Plasma Ghrelin Levels to Predict Severity of Pulmonary Arterial Hypertension Ghrelin in Relation to Pulmonary Hypertension

Plazma Ghrelin Seviyeleri ile Pulmoner Arteriyel Hipertansiyon Ciddiyetinin Öngörülmesi Ghrelin ve Pulmoner Hipertansiyon İlişkisi

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ABSTRACT

Background: Pulmonary arterial hypertension (PAH) causes right ventricular (RV) remodeling and dysfunction, which results in clinical deterioration and mortality. Ghrelin which is proposed as a possible biomarker for PAH, is a peptide mostly secreted from gaster, and has various effects on the cardiovascular system. We aimed to determine ghrelin plasma levels in PAH patients and its correlation with RV function and N-terminal pro b-type natriuretic (NT-proBNP) levels.
Methods: Eighteen patients (37±15y,17f) with different etiologies and a matched control group of 20 volunteers were included. Plasma ghrelin levels were studied. RV dimensions, tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC), global (G) longitudinal strain (LS) as well as left ventricular (LV) ejection fraction (EF) and LVGLS were measured.
Results: The plasma ghrelin levels did not significantly differ between groups. (1067±489 pg/ml vs. 860±240 pg/ml, P = 0.232). PAH patients had similar LVEF, RVFAC and TAPSE (all, p>0.05), however PAH group had lower RVGLS, RV free-wall LS and LV GLS (all, p<0.05). Plasma ghrelin level showed no statistically significant correlations with plasma NT-proBNP level, RVGLS, RVLS and LVGLS. Contrarily, plasma NT-proBNP levels showed significant correlations with RVGLS, RVLS and LVGLS (all P < 0.001).
Conclusion: Ghrelin might play a role in PAH pathogenesis but current data is insufficient to verify the exact relationship between ghrelin and PAH. The results of this study demonstrate that ghrelin levels are not suited to predict the clinical outcome of PAH since it does not represent the actual clinical situation of the patient.

Keywords: Ghrelin, right ventricle, strain,

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ÖZET

Giriş: Pulmoner arter hipertansiyonu (PAH), sağ ventrikül (RV) disfonksiyonu ve yeniden şekillenmesi yaratarak klinik bozulma ve mortaliteye neden olur. PAH için olası bir biyobelirteç olarak önerilen ghrelin, çoğunlukla mideden salgılanan bir peptittir ve kardiyovasküler sistem üzerinde çeşitli etkileri vardır. PAH hastalarında ghrelin plazma düzeylerini ve bu düzeylerin RV fonksiyonu ve N-terminal pro B-tipi natriüretik (NT-proBNP) düzeyleri ile korelasyonunu belirlemeyi amaçladık.
Metodoloji: Farklı etiyolojilere sahip 18 hasta (37 ± 15y, 17f) ve 20 gönüllüden oluşan kontrol grubu dahil edildi. Plazma ghrelin düzeyleri çalışıldı. RV boyutları, triküspid anüler düzlem sistolik hareketi (TAPSE), fraksiyonel alan değişimi (FAC), global (G) boyuna strain (LS) ve sol ventrikül (LV) ejeksiyon fraksiyonu (EF) ve LVGLS ölçüldü.
Bulgular: Plazma ghrelin seviyeleri gruplar arasında anlamlı farklılık göstermedi. (1067 ± 489 pg / ml, 860 ± 240 pg / ml, P = 0.232). PAH hastalarında benzer LVEF, RV-FAC ve TAPSE (tümü, p> 0.05) saptandı, ancak PAH grubunda daha zayıf RV-GLS, RV serbest duvar LS ve LV GLS bulundu (tümü, p <0.05). Plazma ghrelin düzeyi, plazma NT-proBNP düzeyi, RVGLS, RVLS ve LVGLS ile istatistiksel olarak anlamlı bir ilişki göstermedi. Aksine, plazma NT-proBNP seviyeleri RVGLS, RVLS ve LVGLS ile anlamlı korelasyon gösterdi (tümü P <0.001).
Sonuç: Ghrelin, PAH patogenezinde rol oynayabilir, ancak mevcut veriler, ghrelin ve PAH arasındaki tam ilişkiyi doğrulamak için yetersizdir. Bu çalışmanın sonuçları, ghrelin seviyelerinin, PAH'ın klinik sonucunu tahmin etmek için uygun olmadığını, çünkü hastanın gerçek klinik durumunu belirtmediğini göstermektedir.

