values of LDL and sdLDL among the different trimesters. P value < 0.05 was considered statistically significant. Normal pregnancy was associated with a progressive and significant rise in both LDL and sdLDL levels, peak values being attained in the third trimester. Post partum, LDL levels returned to the base line, whereas the sdLDL levels still remained significantly higher than the baseline. Normal pregnancy is associated with an increase in the levels of small dense sub fractions of LDL, which is not fully reversed by 3-4 months postpartum.

Keywords: Small dense LDL, LDL, Normal Pregnancy, Atherogenicity, Hyperlipidemia.

INTRODUCTION

Normal pregnancy is associated with numerous physiological changes; including significant alterations in the lipid profile [1]. Lipid/ lipoprotein abnormalities are common in the general population, and are well known as a modifiable risk factor for cardiovascular disease. Confusion still exists regarding the atherogenic potential of this pregnancy induced hyperlipidemia.

The physiological changes occurring during normal pregnancy are essential to support the developing fetus and prepare the mother for parturition [2]. Alterations in lipid and lipoprotein metabolism play a major role. Maternal fat stores are accumulated in early and mid pregnancy, so that they can be mobilized more in late pregnancy [3]. Lipid profile alterations include progressive elevation in the levels of serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and apolipoprotein B [3]. The extent of increase ranges from 25-50% in case of TC, and 200-400% in case of TGs [4, 5]. LDL levels reach their maximum around mid third trimester [6]. Increased estrogen and

progesterone levels during pregnancy are responsible for this hyperlipidemia, which ensures adequate maintenance of supply of nutrients to the pregnant mother and fetus [4].

Low density lipoprotein particles in the plasma of normal individuals exist as two major sub-types; pattern A, (density 1.019-1.014 gm/ml), with a higher proportion of large, less dense LDL particles, and pattern B, (density 1.044-1.060 gm/ml), with a predominance of small denser LDL (sdLDL) particles [7]. Properties like higher penetration into the arterial wall, lower binding affinity for the LDL receptor, prolonged plasma half-life and lower resistance to oxidative stress account for the higher atherogenic potential of sdLDL particles compared to pattern A. The LDL profile is significantly different in men and women. Pre-menopausal women, maybe due to the influence of estrogen tend to have higher concentration of pattern A [8]. Pregnancy however provides a unique state comprising of increased levels of estrogens, progesterone, and human placental lactogen [9], which may alter the LDL profile in the latter part of pregnancy [10].

Though pregnancy related hyperlipidemia is well documented, confusion still exists regarding its atherogenic potential [11]. The Framingham Heart Study (1993) reported that women with a history of more than 6 pregnancies had a significantly elevated risk of developing cardiovascular disease when compared with nulliparous women, at a relative risk of 1.6 [12]. Taking into consideration the atherogenic potential of sdLDL, it has been suggested that monitoring the sdLDL levels during pregnancy might be used to identify women who will develop atherogenic changes later in life [13].

Given the increasing incidence of coronary artery disease in the Indian population along with the high morbidity and mortality associated with the condition, it is necessary to identify additional factors which may contribute to the development of the above. In this study we attempted to evaluate the LDL and sdLDL levels of pregnant ladies in different trimesters, and to establish whether a shift to a more atherogenic profile is an inevitable consequence of normal pregnancy.

AIMS AND OBJECTIVES

To study the levels of LDL and small dense LDL cholesterol in the different trimesters of normal pregnancy and post partum, and detect, if any, shift towards a more atherogenic lipid profile.

MATERIALS AND METHODS

The present study was a non interventional follow up study, conducted in the Department of Biochemistry and the Department of Obstetrics and Gynecology, Christian Medical College and Hospital, Ludhiana. The study group consisted of 100 ladies with uncomplicated pregnancy. Pregnant ladies attending the ante natal clinic were enrolled in the first trimester, irrespective of their age and parity. Those with known pre existing Dyslipidemias or any co-morbid conditions i.e. diabetes mellitus, hypertension, heart disease, chronic renal failure or hypothyroidism were excluded from the study. Patients who developed pregnancy induced hypertension or gestational diabetes during the course of the study were also excluded. This research project was cleared by the institutional ethics board. Informed consent was taken from all those included in the study.

