Clinical and Experimental Obstetrics & Gynecology

IMR Press

Original Research

A dilemma for women: having many children risks deterioration of diastolic functions

Mehmet Ozgeyik[1,*], Ozge Turgay Yildirim[1], Mevlut Serdar Kuyumcu[2], Mehmet Ali Astarcioglu[3]

[1] Department of Cardiology, Eskisehir City Hospital, 26100 Eskisehir, Turkey

[2] Department of Cardiology, Faculty of Medicine, Suleyman Demirel University, 32000 Isparta, Turkey

[3] Department of Cardiology, Faculty of Medicine, Kutahya Dumlupinar University, 43000 Kutahya, Turkey

*Correspondence: mehmetozgeyik@hotmail.com (Mehmet Ozgeyik)

DOI:10.31083/j.ceog.2021.03.2348

Submitted: 4 November 2020 Revised: 10 December 2020 Accepted: 11 December 2020 Published: 15 June 2021

Background: Echocardiography is the most widely used diagnostic tool for detecting changes in cardiac function. Pregnancy is a dynamic process that affects the cardiovascular system and recent studies have shown that increased parity may cause irreversible changes in the cardiovascular system. In this study, we aimed to evaluate echocardiographic changes in women, especially grand multiparous (6 to 9 parities) and great grand multiparous (more than 9 parities) women, after all their pregnancies had finished. *Methods*: This was a cross-sectional study and contained 195 female patients. Women with one delivery were defined as primiparous (PP), 2 to 5 deliveries were defined as multiparous (MP), 6 to 9 deliveries were defined as grand multiparous (GMP) and more than 9 deliveries were defined as great grand multiparous (GGMP). *Results*: The mean age at cardiac evaluation was 50.6 $\pm$ 16.3 and mean parity was 6.5 $\pm$ 4.2. Diastolic dysfunction was grouped as grade 1–3 and this was determined according to the E/e' ratio. Spearman correlation analysis showed that diastolic dysfunction had positive correlations with parity, age, hypertension, and diabetes mellitus. Receiver-operating curve (ROC) analysis showed that the best cut-off value of the parity number for predicting left ventricular diastolic dysfunction was 6.5, with 66.3% sensitivity and 66.7% specificity. *Discussion*: In the present study, we showed that diastolic dysfunction significantly increased as the number of pregnancies increased. Additionally, the cut-off value of parity for diastolic dysfunction was 6.5 which is higher than other studies.

Keywords

Pregnancy; Echocardiography; Left ventricular dysfunction

1. Introduction

Echocardiography is the most widely used diagnostic tool for detecting changes in cardiac function [1] and innovations in assessment of cardiac ventricular functions are ongoing [2]. The systolic and diastolic functions of the heart can be affected by many variables [3, 4] and systemic chronic diseases (diabetes mellitus, hypertension, hyperlipidemia) in particular negatively affect these functions. However, in healthy populations, some conditions such as pregnancy may also change cardiovascular mechanisms.

Pregnancy is a dynamic process that affects the cardiovascular system. During pregnancy maternal cardiac output, preload and maternal blood volume increase and systemic vascular resistance decreases [5]. These changes are necessary for the continuation of pregnancy and the health of the fetus. Most of the changes that occur during pregnancy return to normal after pregnancy [6].

Recent studies have shown that increased parity may cause irreversible changes in the cardiovascular system [7, 8]. Left ventricular diastolic functions deteriorate during pregnancy and this is associated with increased cardiovascular mortality [9–11]. Diastolic function and other cardiovascular changes tend to return to normal postpartum; however as parity increases, diastolic parameters are affected and these reversible changes may become permanent [6, 12].

In this study, we aimed to evaluate echocardiographic changes in women, especially grand multiparous (6 to 9 parities) and great grand multiparous (more than 9 parities) women, after all their pregnancies had finished.

2. Patients and methods

This was a cross-sectional study and contained 195 female patients. Exclusion criteria were patients under 18 years-of-age, a history of coronary artery disease, heart failure, structural heart diseases, rhythm disorders, renal or hepatic disorders and women who were currently pregnant. Inclusion criteria were patients with a history of one or more deliveries and completion of their pregnancy with a living birth. Also, patients with a history of hypertension and diabetes mellitus that may affect left ventricular diastolic functions were included in the study. For all patients, the time since the last birth was at least one year. A written consent form was signed by all the participants. The study was designed in accordance with the Helsinki Declaration's ethical standards.

