MATERNAL SERUM LEPTIN LEVELS IN SUBJECTS WITH GESTATIONAL DIABETES MELLITUS

Phan Thi To Nhu[1,2], Hoang Trung Vinh[2]*
Vu Ba Quyet[3], Pham The Tai[4]

Abstract

Objectives: To determine serum leptin levels and its correlation with pre-pregnancy BMI, BMI, and insulin resistance at 24 - 28 weeks of gestation and before delivery in subjects with gestational diabetes mellitus (GDM). ***Methods:*** A cross-sectional descriptive, longitudinal follow-up study with a control group on 115 pregnant women with GDM and 115 pregnant women with normal glucose tolerance (NGT) enrolled at 24 - 28 weeks of gestation and completed the study. The subjects' serum leptin levels were measured using the enzyme-linked immunosorbent assay (ELISA) method at 24 - 28 weeks of pregnancy and before delivery. The clinical characteristics, serum insulin levels, and homeostatic model assessment of insulin resistance (HOMA-IR) were also performed at both study time points. ***Results:*** Serum leptin levels in subjects with GDM were significantly higher than those in normal pregnant women at 24 - 28 weeks of gestation and before delivery ($p < 0.001$). At both study time points, there was a positive correlation between serum leptin levels and pre-pregnancy BMI, maternal weight, serum insulin levels, and HOMA-IR index in subjects with GDM ($p < 0.05$). Multivariable logistic regression showed that pre-pregnancy BMI and serum insulin levels were associated with hyperleptinemia ($p < 0.05$). ***Conclusion:*** Serum leptin levels were higher in GDM and had a positive correlation with pre-pregnancy BMI, BMI, and insulin resistance at the time of the study. Pre-pregnancy BMI and serum insulin levels were independent factors related to increased serum leptin levels.

Keywords: Gestational diabetes mellitus; Leptin; Insulin resistance; Adipokines.

[1]**Hanoi University of Pharmacy**

[2]**Military Hospital 103, Vietnam Military Medical University**

[3]**National Hospital of Obstetrics and Gynecology**

[4]**Institute of Biomedicine and Pharmacy, Vietnam Military Medical University**

[*]**Corresponding author: Phan Thi To Nhu (phantonhu.hup@gmail.com)**

Date received: 13/12/2023

Date accepted: 20/3/2024

http://doi.org/10.56535/jmpm.v49i4.584

INTRODUCTION

Pregnancy is a hyperglycemic period of life, with hyperglycemia serving a highly important role in the nutrition and development of the fetus by providing it with adequate glucose levels. GDM is a condition associated with maternal hyperglycemia to a lesser degree than overt diabetes but is associated with an increased risk of adverse obstetric outcomes [1]. Like all forms of hyperglycemia, GDM is characterized by insulin levels that are insufficient to meet insulin demands.

Adiposity is an important modifiable risk factor for the development of GDM, although mechanisms linking excess adiposity to elevated risk of GDM are not completely understood. Leptin is one of the adipocyte-derived hormones involved in energy homeostasis and plays an important role in insulin and glucose metabolism [2]. Most of the literature data have associated hyperleptinemia with the development and progression of GDM, while only a few studies have shown reduced or unchanged levels of this protein in affected women. Such varieties might be ascribed to the utilization of a one-step bio-sample together with immense contrasts within leptin estimation circumstances, which are liable to the active variations in leptin levels during pregnancy. However, very few studies described the longitudinal changes in leptin concentration related to GDM. A longitudinal monitoring of maternal leptin concentrations in relation to insulin resistance during pregnancy may help to gain a better understanding of the development of GDM. Therefore, we conducted this study: *To determine serum leptin levels and its correlation with pre-pregnancy BMI, BMI, and insulin resistance at 24 - 28 weeks of gestation and before delivery in GDM subjects.*

MATERIALS AND METHODS

1. Subjects

230 pregnant women with a single fetus, at 24 - 28 weeks gestation, examined at the Hanoi Obstetrics and Gynecology Hospital from 2019 to 2021, who have not been diagnosed with diabetes before.

** Inclusion criteria:*

- GDM group: Single pregnant women, gestational weeks 24 - 28, diagnosed with GDM according to the criteria of the American Diabetes Association 2011 guidelines.

