QUANTITATIVE MORPHOMETRIC ANALYSIS OF UTERINE SPIRAL ARTERY IN NORMAL AND PREECLAMPTIC PLACENTAE VIA FLUORESCENCE MICROSCOPY

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ABSTRACT:

Back ground: Preeclampsia is a syndrome restricted to pregnancy. It affects approximately 10% of pregnancies throughout the world. Although a definite cause of preeclampsia has not been identified, compelling evidence implicates that factors indirectly affecting implantation of the placenta are involved in the etiopathogenesis. **Objective:** The aim of this study was to determine the association of abnormal remodeling of spiral arteries in pregnancy induced hypertension by comparing the parameters of uterine spiral arteries with the control group. **Design:** Case-Control Place of Study: The histological analysis was done at MDRL-2, Ziauddin University. Duration of study: April 2019- June 2020 **Methodology:** We took 21 normal placentae and 21 diagnosed preeclamptic placentae from Ziauddin Hospital, Kemari Campus. Histological analysis was done following standard protocols. On histological examination, the changes in lumen diameter and area, wall area and wallthickness via fluorescence microscopy were measured between the two groupsby using Nikon NIS-elements D software. Wall / lumen area ratio was also calculated accordinglybetween the two groups. **Results:** Our results demonstrated a statistically significant reduction in the lumen diameter and lumen area of the spiral arteries among the PIH group as compared to the control. We have also observed statistically significant increased wall thickness and wall area of the spiral arteries among PIH group as compared to the control. **Conclusion:** The results of our study suggest a significant alteration in the normal remodeling of spiral arteries due to decrease in the luminal parameters, and increased in wall thickness parameters of spiral artery which could be the reason for the development of pregnancy induced hypertension.

Key words: Placenta, Preeclampsia, Spiral Artery, Cytotrophoblasts.

How to cite this article: Baloch GKR[1], Khan BW[2], Siddiqui RA[3], Borges KJJ[4], Shah SNN[5], Inayatullah[6], Urooj T[7]. **QUANTITATIVE MORPHOMETRIC ANALYSIS OF UTERINE SPIRAL ARTERY IN NORMAL AND PREECLAMPTIC PLACENTAE VIA FLUORESCENCE MICROSCOPY.** *JPUMHS; 2021:11:01,73-79.*
http://doi.org/10.46536/jpumhs/2021/11.01.294

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Received on Dec 23, 2020, Accepted On 15 March 2021, Published On 31 March 2021

INTRODUCTION

Pregnancy-induced hypertension (PIH) is one of the leading causes of maternal, fetal, and neonatal mortality and morbidity[1]that complicates about 10% of all pregnancies[2]. It has been estimated that hypertensive diseases in pregnancy cause 30,000 maternal deaths annually[3] with 303 000 maternal mortality worldwide in 2015[4] accounts for about 14% of Global causes of maternal deaths[5]. Data from Pakistan implies that up to 40% of women who died from postpartum hemorrhage complained of symptoms of preeclampsia or had seizures before the onset of their fatal hemorrhage [3]. In such cases, the histological examination of the placenta should always be performed as up to 60% of fetal deaths have been attributed to placental abnormalities[6].

The placenta contains approximately 80 – 100 spiral endometrial arterioles [7]. Uterine spiral arteries in non-pregnant women have elastic and muscular walls that are highly responsive to changes in hemodynamic status, sothe smooth musclesin the arterial wall contract actively to modulate the uterine hemodynamics[8,9]

During pregnancy,the muscular vascular walls of the spiral arteries are invaded by the trophoblast[10]. This process involves the sequential mechanisms of vasculogenesis, angiogenesis, and pseudo-vasculogenesis[11]. The physiological vascular adaptation of vessel renders a low-resistance, high-flow patternessential for the proper establishment of the placenta[10]. Iteventually resulting in increased blood perfusion[12] which in turn ensures a constant jet of maternal blood supply in intervillous space of placenta with low resistance and a high flow system for the developing fetus. The strength of the jet is regulated by the lumen size and shape of the

uterine spiral artery[13].
In placentas destined to develop pregnancy induced hypertension (PIH), cytotrophoblasts fail to transform from the proliferative epithelial subtype to the invasive endothelial subtype which causes incomplete remodeling of the spiral artery[14]. So endovascular trophoblast invasion is restricted to the peripheral, decidual segments of the spiral arteries, which are incompletely remodeled and retain their smooth muscle and elastic lamina[15]. Inadequate spiral arteriolar remodeling leads to narrow maternal vessels[14] and the mean diameter of the uterine spiral arterioles will become less than one-third of similar vessels in normal or uncomplicated pregnancies[16].
Our study was conducted to compare and quantify the parameters of uterine spiral arteries between normal and preeclamptic placentae.