Women with a history of one delivery were defined as primiparous (PP), 2 to 5 deliveries were defined as multiparous (MP), 6 to 9 deliveries were defined as grand multiparous (GMP) and more than 9 deliveries were defined as great grand multiparous (GGMP).

Table 1. Characteristics of the study population.

	Primiparous 1 delivery n = 16	Multiparous 2 to 5 deliveries n = 73	Grand multiparous 6 to 9 deliveries n = 46	Great grand multiparous >9 deliveries n = 60	*P*
Age, years (± SD)	30.8 ± 9.0	39.4 ± 8.8	52.8 ± 11.9	67.7 ± 9.5	<0.0001
Parity number, n	1.0 ± 0.0	2.9 ± 1.0	7.5 ± 1.2	11.7 ± 1.8	<0.0001
Diabetes mellitus, n (%)	2, 12.5%	3, 4.1%	8, 17.4%	19, 31.7%	<0.0001
Hypertension, n (%)	4, 25.0%	19, 26.0%	32, 69.6%	55, 91.6%	<0.0001
Systolic BP, mm Hg	105.6 ± 15.9	115.6 ± 19.4	132.4 ± 22.0	137.5 ± 23.1	<0.0001
Diastolic BP, mm Hg	67.1 ± 9.6	75.5 ± 12.0	83.0 ± 11.3	83.2 ± 12.9	<0.0001

Abb. BP, blood pressure; SD, standard deviation.

Table 2. Echocardiographic parameters of the study groups.

Variables	PP	MP	GMP	GGMP	*P*
E, cm/s	87.7 ± 15.4	89.1 ± 26.2	102.4 ± 19.8	90.7 ± 25.3	**0.017**
A, cm/s	66.6 ± 11.8	78.2 ± 20.4	101.6 ± 21.4	101.6 ± 20.1	**<0.0001**
Lateral e', cm/sn	14.0 ± 3.3	12.7 ± 3.4	10.6 ± 3.0	8.6 ± 2.7	**<0.0001**
Lateral s', cm/sn	10.1 ± 0.9	10.3 ± 2.0	9.7 ± 2.1	9.3 ± 2.4	**0.027**
Septal e', cm/sn	10.5 ± 2.3	9.5 ± 2.4	7.4 ± 2.1	6.2 ± 1.7	**<0.0001**
Septal s', cm/sn	8.1 ± 1.1	8.2 ± 1.5	8.1 ± 2.0	7.0 ± 1.8	**<0.0001**
EDD, mm	44.7 ± 2.4	44.4 ± 3.5	44.8 ± 4.4	44.5 ± 6.0	0.964
ESD, mm	28.7 ± 2.3	27.4 ± 3.8	27.1 ± 4.9	27.2 ± 6.5	0.733
EF, %	62.3 ± 3.1	62.4 ± 3.0	58.9 ± 5.4	56.2 ± 6.7	**<0.0001**

The bold *P* values are statistically significant.

Echocardiographic (Vivid 7 system with 3S echocardiography probe, GE Vingmed Ultrasound AS, Horten, Norway) evaluation was done by trained cardiology specialists for pateints that referred to cardiology clinic with cardiac complaints. The evaluated parameters were peak early filling velocity before atrial systole (E), peak filling velocity during atrial systole (A), left ventricular ejection fraction (LVEF), left ventricular end-systolic diameter, left ventricular end-diastolic diameter, lateral e' velocity, lateral s' velocity, septal e' velocity, septal s' velocity, tricuspid S velocity. E/A was calculated as the ratio of E to A. E/e' ratio was calculated as the ratio of E velocity to mean e' (as average of lateral e' wave and septal e' wave).

Septal e' ≥ 8 cm/sec, lateral e' ≥ 10 cm/sec were designated as normal diastolic function. Diastolic dysfunction was determined as septal e' < 8 cm/sec, lateral e ' < 10 cm/sec. Stage 1 diastolic dysfunction was defined as the mitral E and A wave velocity ratio (E/A) < 0.8 and the ratio of E to the mean early diastolic mitral annular velocity (E/e') ≤ 8. Stage 2 diastolic dysfunction was defined as the E/A between 0.8–1.5 and the E/e' ratio between 9 and 12. Stage 3 diastolic dysfunction was defined as the E/A ratio being ≥ 2 and the E/mean e' ratio ≥ 13. All these parameters were obtained from the American Society of Echocardiography and European Association of Cardiovascular Imaging (ASE/EACVI) guidelines recommendations [13].