- NGT group: Single pregnant women, gestational weeks 24 - 28 with NGT according to the criteria of the American Diabetes Association 2011 guidelines.

** Exclusion criteria:*

- GDM group: Multiple pregnancies, diagnosed with diabetes before 24 - 28 weeks of gestation, GDM requiring insulin treatment, currently suffering from endocrine disorders, currently using

drugs that affect glucose metabolism, currently suffering from infectious diseases acute or malignant disease.

- NGT group: Multiple pregnancies, diagnosed with diabetes before 24 - 28 weeks of gestation, history of GDM in a previous pregnancy, family history of diabetes, bad obstetric history (stillbirth, multiple miscarriages, giving birth to babies over 4000g), currently suffering from endocrine disorders, currently using drugs that affect glucose metabolism, currently suffering from infectious diseases acute or malignant disease.

2. Methods

* *Study design:* A cross-sectional descriptive, longitudinal follow-up study with a control group.

* *Sample size and sample selection:* This study is part of a project investigating the levels of some adipocytokines in subjects with GDM. According to the results of the previous study [3], the estimated number of subjects needed for each group was 113. This sample size still ensures representativeness and reliability when studying leptin levels. In fact, we selected 115 pregnant women for each group.

* *Research tools and information-gathering techniques:*

- Eligible participants were studied longitudinally during the 24 - 28 weeks and prenatal period (≥ 37 weeks, 1 week prepartum) of pregnancy. Each participant carried out a 75g oral glucose tolerance test under a fasting state (> 8h) at 24 - 28 weeks. Pregnant women with normal glucose tolerance were assigned to the NGT group. Participants who were diagnosed with GDM received individual nutritional counseling with instructions on the appropriate restriction of energy intake, daily moderate exercise, and self-monitoring of blood glucose for 2 weeks. If blood glucose cannot be controlled, pregnant women will be hospitalized for insulin treatment and will be removed from the study.

- Demographic information from each participant, including maternal age, parity, gestational weeks, personal, and familial medical history, pre-pregnancy weight (before 1 month), was collected at 24 - 28 weeks gestation.

- During the observation period, blood pressure, heart rate, height, and body weight were measured at 24 - 28 weeks gestation and before delivery.

- Laboratory methods: At 24 - 28 weeks of gestation and before delivery, maternal venous blood samples of 5mL were collected after fasting at least 8h. Samples were processed within one hour of collection. The serum was separated by centrifugation at 4000 rpm for 15 minutes after clotting, then was separated into appropriate tubes and stored at -80°C until leptin and insulin were quantified.

+ Fasting plasma glucose, glucose after 1 hour, and 2 hours after drinking glucose were measured immediately after venous blood collection using colorimetric enzymatic method with reagents and standards from Roche.

+ Serum leptin levels were determined by the ELISA method, using the Human Leptin ELISA Kit (Austria).

+ Serum insulin levels were determined by solid-phase chemiluminescence immunoassay.

- Serum insulin and serum leptin were measured at the Institute of Biomedicine and Pharmacy, Vietnam Military Medical University.

- Standards and assessments used in research:

+ Diagnostic criteria for GDM according to the criteria of American Diabetes Association 2011 guidelines (fasting plasma glucose (FPG): 5.1 - 6.9 mmol/L, 1-hour glucose ≥ 10.0 mmol/L, or 2-hour glucose ≥ 8.5 mmol/L, if any of the three criteria met or exceeded), using a 75-g OGTT [1].

+ BMI pre-pregnancy and at each time of the study is calculated according to the formula: Weight (kg)/(height)^2 (m). It is called overweight/obese when BMI ≥ 23 kg/m^2.

+ Increased leptin is identified when the leptin level is greater than the 75% quartile of the NGT group in this study.

+ Hyperinsulinemia is identified when the insulin level is greater than the 75% quartile of the NGT group in this study.

+ Insulin resistance was calculated using the homeostasis model assessment of insulin resistance index (HOMA-IR), using the formula [fasting glucose (mmol/L) x fasting insulin (µU/mL)]/ 22.5. It is called insulin resistance when HOMA-IR is greater than the 75% quartile of the NGT group in this study.

* *Statistical analysis:* Results are expressed as mean and standard deviation or median, and interquartile range. The data was compiled and analyzed by SPSS 27.0 using appropriate statistical methods. For comparisons of means, Student's T-test was used to determine the significance between GDM and controls. For the assessment of the correlation between variables, Pearson's correlation was used; $p < 0.05$ were considered statistically significant.