MATERIALS AND METHODS

This study was a case-control study held in Dr. Ziauddin Hospital, Kemari and Ziauddin University, Clifton.
Purposive sampling technique was used to collect 42 placentae which comprised of 21 cases and 21 normal. It was calculated by using OpenEpi calculator for sample size estimation by keeping a confidence interval of 95% power 80 and Odd ratio: 2.
In the inclusion criteria for the normal and healthy group, we recruited non-preeclamptic pregnant females of 20 years or above with or without a family history of preeclampsia. In addition, we also included diagnosed preeclamptic pregnant females 20 years and above. In exclusion criteria we excluded cases having liver disease, myocardial infarction, endocrine, autoimmune and renal diseases. All other diseases or medical conditions related to the uterus like uterine fibroid, uterine cancer, and endometrial cancer were also excluded from our study.

PLACENTAE SAMPLES COLLECTION

Following delivery, placentae were cleaned to remove any clotted blood. Each placenta was cut into two halves. Samples of 2 × 2 × 1 cm were taken from two randomly selected halves. The site of selection was from the center and peripheral parts of the maternal side of each half.

HISTOLOGY PROCEDURE

Small pieces were cut from the placental tissue and transferred into bottles containing Bouin's fixative for four hours. The samples were then washed 3 times by distilled water and then immersed in 70% (v/v) isopropyl alcohol (2-propanol) about 20% greater volume than the tissue and kept overnight.
On the next day, the samples were dehydrated in graded alcohol and embedded in paraffin wax in the following manner. 70% isopropyl alcohol for one hour; 3 times (3 hours), then 90% isopropyl alcohol for one hour; one time, after that 100% isopropyl alcohol for one hour; 3 times (3 hours), Xylene for one hour: 3 times (3 hours), then Xylene + paraffin wax (1:1) for 20-30 min at 68°C in hot air oven and in the last paraffin wax (100%) for overnight at 68°C in hot air oven.
On the third day, samples were embedded in the paraffin wax and tissue blocks were made using tissue mold and tissue embedding cassettes. For the preparation of slides 5 mm, thin tissue sections were cut with the help of a microtome. Sections were placed in a water bath having a temperature of 42oC for 5 minutes to remove the wrinkles. The sections were fished out on gelatin-coated glass slides and kept on slide-warmer at 42oC overnight to allow the proper attachment of the sections on the slides(17). On the next day, the slides were ready for staining.

H&E STAINING

Tissues were processed and stained withHematoxylin and Eosin (H&E) Stain (Carl Roth, Germany) in following steps.
Slides were first deparaffinized in xylene and rehydrated with graded alcohol (100, 90, and 70%). Afterward, sections were stained with hematoxylin for 3 minutes and washed thoroughly with distilled water followed by eosin staining for 30 sec. Then, slides were washed and dehydrated with graded alcohol (70, 90, and 100%) and mounted with DPX (Dibutylphthalate Polystyrene Xylene) mounting media.

BRIGHTFIELD AND FLUORESCENCE MICROSCOPY

H&E stained slides were examined under Nikon ts2r-fl inverted research microscopy with bright field modulatewhereas, the same sections of H&E stained slides were also examined under the fluorescence modulate of the same microscope using a dual channel (FITC/Texas-Red) filter cube. Microscopic images were taken with the software provided by the manufacturer i.e. Nikon NIS-elements D software. Images were analyzed and morphometry was performed by using NIS Elements software. Observations were made from 10 randomly selected fields per slide from each maternal side of placenta. At least 10 – 15 spiral arteries were viewed in every placenta under a magnification of 200X. Only one randomly selected spiral artery, respectively as shown in figure 1 parameters were assessed for changes in lumen area (µm²), lumen diameter (µm), wall thickness (µm), wall area (µm²) and then wall / lumen area ratio (µm) was calculated.