Anahtar Sözcükler: Ghrelin, strain, sağ ventrikül

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INTRODUCTION

Right ventricular (RV) dysfunction is strongly associated with clinical deterioration and mortality in patients with pulmonary arterial hypertension (PAH).(1,2) Echocardiography is used as the first line imaging modality to evaluate RV in daily practice. However, the assessment of RV function with 2D echocardiography is a complicated and mostly inaccurate technique because of the RV's complex anatomical shape and position. In spite of the development of many quantitative and qualitative echocardiographic methods to assess right heart morphology and function, such as dimensions of RV, RV fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE) or tissue Doppler imaging modalities, echocardiography still has some disadvantages for a satisfactory RV assessment.(3-5) 2D speckle tracking was recently successfully applied to the RV in several populations including PAH patients, providing more accurate data about quantitative systolic and diastolic RV function. This technique is able to investigate the displacement and strain patterns of the RV in different stages. Moreover, it has been shown that impaired RV free-wall (FW) longitudinal strain (LS) and RV global (G) LS are associated with poor clinical outcome and all-cause mortality together with increased levels of N-terminal pro b-type natriuretic peptide (NT-proBNP).(1,6-9)

Ghrelin is a 28 amino-acid polypeptide that is mostly secreted by the gastric fundus. It is first produced as preproghrelin than spliced to proghrelin and at last to ghrelin.(10) Ghrelin shows its effect via a splice-variant of the growth hormone secretagogue receptor, which is a G protein-coupled receptor expressed in the brain as well as other parts of the body. Ghrelin is mostly known as a hormone regulating hunger, but it also plays an important role in energy distribution and regulating the rate of energy use. Ghrelin secretion could be influenced by many factors, such as food intake, hormonal status, body composition, etc. In addition, ghrelin has various effects on human physiology, such as the cardiovascular system. It is claimed to have a beneficial effect on the cardiovascular system by inhibiting pro-inflammatory cytokines (IL-1, IL-6, TNF-α), improving energy balance and regulating the autonomic nervous system. Beyond these effects it is stated that ghrelin is a potent peripheral vasodilator and decreases systemic arterial pressure.(11-13) Furthermore, ghrelin levels were found to be higher in idiopathic PAH patients(14) and it is able to attenuate vascular remodeling and RV hypertrophy in an animal model when administered intravenously.(15) It is suggested that ghrelin may not only provide a novel prognostic biomarker for pulmonary hypertension, but it also might become a new therapeutic option for cardiovascular diseases in the light of recent findings. Therefore, this study was conducted to investigate the ghrelin levels in patients with PAH and to evaluate the association of ghrelin levels with echocardiographic parameters and NT-proBNP levels.

METHODS

Study Population

Among patients who were followed by the Cardiology Department of Gazi University Hospital Ankara between January 2012 and 2015, 18 patients over 18 years of age with pulmonary hypertension were enrolled. The diagnosis of PAH was identified according to the recent guidelines. Twenty healthy volunteers were included as a matching control group. Inclusion criteria were being over 18 years old and clinically stable for at least six months. Personal and general information such as age, gender, height, weight, body mass index, medical history, medication and blood tests were recorded. Patients were informed about the research and written consent form was taken. The present study was approved by the local ethical committee.

Blood sample collection and NT-proBNP and Ghrelin Measurements

Biochemical and hematological parameters were measured from venous blood samples taken after a 12-hour fasting period. Aprotinin was added to the blood samples, which were immediately centrifuged at 1600 beats / min for 15 minutes. Plasma ghrelin concentration was measured using a radio-immune assay kit in accordance with the manufacturer's instructions (DIAsource Immuno Assays, Louvain-la-Neuve, Belgium). For NT-proBNP measurements, blood was collected into lithium-heparinized tubes and the NT-proBNP plasma levels were measured by chemiluminescent immunoassay (Immulite 2000 System, Siemens Healthcare Diagnostics). The analytical sensitivity of the Siemens Immulite 2000 Kit used in the assay is 10 pg / mL. The values of the other laboratory parameters were determined using standard methods.