3. Ethics

The study has been approved by Hanoi Obstetrics and Gynecology Hospital for implementation. All research subjects were specifically explained about the purpose and content of the study and agreed to voluntarily participate in the study. All research subject information is kept confidential and used for research purposes only.

RESULTS

230 pregnant women were selected for the study, including 115 pregnant women with GDM and 115 pregnant women with NGT.

Table 1. General characteristics of research subjects.

Parameter	GDM group (n = 115)	NGT group (n = 115)	p
Maternal age (year) ($\overline{X}$ ± SD)	31.48 ± 5.09	29.37 ± 4.94	0.002
Pre-pregnancy BMI (kg/m^2) ($\overline{X}$ ± SD)	21.24 ± 2.68	20.59 ± 2.21	0.045
24 - 28 weeks of gestation			
Maternal weight (kg) ($\overline{X}$ ± SD)	58.8 ± 8.3	56.5 ± 5.8	0.016
Fasting plasma glucose (mmol/L)	5.01 ± 0.41	4.68 ± 0.25	< 0.001
Glucose after 1h of OGTT (mmol/L)	9.99 ± 1.52	7.48 ± 1.25	< 0.001
Glucose after 2h of OGTT (mmol/L)	8.91 ± 1.55	6.84 ± 0.96	< 0.001
Insulin (µU/mL) (Median) (Quartile)	14.08 (8.4)	10.79(6.2)	< 0.001
HOMA-IR (Median) (Quartile)	3.08 (1.82)	2.23 (1.32)	< 0.001
Leptin (ng/mL) (Median) (Quartile)	9.03 (9.25)	4.27 (3.41)	< 0.001
Before delivery			
Maternal weight (kg) ($\overline{X}$ ± SD)	65.4 ± 8.5	62.9 ± 5.9	0.01
Fasting plasma glucose (mmol/L)	4.88 ± 0.76	4.63 ± 0.56	0.005
Insulin (µU/mL) (Median) (Quartile)	18.36 (8.68)	15.05 (6.44)	< 0.001
HOMA-IR (Median) (Quartile)	3.93 (2.27)	3.02 (1.36)	< 0.001
Leptin (ng/mL) (Median) (Quartile)	10.55 (13.17)	6.13 (4.76)	< 0.001

Pregnant women in the GDM group had significantly higher maternal age, pre-pregnancy BMI than the NGT group, $p < 0.05$.

The GDM group had higher blood glucose levels, serum leptin levels, insulin levels, and HOMA-IR index than the NGT group ($p < 0.05$) at 24 - 28 weeks of gestation and before delivery.

Table 2. The proportion of patients with increased leptin levels of study subjects.

Characteristic	GDM group (n = 115)		NGT group (n = 115)		p
	n	%	n	%	
24 - 28 weeks of gestation					
Increased serum leptin	84	73.0	28	24.3	< 0.001
Before delivery					
Increased serum leptin	76	66.1	28	24.3	< 0.001

The GDM group had a higher rate of patients with increased leptin than the NGT group ($p < 0.001$) at 24 - 28 weeks of gestation and before delivery.

Table 3. Serum leptin levels in the GDM group according to assessment indicators.

Evaluation index		Serum leptin levels (ng/mL)	p
24 - 28 weeks of gestation			
Pre-pregnancy BMI (kg/m^2)	≥ 23 (n = 42)	17.46 (13.38 - 20.5)	< 0.001
	< 23 (n = 73)	7.22 (4.92 - 9.58)	
Increased serum insulin	Yes (n = 55)	15.06 (9.11 - 19.43)	< 0.001
	No (n = 60)	6.99 (4.34 - 9.23)	
Insulin resistance	Yes (n = 62)	13.82 (8.38 - 18.99)	< 0.001
	No (n = 53)	6.67 (3.99 - 9.31)	
Before delivery			
Pre-pregnancy BMI (kg/m^2)	≥ 23 (n = 42)	23.22 (17.57 - 26.26)	< 0.001
	< 23 (n = 73)	9.03 (6.99 - 12.12)	
Increased serum insulin	Yes (n = 55)	17.98 (9.94 - 24.62)	< 0.001
	No (n = 60)	8.19 (6.53 - 11.35)	
Insulin resistance	Yes (n = 62)	16.72 (9.34 - 24.22)	< 0.001
	No (n = 53)	7.98 (5.97 - 11.94)	

Serum leptin levels increased significantly in GDM subjects with pre-pregnancy overweight/obesity, increased serum insulin and insulin resistance ($p < 0.001$), at 24 - 28 weeks of gestation and before delivery.