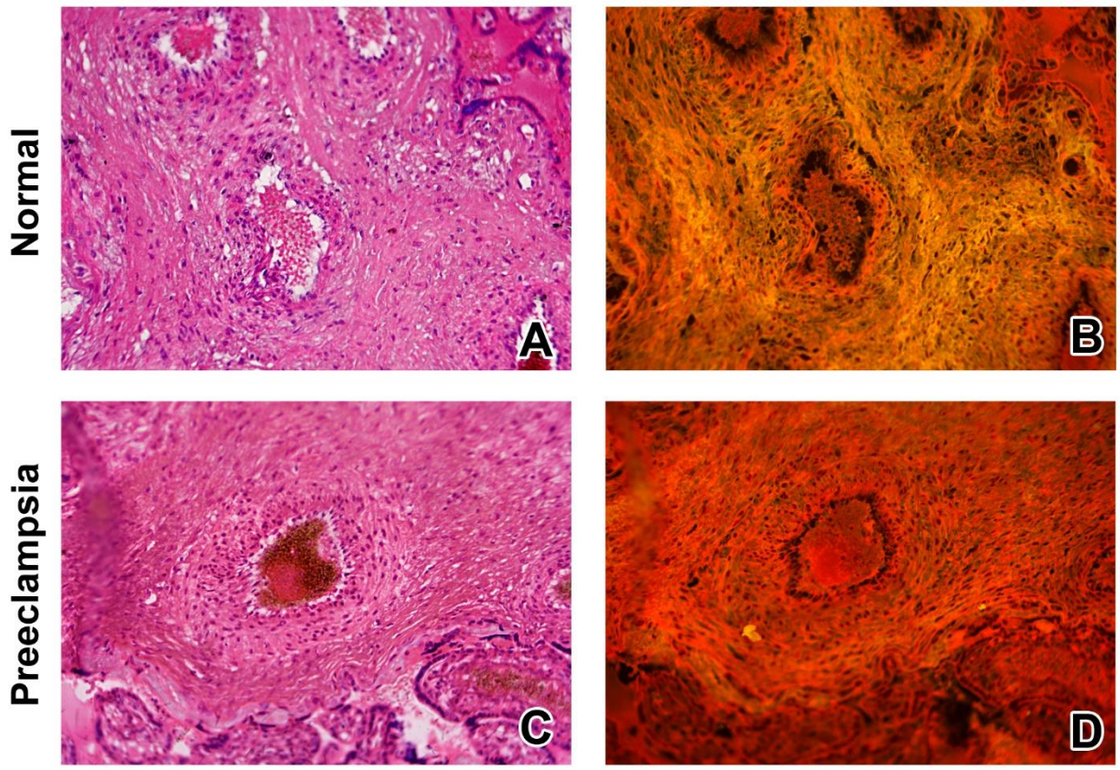


Figure 1: Uterine spiral artery in normal pregnancy group (A) H&E stained slide in bright field microscopy and (B) in fluorescence microscopy using dual channel (FITC/Texas-Red) filter cube. Uterine spiral artery in preeclamptic pregnancy group (C) shows the spiral artery H&E stained slide and (D) shows the same image taken by fluorescence microscopy using dual channel (FITC/Texas-Red) filter cube under Nikon ts2r-fl inverted research microscope.

STATISTICAL ANALYSIS

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.
All quantitative variables were represented as mean and standard deviation.
A pooled t-test was applied to find the difference in histomorphometry of uterine spiral artery between normal and preeclamptic placentae.
In all analyses, a p-value of <0.05 was considered to be significant.