Conventional Echocardiography

GE Vivid 7 Dimension ultrasonography machine equipped with a 3.5 MHz transducer was used for echocardiographic examinations with electrocardiogram and respiration monitorization. Echocardiographic images were recorded with three cardiac cycles at the end of expiration. The images were analyzed with the vendor-specific workstation EchoPAC, BT 13 (GE Vingmed Ultrasound, Norway). Parasternal long axis views were obtained to measure LA and LV dimensions, volumetric measurements and ejection fraction of the LV were calculated from the apical four- and two-chamber views by biplane method of disks summation. RV linear dimensions, systolic and diastolic areas were obtained from the RV focused apical four-chamber view. The thickness of the RV free wall (FW) was measured in the sub-costal view by M-mode in diastole. The RVFAC was calculated as the percentage of change between end-diastolic and end-systolic areas of RV. TAPSE was measured by placing a longitudinal M-mode cursor on the lateral annulus of the tricuspid valve in the apical four-chamber window.

2D Speckle Tracking Strain Analysis

Image acquisitions from apical windows which are suitable for speckle tracking were used. Frame rate was between 40 fps and 70 fps, on average 45 fps. Although speckle tracking has been primarily used for the assessment of the LV, several studies have shown its feasibility and accuracy in evaluating RV function as well. RV focused apical four-chamber views were used to analyze RV GLS and RV FW LS. LS is defined as the fractional change in length of a region of interest (ROI) relative to its original length, and is expressed as a negative percentage.
The ROI on the endocardial surface of the RV was defined by placing at least 15 markings, starting from the lateral annulus and ending at the septal annulus of the tricuspid valve. LV GLS was measured from at least two of the three apical views, similarly by placing at least 15 markings on the LV endocardial border. ROI for both ventricles was assessed visually and corrected manually to obtain a better fit to the myocardial motion.

Statistical Analysis

Continuous variables were examined by the Shapiro-Wilk test to check for normality of distribution. Continuous variables are presented as mean ± standard deviation and standard error, and categorical data are presented as percentages or frequencies. Student t-test / one-way analysis of variance and Mann-Whitney U / Kruskal Wallis-H tests were used to compare parametric and nonparametric continuous variables, respectively. Categorical variables were compared by Chi-square ($\chi 2$) test. Pearson correlation coefficients were used to show relation among plasma ghrelin, NT-proBNP, RV GLS, RV FW LS and LV GLS. A two-tailed P-value of <0.05 was considered as statistically significant. All data were analysed using SPSS version 23.0.

Table 1. Baseline characteristics of the participants

Parameters	PAH Group (n:18)	Control Group (n:20)	P
Age (year)	37±15	37±14	0.172
Gender (Female/Male)	17/1	19/1	0.277
BMI (kg/m²)	21.1 ± 3.90	22.9 ± 4.21	0.100
Etiology			0.040
Congenital heart disease	12		
Connective tissue disorders	3		
Idiopathic	3		
6 MWD (m)	444 ± 108	630 ± 120	0.031
NYHA functional class			
I	2	20	
II	10		
III	6		
Treatment			
ERAs	6		
PGE1 analogue	2		
ERAs - PDE-5 inhibitors	2		
ERAs - PGE1 analogue	6		
ERAs - PGE1 analogue - PDE-5 inhibitors	2		

6MWD, 6-min walking distance; NYHA, New York Heart Association; BMI; body mass index, ERA; Endothelin receptor antagonist, PDE-5; Phosphodiesterase Type 5, PGE1; Prostaglandin E1

Table 2. Conventional echocardiographic measurements before and after hemodialysis.