The women were advised to come after overnight fasting of 12 hours and samples were drawn between 8.30 to 9.30 AM. Blood samples of each woman was taken four times, first sample in 1st trimester (8-12 weeks), and followed through 2nd (22-26 weeks) and 3rd trimesters (32-36 weeks), and postpartum between 3-4 months. After taking consent, 5ml of fasting venous blood was drawn from each participant, via venipuncture from the ante-cubital vein under aseptic precautions. The samples were collected in plain vials. Serum was separated and stored in aliquots at 4° C to be processed within 7 days.

LDL estimation was done by an enzymatic colorimetric test based on the CHOD-PAP principle using ROCHE kits on Modular P-800 auto-analyzer.

sdLDL was estimated on Hitachi 902 auto-analyzer using the sdLDL kit provided by Randox. The assay was performed according to the instructions of the manufacturer. It consists of a two step reaction using 5 µL of serum. The first reaction involves degradation of non sdLDL lipoproteins and enzymatic degradation of released cholesterol. In the second reaction cholesterol from sdLDL is released and subjected to an enzymatic reaction leading to the formation of purple red colored complex. Readings were taken at 600nm. This method has a detection limit of 1mg /dl and linearity upto 100mg/dl. Correlation coefficient (r) of this method vs the standard method, ultra centrifugation is 0.954. Quality was checked by running of a control along with each batch.

RESULTS AND ANALYSIS

123 pregnant women were enrolled in the study. 9 women developed pregnancy induced hypertension, 2 developed gestational diabetes, and 12 were lost to follow up. Analysis was performed for 100 women.

The demographic profile of the women is described in Table-1, Fig-1. Out of total 100 women, 56 % were between 20-24 years of age, 38% were 25-29 years of age and only 6 were ≥30 years. The mean age of the women was 24.63 years with a standard deviation of 2.68 and range of 20-30. 74% of the patients were primigravidae, 26% were multigravidae.

Table-1: Distribution of patients according to age and parity

Age (yrs)	20-24		25-29		≥30		Total
N	56		38		6		100
	Primis	Multis	Primis	Multis	Primis	Multis	
	49	7	23	15	2	4	
Mean age (yrs)	24.63 ± 2.68						

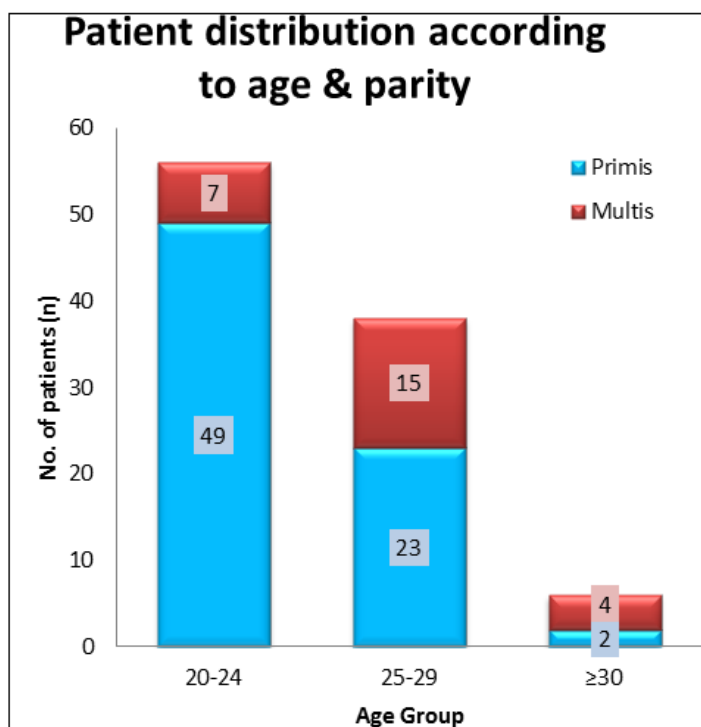


Fig-1: Distribution of patients according to age and parity

The values of LDL and sdLDL obtained in the different trimesters, and the percentage of sdLDL out of total LDL are shown in Table-2. First trimester values of each parameter were considered as baseline values. Repeated Measures ANOVA was used to compare the

mean values of LDL and sdLDL among the different trimesters. Post hoc analysis with Bonferroni correction was done to compare between the mean values of each trimester. P value < 0.05 was considered statistically significant.