Table 4. The correlation between increased serum leptin and some indicators in GDM group.

Evaluation index		Increased serum leptin (n, %)	p
24 - 28 weeks of gestation			
Pre-pregnancy BMI (kg/m^2)	≥ 23 (n = 42)	40 (95.2)	< 0.001
	< 23 (n = 73)	44 (60.3)	OR = 13.18
Increased serum insulin	Yes (n = 55)	50 (90.9)	< 0.001
	No (n = 60)	34 (56.7)	OR = 7.65
Insulin resistance	Yes (n = 62)	55 (88.7)	< 0.001
	No (n = 53)	29 (54.7)	OR = 6.5
Before delivery			
Pre-pregnancy BMI (kg/m^2)	≥ 23 (n = 42)	41 (97.6)	< 0.001
	< 23 (n = 73)	35 (47.9)	OR = 44.51
Increased serum insulin	Yes (n = 61)	52 (85.2)	< 0.001
	No (n = 54)	24 (44.4)	OR = 7.22
Insulin resistance	Yes (n = 70)	57 (81.4)	< 0.001
	No (n = 45)	19 (42.2)	OR = 6.0

In the GDM group, there was overweight and obesity before pregnancy, increased serum insulin levels and insulin resistance. The rate of increase in serum leptin levels was higher than in the normal weight group; there were no increase serum insulin levels and no insulin resistance statistically significant ($p < 0.05$).

Table 5. The correlation between leptin levels and some indicators in the GDM group.

Evaluation index	Leptin levels at 24 - 28 weeks		Leptin levels before delivery	
	r	p	r	p
Pre-pregnancy BMI	0.735	< 0.001	0.747	< 0.001
BMI at the time of the study	0.723	< 0.001	0.71	< 0.001
Maternal weight at the time of the study	0.653	< 0.001	0.643	< 0.001
FPG at the time of the study	0.123	0.189	- 0.02	0.835
Insulin levels at the time of the study	0.763	< 0.001	0.734	< 0.001
HOMA-IR at the time of the study	0.774	< 0.001	0.695	< 0.001

At gestational weeks 24 - 28 and before delivery: There were a significant, fairly close positive correlation between serum leptin levels and BMI, maternal weight, serum insulin levels and HOMA-IR index in patients with GDM, $p < 0.001$.

There were no significant correlation between serum leptin levels and fasting plasma glucose levels at the time of the study ($p > 0.05$).

Table 6. The correlation between increased serum leptin and some indicators in GDM group.

Evaluation index	Increased leptin at 24 - 28 weeks		Increased leptin before delivery	
	OR	p	OR	p
Pre-pregnancy BMI	1.75	0.006	1.63	0.007
Maternal weight gain	1.09	0.541	0.95	0.648
Fasting plasma glucose	4.81	0.31	1.33	0.42
Insulin levels at the time of the study	2.85	0.039	1.23	0.047
Insulin resistance at the time of the study	0.43	0.369	0.42	0.304

Among the related factors, pre-pregnancy BMI and serum insulin levels were independent factors related to increased serum leptin levels, $p < 0.05$.