Parameters	Before HD	After HD	P
RV basal diameter (cm)	**5.1 ± 0.7**	**3.5 ± 0.6**	**<0.001**
RV mid-cavity diameter (cm)	**4.3 ± 0.7**	**2.5 ± 0.6**	**<0.001**
RV longitudinal diameter (cm)	**7.0 ± 0.7**	**6.1 ± 0.8**	**<0.001**
RA longitudinal axis (cm)	**31.2 ± 10**	**40.4 ± 9**	**<0.001**
RV FAC (%)	**16 ± 3.7**	**19 ± 3.1**	**0.389**
TAPSE (cm)	**2.1±0.4**	**1.7±0.3**	**<0.001**

FAC; fractional area change, RV; right ventricle, TAPSE; tricuspid annular plane systolic excursion

Table 3. 2D speckle tracking strain measurements

Strain measurements			
RV GLS (%)	**16.1 ± 4.8**	**25.9 ± 4.5**	**<0.001**
RV FW LS (%)	**15.7 ± 6.9**	**20.0 ± 5.0**	**<0.001**
LV GLS (%)	**16.3 ± 7.2**	**25.0 ± 5.0**	**<0.001**

FW; free-wall, GLS; global longitudinal strain, LS; longitudinal strain, LV; left ventricle, RV; right ventricle

RESULTS

Baseline demographic and clinical characteristics are presented in Table 1. There was no difference in age, sex and relating medical history between groups. The case population was mostly comprised of congenital heart disease associated PAH.

Patients with PAH had enlarged RVs compared to control group. However, conventional echocardiographic methods (LV EF, TAPSE and RV FAC) did not show significant differences between patients and the control group. 2D speckle tracking strain analysis revealed an impaired systolic function in both ventricles in the PAH group compared to control group.

Measurements of conventional echocardiographic parameters and deformation imaging echocardiographic parameters are presented in Table 2 and 3, respectively.

The plasma ghrelin levels did not show a significant difference between patients and control group (Figure 1). There was no significant correlation between plasma NT-proBNP and ghrelin levels (r = -0.128, P = 0.68) (Figure 2).

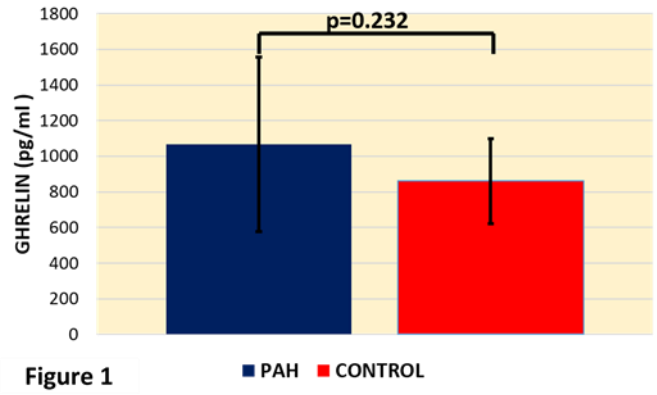

Figure 1: Comparison of plasma ghrelin level between pulmonary arterial hypertension patients and control group.

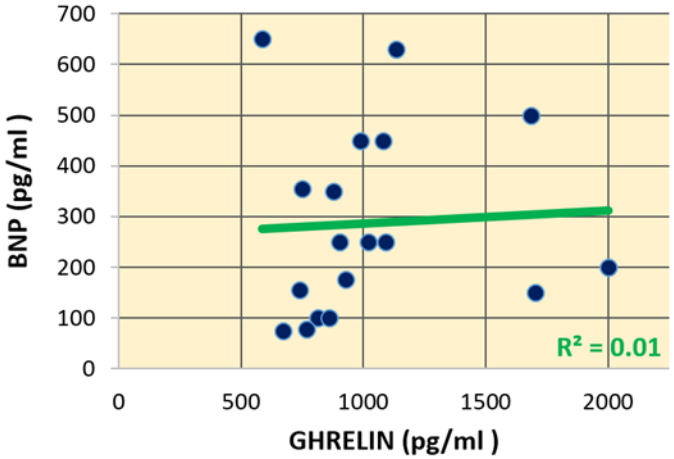

Figure 2: Correlation plot of plasma ghrelin and NT-proBNP levels. R 2 value is indicated (p:0.82).