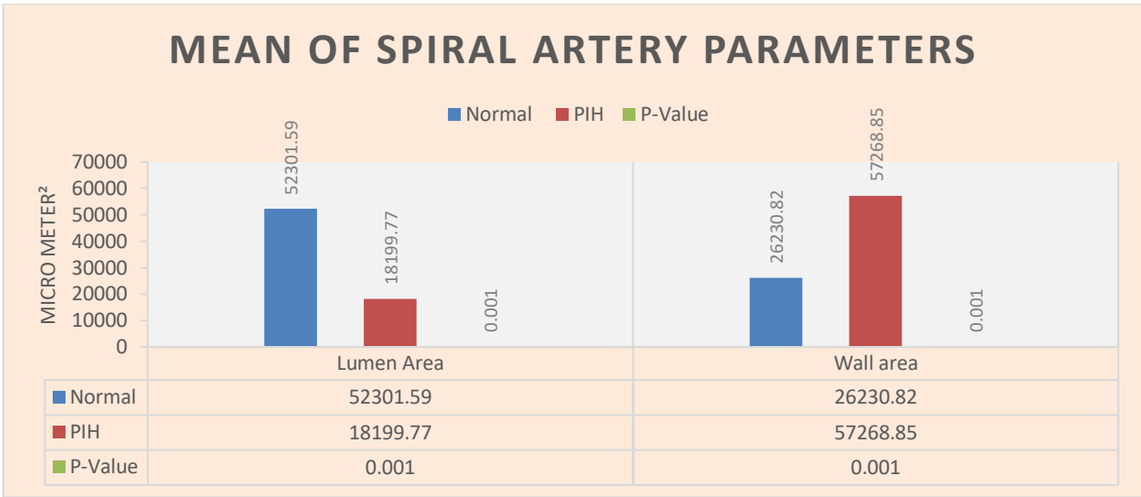
RESULTS

As shown in Table 1, when we compared the spiral arteries lumen areas between the two groups, we found that the luminal area of the PIH group (18199.77±6707.62 μm²) was significantly smaller than that of the normal group which was found to be (52301.59 ±11714.94 μm²). When we compared the spiral artery wall areas between control and PIH groups, we have found statistically significant smaller mean of spiral arteries wall area found amongst the normal pregnancies (26230.82 ± 8721.74 μm²) as compared to the PIH group (57268.85±14694.868 μm²).

Table-1: Comparison of Mean differences of both groups.

Parameters	**Study Groups**	**Mean (μm^2)**	**Std. Deviation (μm^2)**	**p-value**
Lumen Area	Normal	52301.59	±11714.946	0.001*
	Preeclampsia	18199.77	±6707.629	
Wall area	Normal	26230.82	±8721.743	0.001*
	Preeclampsia	57268.85	±14694.868	

* Significance of the mean difference is at 0.05 level. Independent t-test was applied



Graph 1: Shows the values for the Mean difference between the lumen and wall area of spiral arteries in normal and PIH pregnancies.

Table-2: Comparison of Mean differences of both groups

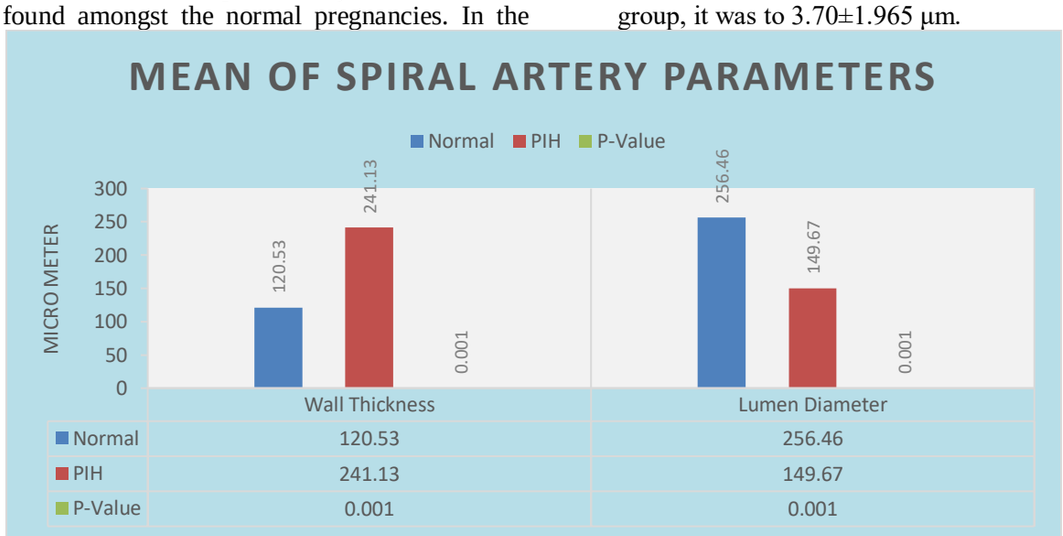
Parameters	Study Groups	Mean (µm)	Std. Deviation (µm)	p-value
Lumen Diameter	Normal	256.46	± 29.277	0.001*
	Preeclampsia	149.67	± 28.426	
Wall thickness	Normal	120.53	±11.176	0.001*
	Preeclampsia	241.13	±20.516	
Wall / lumen area ratio	Normal	0.56	±0.292	0.001*
	Preeclampsia	3.70	±1.965	