Table-2: Biochemical parameters in successive trimesters and post partum

Group	1 st Trimester	2 nd Trimester	3 rd Trimester	Post Partum	P Value
sdLDL mg/dl	14.11 ± 8.279 ^a	27.86 ± 11.199 ^b	38.10 ± 19.644 ^c	25.14 ± 14.182 ^d	<0.0001
LDL mg/dl	100.86 ± 26.709 ^a	129.57 ± 44.072 ^b	145.87 ± 58.228 ^c	106.89 ± 36.77 ^a	<0.0001
sdLDL/LDL %	13.9%	21.5%	26.11%	23.5%	

Note

1. All the parameters in this table are expressed as mean ± standard deviation of mean
2. Means with different superscript letters (a,b) are significantly different for the respective parameters at p < 0.05
3. Means with same superscript letters (b, b) are not significantly different for the respective parameters at p > 0.05

There was a progressive rise of mean LDL levels from the 1st to the 3rd trimester, followed by a fall post partum (Fig-2). The mean LDL levels rose by 28.5% from the 1st to 2nd trimester (p value < 0.0001),

and 12.5 % from the 2nd to 3rd trimester (p value < 0.0001). Though the mean LDL levels post partum was higher than the 1st trimester levels by 6.0%, the rise was not significant statistically (p=0.7).

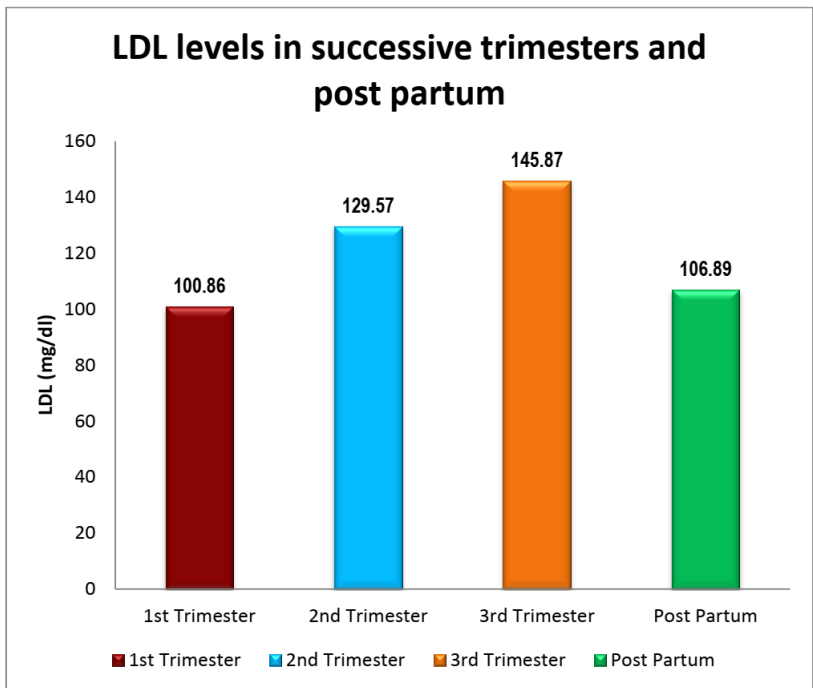


Fig-2: LDL levels in successive trimesters and post partum

The mean sdLDL levels rose from the 1st to the 3rd trimester, followed by a fall post partum (Fig-3). The mean sdLDL levels rose by 97.4% from the 1st to 2nd trimester (p value < 0.0001), and 36.7 % from the 2nd to

3rd trimester (p value < 0.0001). Though there was a fall in the mean sdLDL levels post partum, it was still significantly higher than the 1st trimester levels by 78.1% (p < 0.0001).