Moreover, no clinically relevant correlations of deformation imaging parameters and ghrelin level were found (Figure 3). On the contrary, NT-proBNP levels and deformation imaging parameters showed significant correlations (Figure 4).

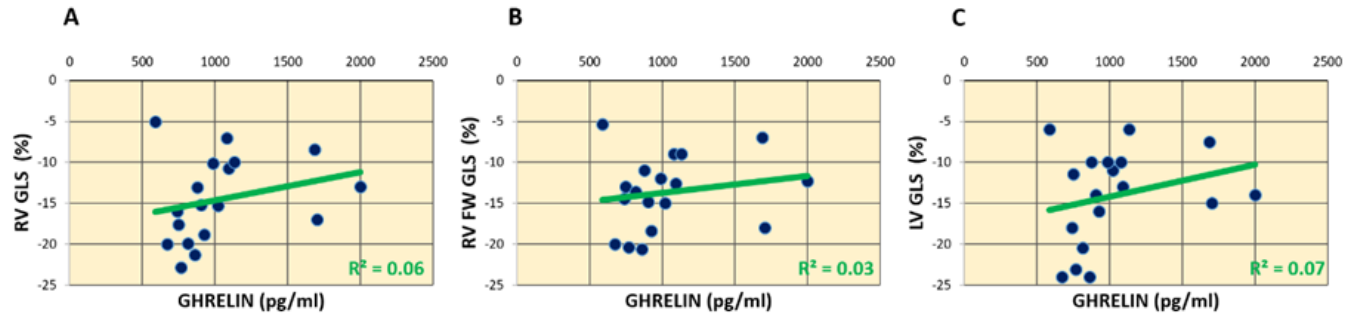

Figure 3: Correlation plot of plasma ghrelin levels and right ventricular global longitudinal strain (A), right ventricle free-wall longitudinal strain (B) and left ventricular global longitudinal strain (C). R 2 values are indicated.

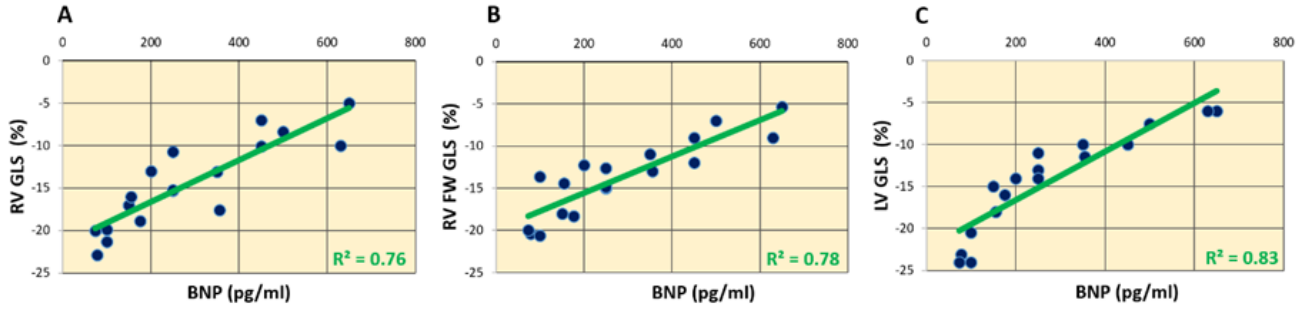

Figure 4: Correlation plot of plasma NT-proBP levels and right ventricular global longitudinal strain (A), right ventricle free-wall longitudinal strain (B) and left ventricular global longitudinal strain(C). R 2 values are indicated.

DISCUSSION

In this study, we investigated plasma ghrelin levels of PAH patients and its relationship with NT-proBNP and echocardiographic parameters. The results revealed that there is no significant elevation of plasma ghrelin levels in PAH patients. In addition, there was no correlation of plasma ghrelin levels with plasma NT-proBNP levels and echocardiographic indices. Moreover, our results demonstrated that 2D speckle tracking derived strain measurements detect RV dysfunction better and earlier than conventional parameters.