* Significance of the mean difference is at 0.05 level. Independent t-test was applied

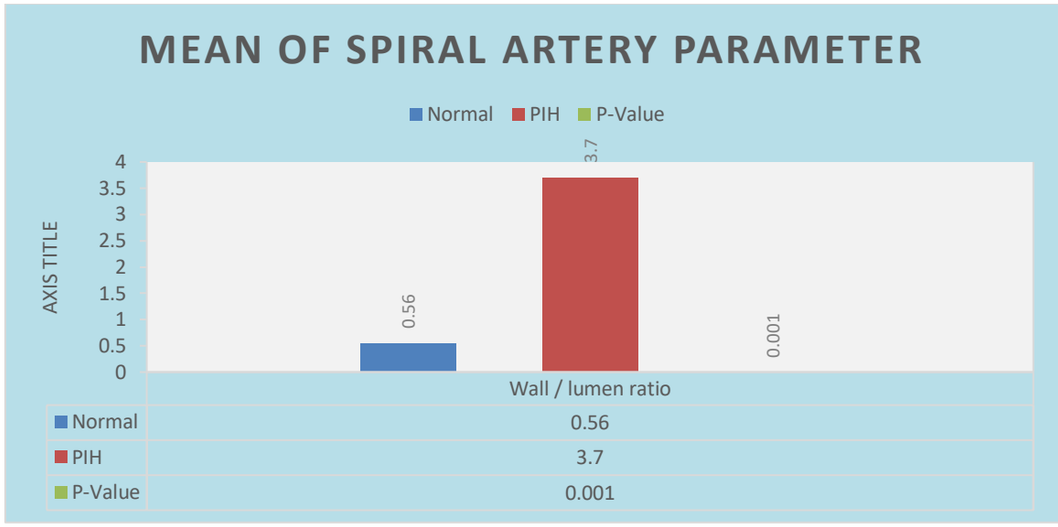
Table 2: The spiral artery lumen diameter was found to be statistically significantly between the groups with greater mean of the lumen diameter was found amongst the normal groups (256.46± 29.277 µm) then the PIH group (149.67± 28.426 µm).

When we compared the uterine spiral artery wall thickness between control and PIH groups, we have found a statistically significant with smaller mean of spiral artery wall thickness found amongst the normal pregnancies. In the control group, spiral arteriole had a mean wall thickness of 120.53±11.176 µm, and in the PIH group, it was to 241.13±20.516 µm.

When the calculated wall/ lumen area ratio was compared between the both groups, we have found a statistically significantratio with smaller mean of spiral artery wall/ lumen area found amongst the normal pregnancies. In the control group, spiral arteriole had a mean wall/ lumen area ratio of 0.56±0.292 µm, and in the PIH group, it was to 3.70±1.965 µm.



Graph 2. Shows the values of mean difference of spiral arteries lumen diameter and wall thickness in normal and PIH pregnancies.



Graph 3: Shows the values for the Mean difference between the wall / lumen ratio and Spiral artery diameter / wall thickness ratio in normal and PIH pregnancies. When results of these parameters of spiral arteries ratios were analyzed the results showed a significant in both parameters.

DISCUSSION

The full-term human placenta is a temporary fetomaternal and complex discoid shaped organ [18] that facilitatesrespiration, nutritional, and hormonal support to the fetus[19]. Abnormality in structure and function of placenta leads to development of gestational hypertensive disorders [20]. Pre-eclampsia (PE) is the most frequently encountered medical complication during pregnancy [21]. There is defective trophoblast invasion in the spiral arteries [9] which results in defective remodeling of uterine spiral arteries and arterioles which leads to placental ischemia and hypertension [22].