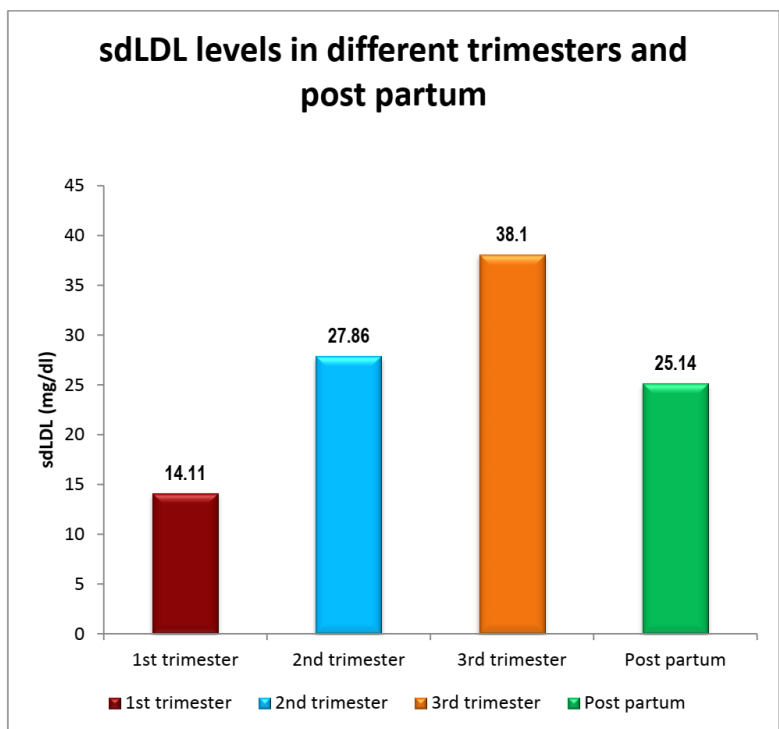


Fig-3: sdLDL levels in successive trimesters and post partum

The percentage of sdLDL out of total LDL which was 13.9% in the 1st trimester rose up to 21.5% in the 2nd (p=0.0001) and 26.1% in the 3rd trimester

(p=0.0002). Though there was a fall in the post partum levels of both LDL and sdLDL, the relative fall was much more in case of LDL, sdLDL still contributing to

23.5% of the total LDL fractions, which was still significantly higher than the baseline percentage

($p=0.0001$) (Fig-4).

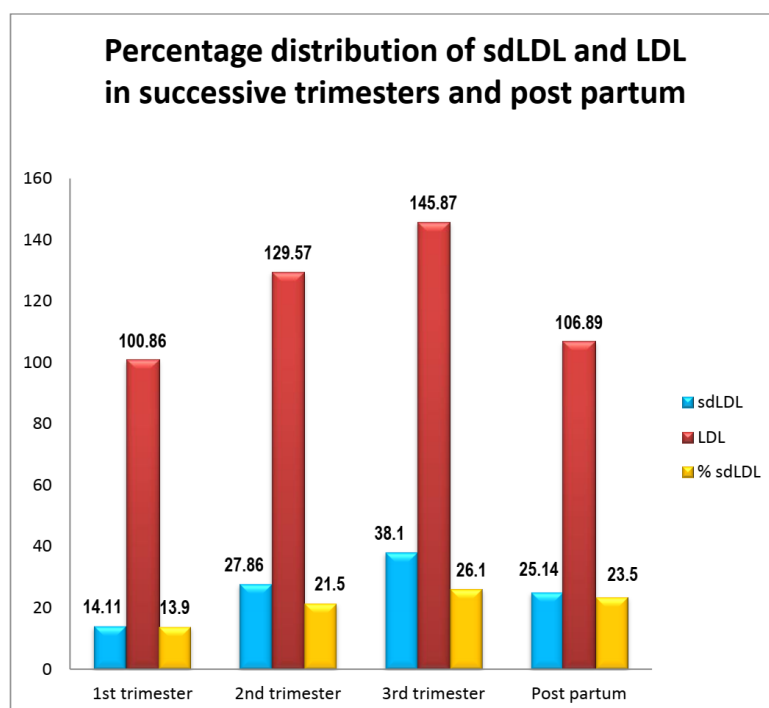


Fig-4: Percentage wise distribution of sdLDL in different trimesters and post partum

DISCUSSION

In this study we aimed to determine the levels of LDL and small dense LDL throughout gestation and post partum in normal, uncomplicated pregnancy. In agreement with earlier studies, we found a progressive rise in the levels of both LDL and sdLDL throughout gestation [14]. When the post partum levels were measured, we found the LDL levels returned to normal, while the sdLDL levels still remained high.