DISCUSSION

Leptin is a protein secreted from adipocytes, which is believed to play a role in the pathogenesis of GDM [2]. Except for adipocytes, leptin can also be produced by non-adipose tissues such as the stomach, intestine, and, in particular, the placenta in humans. As pregnancy progresses, because of the increased fat mass and the presence of placenta, maternal leptin levels increase 2 to 3-fold above non-pregnant concentration, with the peak occurring around 28 weeks of gestation [2]. However, GDM induces more weight gain as it creates chronic hypoxia in the placental environment, leading to compensatory hyper-perfusion and thereby increasing the placental weight more as compared to normal pregnancy [4]. As a result, the serum leptin levels of women with GDM were higher than that of healthy pregnant women. This is in concordance with our findings in this study. Our study shows that pregnant women with GDM have higher serum leptin levels (*Table 1*) and a significantly higher rate of serum leptin increase (*Table 2*) compared to normal pregnant women, in both study times ($p < 0.001$). These results are generally consistent with studies that assessed maternal serum leptin levels in pregnancies complicated by GDM. In a case-control study, Fatima et al. [5] reported that maternal serum leptin levels were higher in GDM women compared with the control group (20.38 ± 12.7 ng/mL vs. 3.41 ± 2.17 ng/mL, $p < 0.001$). Andleeb et al. [6] and Yang Mei et al. [7] also showed similar results of elevated values of leptin in GDM at 24 - 28 weeks of gestation. Florian et al. [8] found significantly increased leptin levels in pregnant women with GDM and a higher probability of developing GDM in women with serum leptin concentrations > 16 ng/mL at 11 - 13 weeks of gestation ($p < 0.001$). However, in contrast to the findings reported above, Mosavat et al. [9] reported a decrease in leptin levels and soluble leptin receptor in 53 GDM women compared with 43 women with NGT. These inconsistencies among the results might arise from the different sample sizes of the population and timing of maternal blood collection: Many previous studies were conducted in non-pregnant women, but the regulation of leptin might be very different during pregnancy.

The classic perception of adipose tissue merely as a lipid storage site has changed significantly over the past decade. Now, adipose tissue can act as an endocrine organ that regulates systemic metabolism. Leptin is produced mainly in mature cells of white adipose

tissue. Leptin biosynthesis and secretion depend on white adipose tissue mass and adipocyte size. In obese people, there is an increase in adipose tissue mass and fat cell size. Hypertrophied and excess adipose tissue tends to enhance leptin release. Therefore, leptin levels is closely correlated with the body's BMI; the higher the body fat mass, the higher the leptin levels. In pregnant women, increased body weight and accumulation of visceral fat can increase adipose tissue mass, thereby stimulating leptin production. As a result, serum leptin levels increased in overweight and obese pregnant women. This is consistent with our research results. In this study, women with GDM had significantly higher pre-pregnancy BMI than the NGT group ($p < 0.001$) (*Table 1*). We found strong positive correlations between serum leptin levels and pre-pregnancy BMI, BMI, and weight at each study time point in the mother ($p < 0.001$) (*Table 5*). Overweight/obese women with GDM had higher leptin levels (*Table 3*) and higher rates of leptin elevation (*Table 4*) compared with non-overweight/obese pregnant women. This suggests that obesity is a major factor in insulin resistance and increased leptin levels. Our results are supported by Yang Mei et al. [7], who studied 357 GDM at 24 - 30 weeks of gestation and found a positive correlation between leptin levels and maternal pre-pregnancy BMI ($r = 0.45$, $p < 0.001$). Our results are supported by Yang Mei et al. [7], who studied 357 GDM at 24 - 30 weeks gestation and found a positive correlation between leptin levels and maternal pre-pregnancy BMI ($r = 0.45$, $p < 0.001$).

GDM is associated with reduced responsiveness of maternal body tissues to insulin. Therefore, increased serum insulin levels and high HOMA-IR values are expected in GDM patients. The same thing was also described in our study when serum insulin levels and HOMA-IR index in the GDM group were significantly higher than the NGT group ($p < 0.001$) (*Table 1*). As mentioned in previous studies, leptin inhibits insulin secretion from pancreatic beta cells in obese or pregnant people. Chronic increases in leptin levels cause the leptin receptor system on the pancreatic beta cell membrane to become less sensitive to leptin. Therefore, insulin synthesis increased. This is a condition of dysregulation of the endocrine-pancreas adipose tissue axis. If this axis is deregulated, more insulin is secreted. Increased insulin levels have a positive feedback effect on adipose tissue, stimulating fat formation. Increasing fat mass causes more leptin to be

secreted - a vicious cycle. More central obesity is accompanied by a gradual increase in glucose levels and increased insulin response to oral glucose intake. Central obesity increases posthepatic insulin, leading to increased peripheral insulin levels, ultimately causing insulin resistance. According to Fatima et al. [5], leptin levels were positively correlated with serum insulin levels ($r = 0.619$, $p < 0.001$) and HOMA-IR ($r = 0.653$, $p < 0.001$) in GDM women at 24 - 28 weeks of gestation. Yang Mei et al. [7] also noted a positive correlation between serum leptin levels with serum insulin and HOMA-IR. Our study obtained similar results to the above authors. We found that serum leptin levels had a strong positive correlation with serum insulin levels and insulin resistance in GDM patients (*Table 5*). Pregnant women with GDM who have increased serum insulin and insulin resistance have higher serum leptin levels than pregnant women without insulin resistance (*Table 3*).