The prognostic evaluation of PAH patients is an important part of the follow-up visit. Several parameters are being used for this purpose which are well established in recent PH guidelines. Nevertheless, authors state the need for novel biochemical and imaging modalities besides the existing parameters.(1) Ghrelin was proposed as a novel prognostic indicator because it has an endothelium-independent arterial vasodilating effect(13) and its elevation might be a predictor of clinical deterioration. Previous studies showed promising outcomes after ghrelin administration in animal models which made this hormone to be a novel therapeutic target.(15) After recent studies with contradicting results, the presence of elevated ghrelin levels in PAH patients is controversial.(14-18) The main reason for these discrepancies might be the different subtypes of PAH used in these studies.(14,17,18) Moreover, ghrelin could provide additional information over the conventional clinical parameters in terms of diagnosis, prognosis and response to treatment. However, this provided additional information by ghrelin should be validated. Unfortunately, current studies on this field do not provide enough and reproducible data for the use of plasma ghrelin levels in daily clinical practice.

Our study population mainly consisted of patients with PAH due to congenital heart diseases. Two studies evaluated ghrelin levels in adult PAH patients with atrial septal defect(18) and in children with PAH due to varying congenital heart diseases.(17) Both studies were performed in China and showed increased ghrelin levels in adult PAH patients with ASD and a negative correlation of ghrelin and systolic pulmonary arterial pressure in children with PAH due to congenital heart diseases. Both studies claimed that ghrelin had substantial effects on the pulmonary arterial bed.

Furthermore, our study population had higher plasma ghrelin levels than previous studies. The main explanation could be the lower body mass index (BMI) values of PAH patients' due to heart failure. However, there was no statistically significant difference in BMI between the groups. Nevertheless, both groups had lower BMI values compared to previous studies.

The preservation of RV function is vital in pulmonary hypertension patients because two thirds of the patients die as a result of RV failure.(1) Unfortunately, there is no optimal imaging modality for the evaluation of RV function. New biomarkers or imaging modalities are needed to allow a more accurate assessment of RV function.(3) Deformation imaging is a new tool in predicting both left and right ventricular dysfunction. It has already been shown for the LV that speckle tracking strain analysis can detect the impaired function earlier than ejection fraction in diseases such as hypertrophic or dilated cardiomyopathy.(3) Recently, this has also been shown for the RV. Deformation imaging is able to detect the impaired function of the RV in pulmonary hypertension patients, which cannot be detected by standard echocardiography. Decreased strain values of the RV and RV FW are shown to be related with increased mortality and morbidity.(6-8,19) Furthermore, several studies evaluated the relation between longitudinal strain measurements of the RV and Pro-BNP levels, which is a well-defined parameter of prognosis.(6-8) In our study, there was no significant relation between deformation imaging parameters and serum ghrelin levels, whereas higher NT-proBNP levels were associated with impaired RV and RV FW strain measurements, however it cannot be argued for serum ghrelin levels, unfortunately. Moreover, since RV goes through several stages of remodeling in PAH, we claim that most of our patient population were in adaptation phase of RV remodeling, which resulted in almost normal values in standard echocardiographic parameters. Still, large prospective studies must be performed to validate our results. Deformation imaging can also show us the impaired function of right ventricle in pulmonary hypertension patients which cannot be detected by standard echocardiography. Evaluation of the RV with deformation imaging is shown to provide better predictors for the follow up of patients. Still, large prospective studies must be performed to validate our results.

In the present study, patients with PAH due to differing etiologies were enrolled, since ghrelin is proposed as a novel general biomarker in PAH. Previous studies showed increased ghrelin levels in study populations with the same PAH etiologies. Our study population was created with the purpose of having at least similar numbers of subjects compared to previous studies. Until now, most of the studies were performed in 20 patients, however larger number of subjects are needed in this field of research.

CONCLUSION

Our findings discourage the routine measurement of ghrelin levels for the prediction of outcomes in PAH patients. More studies with a larger patient population are needed to verify our findings.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et. al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertensionThe Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorse. Eur Heart J 2016; 37: 67–119.

2. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et. al. Survival in Patients With Idiopathic, Familial, and Anorexigen-Associated Pulmonary Arterial Hypertension in the Modern Management Era. Circulation 2010; 122:156–163.

3. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et. al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015; 16: 233–271.

4. Portnoy SG, Rudski LG. Echocardiographic Evaluation of the Right Ventricle: a 2014 Perspective. Curr Cardiol Rep. 2015; 17: 21

5. Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et. al. Guidelines for the Echocardiographic Assessment of the Right Heart in Adults: A Report from the American Society of Echocardiography. J Am Soc Echocardiogr 2010; 23: 685–713.

6. Sachdev A, Villarraga HR, Frantz RP, McGoon MD, Hsiao JF, Maalouf JF, et. al. Right Ventricular Strain for Prediction of Survival in Patients With Pulmonary Arterial Hypertension. Chest 2011; 139: 1299–1309.

7. Fine NM, Chen L, Bastiansen PM, Frantz RP, Pellikka PA, Oh JK, et. al. Outcome Prediction by Quantitative Right Ventricular Function Assessment in 575 Subjects Evaluated for Pulmonary Hypertension. Circ Cardiovasc Imaging 2013; 6: 711–721

8. Haeck MLA, Scherptong RWC, Marsan NA, Holman ER, Schalij MJ, Bax JJ, et. al. Prognostic Value of Right Ventricular Longitudinal Peak Systolic Strain in Patients With Pulmonary Hypertension. Circ Cardiovasc Imaging 2012; 5: 628–636.

9. Giusca S, Dambrauskaite V, Scheurwegs C, D'hooge J, Claus P, Herbots L, et. al. Deformation imaging describes right ventricular function better than longitudinal displacement of the tricuspid ring. Heart 2009; 96: 281–288.

10. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999 ;402:656–660.

11. Kishimoto I, Tokudome T, Hosoda H, Miyazato M, Kangawa K. Ghrelin and cardiovascular diseases. J Cardiol 2012; 59: 8–13.

12. Tokudome T, Kishimoto I, Miyazato M, Kangawa K. Ghrelin and the Cardiovascular System. Front Horm Res. 2014; 43: 125–133.

13. Callaghan B, Kosari S, Pustovit R V, Sartor DM, Ferens D, Ban K, et. al. Hypotensive effects of ghrelin receptor agonists mediated through a novel receptor. Br J Pharmacol. 2014; 171: 1275–1286.

14. Yang D, Liu Z, Yang Z.. Ghrelin and Its Relation with N-Terminal Brain Natriuretic Peptide, Endothelin-1 and Nitric Oxide in Patients with Idiopathic Pulmonary Hypertension. Cardiology 2013; 124: 241–245.

15. Henriques-Coelho T, Roncon-Albuquerque Junior R, Lourenco AP, Baptista MJ, Oliveira SM, Brandão-Nogueira A, et. al. Ghrelin reverses molecular, structural and hemodynamic alterations of the right ventricle in pulmonary hypertension. Rev Port Cardiol. 2006; 25: 55–63.

16. Zhang M, Fang W, Yuan F, Qu XK, Liu H, Xu YJ, et. al. Plasma ghrelin levels are closely associated with severity and morphology of angiographically-detected coronary atherosclerosis in Chineses patients with diabetes mellitus. Acta Pharmacol Sin 2012; 33: 452–458

17. Li G, Xia J, Jia P, Zhao J, Sun Y, Wu C, et. al. Plasma Levels of Acylated Ghrelin in Children with Pulmonary Hypertension Associated with Congenital Heart Disease. Pediatr Cardiol. 2015; 36: 1423–1428.

18. Li ZF, Zhou DX, Pan WZ, Zhang L, Ge JB, et. al. Circulating ghrelin was negatively correlated with pulmonary arterial pressure in atrial septal defect patients. Chin Med J 2013; 126: 3936–3939.

19. Unlu S, Farsalinos K, Ameloot K, Daraban AM, Ciarka A, Delcroix M, et. al. Apical traction: a novel visual echocardiographic parameter to predict survival in patients with pulmonary hypertension. Eur Hear J – Cardiovasc Imaging 2016; 17: 177–183.