The mean 1st trimester LDL values in our study were in agreement with the findings of Hubel *et al.*, [15] and Winkler *et al.* [3] whereas Neboh *et al.*, in a study on a Nigerian population reported a higher mean 1st trimester value [16]. Some of the studies reported lower mean 1st trimester LDL levels [17]. This difference may be explained by different baseline values among different populations with varying dietary habits, our study being among urban Punjabi women; or due to difference in the time of sampling.

The mean 2nd trimester LDL values in our study were in agreement of the findings of Hubel *et al.*, Belo *et al.*, Basaran and Neboh *et al.*, [14, 17, 16], while the 3rd trimester values corroborated with the findings of Hubel *et al.*, Ogura *et al.*, Belo *et al.*, and Okojie *et al.*, [15, 19-21]. The mean levels of LDL rose significantly between the 1st and 2nd, and 2nd and 3rd trimesters, the Mankuta *et al.*, [22].

The mean LDL levels at 4-6 months post partum obtained in the present study was 106.8 mg/dl, which was almost near the 1st trimester levels of 100.8 mg/dl. Varying results have been obtained in different studies regarding the post partum LDL levels. Belo *et al.*, reported a significant fall in the LDL levels within 24-48 hours post partum [14], whereas Ogura *et al.*, reported LDL levels at 4 weeks post partum to be similar to those at the 37th week of gestation [19]. Hubel *et al.*, reported that though there was a significant fall in LDL levels at 6-12 weeks post partum from the term levels; they still remained significantly higher than the 1st trimester levels [15]. Alvarez *et al.*, studied the LDL levels 60 days after the end of lactation when spontaneous menses resumed (average 9 months post partum) and reported values significantly higher than the 1st trimester levels [23]. Mankuta *et al.*, in a study on lipid profile in consecutive pregnancies reported that LDL levels reach pre-pregnant values within 1 year post partum and continues to decline during the 2nd and 3rd years post partum [22].

We also found that the LDL rise during gestation was accompanied by a concurrent rise in sdLDL levels. The percentage of sdLDL out of total LDL rose from 13.9% in the 1st trimester to 21.5% in the second and 26.11% in the third trimester. This shows that pregnancy is accompanied by a redistribution of the LDL sub-classes. These findings corroborate with the findings of previous studies which

reported that normal pregnancy is associated with shift of the LDL sub-classes to a smaller and denser sub type.

Though previous reports all reported an increase in the sdLDL levels as pregnancy advances, varying results have been obtained regarding the baseline sdLDL levels and the extent of rise in the trimesters. Sattar *et al.*, reported successive levels of 14%, 14% and 21% [4]; Belo *et al.*, reported 30.54%, 34.84% and 36.89% [14]; Belo et al (2004) reported 21%, 31.4% and 39.07% [20]; whereas Basaran reported levels of 14%, 10.9% and 34.8% [17].

The differences in the values obtained in the previous studies with our study may be explained partly by the technique used for sdLDL estimation. In the present study, sdLDL estimation was done by a kit based method provided by Randox. A direct estimation of sdLDL levels was done using a reaction based on surfactant mediated hydrolysis of cholesterol from sdLDL particles, followed by hydroxylation and oxidation, giving a purple red coloured end product.

Sattar *et al.*, used density grade ultracentrifugation for separation and quantification of LDL sub type III in particular [4]. Ogura et al. studied the LDL PPD (peak particle diameter) during pregnancy and found that the LDL PPD at 37 weeks was significantly lower than that of 10 weeks; and increased gradually after delivery [19]. Hubel *et al.*, too reported a significant fall in LDL PPD with advancing gestation after separating LDL subfractions by PAGE [15].