Hypertension was defined as systolic pressure greater than 140 mm Hg or diastolic pressure greater than 90 mm Hg or a history of hypertension with the use of antihypertensive medication [14]. Diabetes mellitus was defined as a fasting blood glucose level of 126 mg/dL, a random glucose measurement of 200 mg/dL, hemoglobin A1c > 6.5%, or a previous diagnosis with any use of anti-diabetic medication [15].

Data were presented as mean ± standard deviation (SD) for continuous variables and as numbers and proportions for categorical variables. Distribution of the data for normality was tested by the Shapiro-Wilk test and homogeneity of group variances were tested by the Levene test. The *t*-test or Chi-square test was used for comparisons of continuous and categorical variables, respectively. For the parameters which are not normally distributed, the Mann Whitney U test was used. More than two independent groups with normal distribution were compared with the ANOVA test. Binary logistic regression analysis was used to identify the associations of diastolic dysfunction presence to other variables. Multinomial regression analysis was used to evaluate the associations of diastolic dysfunction grades to other variables. The data analyses were performed with SPSS 23.0 (IBM SPSS Ver. 23.0, IBM Corp, Armonk, NY, USA). A *P*-value of <0.05 was considered significant.

3. Results

The study population consisted of 195 women with a history of at least one delivery. PM women constituted 8.2% (n = 16), MP women constituted 37.4% (n = 73), GMP women constituted 23.6% (n = 46) and GGMP women constituted 30.8% (n = 60) of the study population. The mean age at cardiac evaluation was 50.6 ± 16.3 and mean parity was 6.5 ± 4.2. Mean height and weight were 159 ± 5.3 centimeter and 64 ± 7.1 kilogram, respectively. Body mass index (BMI) was

Table 3. Binary comparison of parity groups according to parameters.

	PP vs MP	PP vs GMP	PP vs GGMP	MP vs GMP	MP vs GGMP	GMP vs GGMP
E	0.844	0.009	0.779	**0.004**	0.714	0.011
A	0.025	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.987**
Lateral e'	0.147	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**< 0.0001**
Lateral s'	0.623	0.442	**0.026**	0.084	**0.005**	0.350
Septal e'	0.138	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**
Septal s'	0.830	0.907	**0.018**	0.645	**< 0.0001**	**0.003**
EF	0.892	**0.019**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.027**

The bold *P* values are statistically significant.

Table 4. Diastolic function classification among the study population.

	PP (n = 16)	MP (n = 73)	GMP (n = 46)	GGMP (n = 60)	*P*
Normal diastolic function	14 (87.5%)	52 (71.2%)	26 (56.5%)	17 (28.3%)	< 0.0001
DD Grade 1	1 (6.25%)	9 (12.3%)	5 (10.9%)	20 (33.3%)	< 0.0001
DD Grade 2	1 (6.25%)	12 (16.4%)	15 (32.6%)	23 (38.3%)	< 0.0001

Table 5. Spearman correlation analysis between the presence of diastolic dysfunction and parity number, age, hypertension and diabetes mellitus.

	Parity number	Age	Hypertension	Diabetes mellitus
r	0.404	0.614	0.448	0.331
P	**0.000**	**0.000**	**0.000**	**0.000**

The bold *P* values are statistically significant.

Table 6. Binary logistic regression analysis for the presence of diastolic dysfunction.

	Odds ratio	95% CI	*P*
Parity number	0.805	0.692–0.938	**0.005**
Age	1.166	1.103–1.234	**0.000**
Hypertension	0.968	0.359–2.611	0.949
Diabetes mellitus	0.520	0.176–1.536	0.237

The bold *P* values are statistically significant.

25.3 ± 2.5. BMI, height and weight parameters were not statistically significant different among groups. The characteristics of the study population were given in Table 1.

The E velocity ($P = 0.017$), A velocity ($P < 0.0001$), lateral e' velocity ($P < 0.0001$), lateral s' ($P = 0.027$), septal e' ($P < 0.0001$), septal s' ($P < 0.0001$), and EF ($P < 0.0001$), values were significantly different among all parity groups. The results were shown in Table 2. Binary comparison of the study groups evaluating the echocardiographic parameters can be seen in Table 3.