In our study, we found significantly increased spiral artery wall thickness and wall area in the PIH group as compared to normal pregnant females. Similar findings regarding wall thickness of uterine spiral arteries were documented. The findings were also documented a significant increase in wall width of the spiral artery in the preeclamptic group. Another study showed that the non-remodeled arteries were characterized by a sharp thickening of the walls and hypertrophy of smooth muscle. From these findings, we infer that in PIH, there is limited pregnancy associated remodeling of the walls of the spiral artery, specifically the tunica media in the PIH group. This results from the decreased invasion of the media by the syncytiotrophoblast. This defective erosion of the medial layer results in comparatively thicker wall size and wall area in PIH associated spiral arteries data showed a non-significant reduction of the wall area in anemic subjects then the normal subjects and the wall area was significantly reduced in early onset of preeclamptic/IUGR, groups as compared with controls and anemic subjects. In his study, they have found that theearly-onset PE with IUGR was characterized by reduced invasion of the spiral arteries by extravillous (intramural) trophoblast. As compared to his study we have contradicting results regarding the wall area as we have taken the full term placentae of PIH grouped females that showed a significantly increased in the wall area of PIH groups.

We applied the same formula for identifying spiral arteries wall/lumen area ratio as applied according to them which showed contrasting results than ours, no significant changes were observed, although there was an apparent decrease from normal to pathological subjects. This might be because that they have taken the samples with severe early-onset preeclampsia and IUGR (at 29-34 weeks' gestation) and the mean value of their wall area was also decreased. On the other hand, according to Ong, Baker et al. in arteries isolated from women with preeclampsia, the wall/lumen ratio was greater than that seen in normal pregnancy.

Our findings report that the ratio in the PIH group was significantly high when compared to the normal group. The reason we given here is again the same that if the spiral arteries are not invaded properly, their walls which are normally disrupted in pregnancy will not be eroded as much in PIH. Hence, resulting in the numerator value dominating the calculations eventually resulting in greater values in the PIH group. Moreover, in hypoxic conditions fibroblast activity is enhanced thus resulting in excessive deposition of collagen fibers in the tunica media which ultimately will lead to thicker vessel walls.

Spiral artery lumen area was found to be increased amongst the normal pregnancies in our study.These findings are similar to the studies done. While result showed an increase in lumen area in spiral arteries but it also showed a decrease in uteroplacental arteries in normal groups which contradict with our and others mentioned data. We also observed that the standard deviation in the data of is nearly twice

that of the value showing the abnormal distribution of data.According to Hiroshi et al. at the normal gestation, spiral artery showed the large lumen with irregular endothelial cells and fibroblasts in the wall. Spiral artery in preeclampsia showed thick wall and narrow lumen by scanning electron microscopy SEM analysis. From our findings, we infer that as the tunic media of the vessels are eroded by the syncytiotrophoblast, the artery loses its capability to constrict. This in turn leads to a lax, dilated tunica intima henceforth resulting in an increased luminal diameter. In PIH, the muscles in the tunica media are not lost. Hence the vasoconstrictive ability of these vessels is retained eventually leading to comparatively smaller lumen diameter leads to PIH.

CONCLUSION

Our study quantitatively demonstrates differences in the spiral artery morphometry parameters using fluorescence microscope between normotensive and hypertensive pregnancies. Use of this quantifiable eosin fluorescence technique on routine H&E-stained sections on tissues may uncover more valuable findings and improved morphological details. We found significantly thicker spiral artery walls with greater wall areas and higher value of wall-area to lumen-area ratio in the PIH group which we attribute to defective trophoblast invasion of the maternal spiral arteries. We also found significantly smaller spiral artery lumens in the PIH implicatingretaining of the otherwise lost vascular smooth muscles in the arterial walls. This study concludes that fluorescence microscopy as an observational tool may help in the identification and quantification of structures in healthy and diseased conditions.

HUMAN AND ANIMAL RIGHTS

No Animals were used for this research study. Consent for placenta was not needed.

FUNDING

This study is funded by Ziauddin University, Karachi, Pakistan.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We are thankful to our patients who have contributed their placenta as a study subject in this research as without them it would not have been possible to conduct this study.

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