The mean post partum value of sdLDL at 3-4 months obtained in our study was 25.14 mg/dl (23.5%), which though lesser than the term levels was similar to the 2nd trimester (p value = 0.056) and significantly higher than the 1st trimester levels. Belo et al. reported a significant fall in sdLDL levels by 24-48 hours post partum (33.4%) [14]. Hubel *et al.*, reported no change in LDL PPD values 24-48 hours post partum, though the values normalized gradually by 6-12 weeks [15]. Ogura *et al.*, reported that fall in TG levels by 4 weeks post partum was accompanied by increase in the LDL PPD, and that the LDL sub type changes accompanying normal pregnancy were reversible [19]. Due to differences in the time of sampling and also in the method used for estimation, it is difficult to corroborate the findings of this study with previous ones in case of post partum sdLDL values.

The mechanism behind LDL increase in pregnancy is well understood. Estrogen, progesterone and human placental lactogen levels increase during gestation, and have been shown to be positively correlated to TG levels [23]. Hormonal influences cause

the hepatic VLDL synthesis to increase. VLDLs contain high concentrations of TGs. Hepatic and lipoprotein lipase activity on VLDL hydrolyses the TGs and leads to the formation of LDL through IDL. TGs reach their peak value at term. These changes are very favorable during pregnancy as TGs are used as to provide energy to the mother saving glucose for the fetus [24]. This hormone induced hypertriglyceridemia is ultimately responsible for increased LDL levels as a secondary phenomenon. From the maternal point of view, raised LDL levels are also necessary for steroidogenesis. Parker et al. found that hypocholesterolemia due to hypoapobetalipoproteinemia leads to decreased estrogen and progesterone levels in affected pregnant women, which in turn could lead to an adverse outcome of pregnancy [25].

The transient nature of hormonal changes in pregnancy also explains the transient nature of the associated hyperlipidemia. After the baby is delivered, estrogen and progesterone levels rapidly fall. This in turn causes fall in VLDL and TG levels, and as a consequence the LDL levels fall as well. Studies have reported that TG and lipid concentrations fall sharply by 24-48 hours post-partum, with a more gradual reversal to pre-pregnant values continuing beyond 6 weeks [15].

Along with hyperlipidemia, pregnancy is also associated with a redistribution of LDL sub classes. LDL subclasses (1/2/3/A/B) are formed by neutral lipid exchange and lipolysis. The sub class redistribution associated with pregnancy can again be explained hormonally. CETP (cholesterol ester transfer protein) found in the blood normally transfers TGs from VLDL1 and CM remnants into the core of LDL in exchange for cholesterol esters. Advancing gestation is accompanied by increased estrogen levels, increased VLDL and TG synthesis and increased activity of CETP [4]. Though increased activity of CETP is a factor, the rate limiting factor determining rate of TG transfer into the LDL core has been found to be VLDL1 levels. CETP preferentially acts more upon TG rich VLDL1 as a substrate. Hydrolysis of this newly acquired TG in LDL via hepatic lipase leads to a conformational change in Apolipoprotein B, remodeling LDL into smaller denser thermodynamically stable forms [26].

From this study we conclude that normal pregnancy is associated with significant hyperlipidemia. The increase in LDL levels was transient; it returned to baseline values within six months postpartum. There was however a shift in the LDL sub-types redistribution with a significant increase in the smaller denser atherogenic subtype. It is not clear however whether this increase has any detrimental effect or not.

The drawback of this study was that we though considered the first trimester values to be baseline; high

degree of variation was found in the LDL and sdLDL values among different women. Maybe inclusion of a group of age matched healthy non pregnant controls will be helpful. Secondly, we were unable to comment on the increase in atherogenic propensity due to this pregnancy related shift. For this a larger study with prolonged follow up period is necessary to check whether this shift is permanent or reversible in the long term. Women having higher increases in their sdLDL levels have to be followed up for a longer period to check whether they have any increased incidence of cardiac events.

Conflict of Interest

Both the authors declare that they have no conflict of interest in this study.

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