Diastolic dysfunction classification was done according to the echocardiographic parameters. For the PM group, 87.5% (n = 14) had normal diastolic function, 6.25% (n = 1) had grade 1 diastolic dysfunction and 6.25% (n = 1) had grade 2 diastolic dysfunction. For the MP women, 71.2% (n = 52) had normal diastolic function, 12.4% (n = 9) had grade 1 diastolic dysfunction and 16.4% (n = 12) had grade 2 diastolic dysfunction. For the GMP women, 56.5% (n = 26) had normal diastolic function, 10.9% (n = 5) had grade 1 diastolic dysfunction and 32.6% (n = 15) had grade 2 diastolic dysfunction. For the GGMP women, 28.6% (n = 17) had normal diastolic function, 33.2% (n = 20) had grade 1 diastolic dysfunction and 38.2% (n = 23) had grade 2 diastolic dysfunction (Table 4). There were no women with grade 3 diastolic dysfunction among the study population.

Spearman correlation analysis showed that diastolic dysfunction has significant positive correlations with parity, age, hypertension, and diabetes mellitus (Table 5).

Table 6 and Table 7 report the findings of the binary and multinomial logistic regressions. Explanatory variables in both models were age, parity number, hypertension and diabetes mellitus. The differences among the models stems from how the dependent variable is handled. In the binary logistic regression, dependent variables are grouped into two categories: the existence of diastolic dysfunction or normal diastolic function. On the other hand, multinomial logistic regression in this study separates the patients into three groups: patients without diastolic dysfunction, patients with grade 1 and with grade 2 diastolic dysfunction. Both models show that only parity number and age are statistically significant.

ROC analysis showed that the best cut-off value of the parity number for predicting left ventricular diastolic dysfunction was 6.5, with 66.3% sensitivity and 66.7% specificity (Fig. 1).

4. Discussion

In the present study, we have shown that diastolic dysfunction significantly increased as the number of pregnancies increased. Additionally, the cut-off value of parity for diastolic dysfunction was 6.5, which is higher than other studies [6, 12].

Previous studies have shown that cardiovascular mortality increases along with increased parity [7]. Changes in the renin-angiotensin-aldosterone (RAAS) system explain this mechanism [16]. Estrogen secreted by the placenta increases the release of angiotensinogen. Angiotensinogen produces

Table 7. Multinomial logistic regression analysis for diastolic dysfunction grade.

Diastolic dysfunction grades		Odds ratio	95%	*P*
Grade 1	Parity number	0.760	0.625–0.925	**0.006**
	Age	1.198	1.118–1.283	**0.000**
	Hypertension	0.885	0.217–3.614	0.865
	Diabetes mellitus	0.525	0.152–1.815	0.309
Grade 2	Parity number	0.829	0.705–0.976	**0.024**
	Age	1.150	1.084–1.220	**0.000**
	Hypertension	1.006	0.334–3.032	0.991
	Diabetes mellitus	0.516	0.164–1.621	0.257

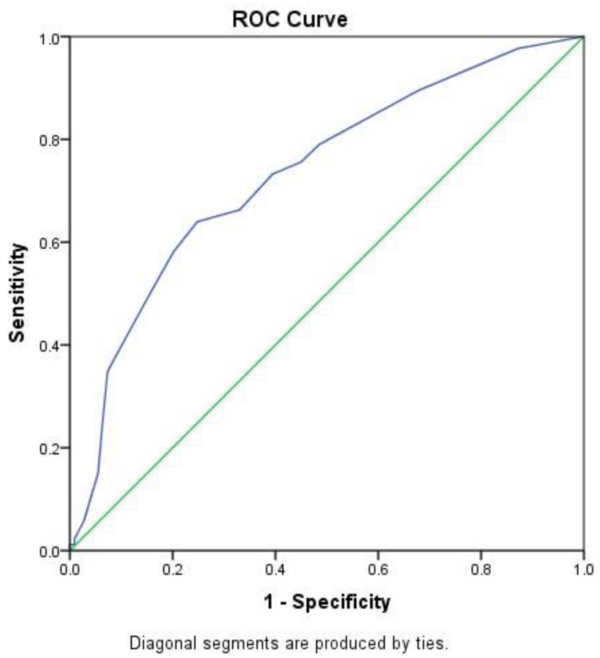

Fig. 1. Receiver-operating curve (ROC) analysis. ROC analysis revealed that the best cut-off value of the parity number for predicting left ventricular diastolic dysfunction was 6.5, with 66.3% sensitivity and 66.7% specificity (Area Under the Curve: 0.734; 95% CI 0.663 to 0.805; $P < 0.000$).