There are several independent factors associated with increased serum leptin, including pre-pregnancy BMI and serum insulin levels (*Table 4, 6*). As is known, white adipose tissue is the main place to synthesize and regulate leptin secretion. Obese people often have increased BMI, so it often causes increased serum leptin. On the other hand, obesity is a disorder that facilitates the development of chronic inflammation. Excess adipose tissue and hypertrophied adipocytes lead to increased proinflammatory cytokines. The resulting chronic inflammation promotes insulin resistance and increases serum insulin. High blood insulin will increase the expression of leptin synthesis genes and increase leptin levels in the blood. Researchers have documented insulin as an important regulatory factor in the synthesis and secretion of leptin from white adipose tissue.

CONCLUSION

Serum leptin levels were higher in patients with GDM and were positively correlated with pre-pregnancy BMI, BMI, and insulin resistance at 24 - 28 weeks of gestation and before delivery. Pre-pregnancy BMI and serum insulin levels were independent factors related to increased serum leptin levels at 24 - 28 weeks of gestation and before delivery.

Limitations of the study: Small sample size; the study quantified leptin levels at 24 - 28 weeks, coinciding with the time of diagnosis of GDM, so the predictive ability of leptin could not be evaluated. Research needs to be conducted with a larger sample size, at many other times during pregnancy, such as 11 - 13 weeks, postpartum

period to clarify the role of leptin in the pathophysiology of GDM.

Acknowledgments: We would like to thank Hanoi Obstetrics Hospital for coordinating to facilitate the implementation of the study and all patients for agreeing to participate in the study. We declare to have no conflicts of interest in this research.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care.* 2011; 34(1):S11-S61.

2. Mallardo M, Ferraro S, Daniele A, Nigro E. GDM-complicated pregnancies: Focus on adipokines. *Molecular Biology Reports.* 2021; 48:8171-8180.

3. Phan Thị Tố Như, Hoàng Trung Vinh, Vũ Bá Quyết, Phạm Thế Tài. Nồng độ adiponectin huyết thanh ở đối tượng đái tháo đường thai kỳ tuần thai 24 - 28 và trước khi sinh. *Tạp chí Y Dược học Quân sự.* 2024; 49(2):205-216.

4. Goel R, Duhan N, Kumar HD. Maternal serum leptin concentration in gestational diabetes mellitus. *Imp J Interdisp Res*. 2016; 2(2):414-422.

5. Fatima S, Alam F, Chaudhry B, Khan TA. Elevated levels of chemerin, leptin, and interleukin-18 in gestational diabetes mellitus. *The Journal of Maternal-Fetal & Neonatal Medicine.* 2017; 30(9):1023-1028.

6. Andleeb J, Mehboob F, Javaid S, Ali M, Khan MI, Ain QU. Effect of Gestational Diabetes on Serum Leptin during Pregnancy and Postpartum Period: Effect of GDM on S. Leptin. *The International Journal of Frontier Sciences*. 2020; 5(1):1-5.

7. Yang M, Peng S, Li W, Wan Z, Fan L, Du Y. Relationships between plasma leptin levels, leptin G2548A, leptin receptor Gln223Arg polymorphisms and gestational diabetes mellitus in Chinese population. *Scientific reports.* 2016; 6(1):23948.

8. Florian AR, Cruciat G, Pop RM. Predictive role of altered leptin, adiponectin and 3-carboxy- 4-methyl-5-propyl-2-furanpropanoic acid secretion in gestational diabetes mellitus. *Experimental and Therapeutic Medicine*. 2021; 21(5):1-7.

9. Mosavat M, Omar SZ, Tan PC, Razif MFM, Sthaneshwar P. Leptin and soluble leptin receptor in association with gestational diabetes: A prospective case-control study. *Archives of Gynecology and Obstetrics.* 2018; 297:797-803.