angiotensin-2 that activates the RAAS system. The RAAS system induces sodium and water retention. As a result, increased afterload is observed during pregnancy. Also, decreased relaxin levels affect cardiovascular mortality during pregnancy [17]. However, these changes continue only during pregnancy and their effects after pregnancy are still not clear. In our study, we hypothesized that repeated pregnancy exposed the cardiovascular system to the above-mentioned mechanisms for longer periods of time. Therefore, even if hormonal levels return to normal after pregnancy, changes in the cardiovascular system can become permanent.

We also found that diastolic function deteriorated as parity increased. Aggarwal *et al.*, performed the first published study on this issue and they found the same results [18]. Other studies similarly showed that diastolic dysfunction increases with parity [6, 12]. However, these studies examined up to 7 pregnancies (grand multiparity). In our study, women that have a history of 9 and more pregnancies (great grand multiparity) were also included. The present study has the highest range of parity numbers in the literature.

There is a lack of evidence about the relationship between parity and the severity of diastolic dysfunction. Kim *et al.* found that a parity number of 2.5 and above significantly increased diastolic dysfunction [6]. A study performed by Keskin *et al.* showed that a parity number of 4 and above significantly increased diastolic dysfunction [12]. In our ROC curve analysis, the cut-off value for diastolic dysfunction severity was 6.5 pregnancies, higher than in previous studies.

Aortic stiffness is a prognostic risk factor for cardiovascular mortality. In the present study, binary logistic regression analysis showed that the presence of hypertension and diabetes mellitus did not make a significant difference in terms of diastolic dysfunction; however, the number of pregnancies and age did make a significant difference. This can be attributed to increasing aortic stiffness. In our study, we showed the same results as previous studies in the literature [19–22].

In the present study, a significant decrease in ejection fraction was observed as the number of pregnancies increased. However, this decrease did not reach the systolic dysfunction range (less than 50%). Although Kim *et al.* found the same results as ours, other studies have not shown this correlation [6, 12, 18]. Our findings on diastolic dysfunction could be attributed to more participants in our study population having longer exposure due to higher pregnancy numbers.

In conclusion, we showed that parity number is significantly correlated with diastolic dysfunction. Therefore, we recommend that physicians discuss with their patients about multiparity's negative effect on the cardiovascular system. In addition, we recommend that patients with a parity of six or greater receive more cardiology screening.

5. Limitations

This study has limitations that should be considered. First, our study population had a limited number of patients. Second, our findings do not represent the healthy population, because our study only studied patients that were referred to the cardiology clinic with cardiac complaints. Third, we performed this study in a lower socio-economic area that could affect cardiovascular status independently. Fourth, we showed by multinomial logistic regression that parity number and age were significant risk factors for diastolic dysfunction. In future studies, patient's age should be selected to be homogeneous among groups. Finally, due to lack of facilities, we did not perform cardiac magnetic resonance measurements or measure brain natriuretic peptides that could give more detailed information about diastolic dysfunction.

Author contributions

Concept and design: MO; Data analysis/interpretation: MO, OTY, MSK; Drafting article: MO, OTY, MSK; Critical revision of article: MO, MAA; Statistics: MO, MAA; Data collection: MO. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Osmangazi University (approval number: 2020-398).

Acknowledgment

We would like to express my gratitude to all those who helped me during the writing of this manuscript. Thanks to all the peer reviewers for their opinions and suggestions.

Funding

This research received no external funding.

Conflict of interest

The authors declare no competing interests.

References

[1] Wood PW, Choy JB, Nanda NC, Becher H. Left ventricular ejection fraction and volumes: It depends on the imaging method. Echocardiography. 2014; 31: 87–100.

[2] Urbano-Moral JA, Patel AR, Maron MS, Arias-Godinez JA, Pandian NG. Three-dimensional speckle-tracking echocardiography: Methodological aspects and clinical potential. Echocardiography. 2012; 29: 997–1010.

[3] Ponikowski P, Voors A, Anker S, Bueno H, Cleland J, Coats A, *et al.* 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European Journal of Heart Failure. 2016; 18: 891–975.

[4] Jeong E-M, Dudley SC, Jr. Diastolic dysfunction. Circulation Journal. 2015; 79: 470–477.

[5] Clapp III JF, Seaward BL, Sleamaker RH, Hiser J. Maternal physiologic adaptations to early human pregnancy. American Journal Of Obstetrics and Gynecology. 1988; 159: 1456–1460.

[6] Kim HJ, Kim MA, Kim HL, Shim WJ, Park SM, Kim M, *et al.* Effects of multiparity on left ventricular diastolic dysfunction in women: Cross-sectional study of the KoRean wOmen'S chest pain rEgistry (KoROSE). BMJ Open. 2018; 8: e026968.

[7] Peters SA, Yang L, Guo Y, Chen Y, Bian Z, Tian X, *et al.* Pregnancy, pregnancy loss, and the risk of cardiovascular disease in Chinese women: Findings from the China Kadoorie Biobank. BMC Medicine. 2017; 15: 148.

[8] Ness RB, Harris T, Cobb J, Flegal KM, Kelsey JL, Balanger A, *et al.* Number of pregnancies and the subsequent risk of cardiovascular disease. New England Journal of Medicine. 1993; 328: 1528–1533.

[9] Kuznetsova T, Herbots L, López B, Jin Y, Richart T, Thijs L, *et al.* Prevalence of left ventricular diastolic dysfunction in a general population. Circulation: Heart Failure. 2009; 2: 105–112.

[10] Mesa A, Jessurun C, Hernandez A, Adam K, Brown D, Vaughn WK, *et al.* Left ventricular diastolic function in normal human pregnancy. Circulation. 1999; 99: 511–517.

[11] Moran AM, Colan SD, Mauer MB, Geva T. Adaptive mechanisms of left ventricular diastolic function to the physiologic load of pregnancy. Clinical Cardiology. 2002; 25: 124–131.

[12] Keskin M, Avşar Ş, Hayıroğlu Mİ, Keskin T, Börklü EB, Kaya A, *et al.* Relation of the number of parity to left ventricular diastolic function in pregnancy. The American Journal of Cardiology. 2017; 120: 154–159.

[13] Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, *et al.* Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. European Journal of Echocardiography. 2016; 17: 1321–1360.

[14] Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, *et al.* 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). European Heart Journal. 2018; 39: 3021–3104.

[15] American Diabetes Association. 2. Classification and Diagnosis of Diabetes. Diabetes Care. 2017; 40: S11–S24.

[16] Irani RA, Xia Y. Renin angiotensin signaling in normal pregnancy and preeclampsia (pp. 47–58). Seminars in Nephrology. Elsevier. 2011.

[17] Teichman SL, Unemori E, Teerlink JR, Cotter G, Metra M. Relaxin: Review of biology and potential role in treating heart failure. Current Heart Failure Reports. 2010; 7: 75–82.

[18] Aggarwal SR, Herrington DM, Vladutiu CJ, Newman JC, Swett K, Gonzalez F, *et al.* Higher number of live births is associated with left ventricular diastolic dysfunction and adverse cardiac remodelling among US Hispanic/Latina women: results from the Echocardiographic Study of Latinos. Open Heart. 2017; 4: e000530.

[19] Mahendru AA, Everett TR, Wilkinson IB, Lees CC, McEniery CM. A longitudinal study of maternal cardiovascular function from preconception to the postpartum period. Journal of Hypertension. 2014; 32: 849–856.

[20] Fujime M, Tomimatsu T, Okaue Y, Koyama S, Kanagawa T, Taniguchi T, *et al.* Central aortic blood pressure and augmentation index during normal pregnancy. Hypertension Research. 2012; 35: 633–638.

[21] Mitchell GF, Guo C-Y, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, *et al.* Cross-sectional correlates of increased aortic stiffness in the community. Circulation. 2007; 115: 2628–2636.

[22] Ungvari Z, Kaley G, de Cabo R, Sonntag WE, Csiszar A. Mechanisms of vascular aging: new perspectives. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 2010; 65: 1028–1041.