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Research Article

Relationship Between Chordin Like-1 Protein Level and Patients with Pulmonary Arterial Hypertension Disease

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ABSTRACT

Pulmonary arterial hypertension (PAH) is a serene executioner, which can incite irreversible changes in pneumonic vascular structure and point of confinement, developing pulmonary vascular resistance (PVR), right ventricular disappointment, and death. Background and Objective: This study was done at the Cardiac Centre of Al-Sader Medical City Hospital in Al-Najaf Al-Ashraf province for the period from December 2016 to May 2017 to investigate the level of biomarker of Chordinlike-1for patients with pulmonary arterial hypertension as predictive indicators of the disease for detection, diagnosis and early treatment of patients. Materials and Methods: The study was applied on 88 people aged 30-69 years; 67 patients group with pulmonary arterial hypertension (PAH) and 21 healthy group. The group of patients was divided into subgroups based on gender, age, body mass index (BMI), waist circumference, smokers and non-smokers, type of primary and secondary arterial hypertension, types of secondary arterial hypertension, and disease grade. The healthy group was divided according to gender and age. Results The study emerged a significant decrease ($p < 0.05$) in the level of Chordin like-1 in the group of pulmonary arterial hypertension patients compared to the healthy group, the female group compared to the males, the age group at the age of 30 to 69, Secondary compared with a primary and the disease grade group was found that sever lower than moderate and mild.While the level of Chordin like-1 in the healthy group divided by gender and age have no significant differences.The study produced that in comparing secondary PAH types, there was a significant decrease ($p < 0.05$) in the level of Chordin like-1 for both COPD and Left Heart Disease systolic dysfunction or diastolic dysfunction (LHD sys or dia) compared with Valvular, Congenital, and Pulmonary embolism. While there was no significant difference in the level of Chordin like-1between Valvular, congenital and pulmonary embolism. The study revealed no significant difference in the level of Chordin like-1 for the patients group of pulmonary arterial hypertension divided according to BMI group (normal weight, overweight, obese weight), waist circumference group (70-80 cm, 81-90 cm, 91-100 cm, 101-110 cm, 111-120 cm) and the group of smokers compared to non-smokers. conclusion: The biomarker Chordin like-1consider cautioning sign as a prognostic marker for determination PAH patients.

Keywords: Chordin like-1, PAH, BMI, Chronic obstructive pulmonary disease.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a serene executioner, which can incite irreversible changes in pneumonic vascular structure and point of confinement, developing pulmonary vascular resistance (PVR), right ventricular disappointment, and death[1]. PAH is known by a resting mean pulmonary artery pressure (PAP) ≥ 25 mmHg,the normal mean PAP still is 14± 3 mmHg, and a high most extreme of run of the mill 20 mmHg[2,3,4] Biomarker is enchanting non-intrusive mechanical congregations to check and screen of PAH infection and expect survival in patients with PAH, along these lines various examinations requested to research new biomarkers to working environments finding of PAH[5]. Chordin-like 1 is a protein encoded by the CHRDL1 gene. It is an antagonist of bone morphogenetic protein 4 by binding to BMP4 and preventing its interaction with receptors also play an important role in regulating retinal angiogenesis through modulation of BMP4 actions in endothelial cells[6].

MATERIALS AND METHODS

Patients and healthy group

The current study included sixty seven (67) patients suffered from pulmonary arterial hypertension disease. The samples were collected from Echocardiography unit in Cardiac Centre of AL-Sader Teaching Hospital in AL-Najaf AL-Ashraf province /Iraq, during the period from December 2016 to May 2017. The patients group are divided into subgroups according to gender, age, body mass index types, waist circumferences types, smoking, primary and secondary of pulmonary arterial hypertension, types of secondary pulmonary arterial hypertension, and grade. The healthy group are composed

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of twenty one (21) appear healthy; they are divided into subgroups according to gender and age, a full history of each subjects was recorded.

Exclusion criteria

They should have no history of heart disease, PAH, thyroid disorders, chronic liver disease, diabetes mellitus, cancer, renal disorders, anemia, myocardial infarction (MI) and acute infections. The healthy group also entered to Echocardiography unit to evaluate the presence of pulmonary arterial hypertension or any disease related with PAH.

Ethical statement

The ethics committee for human of AL-Sader Teaching Hospital in AL-Najaf AL-Ashraf province /Iraq approved protocol.

Collection of Blood samples

Five milliliters of venous blood are drawn from pulmonary arterial hypertension patients and healthy group among 9-11 A.M from ante cubital venipuncture using a disposable needle and plastic syringes blood was left at room temperature for 10 minutes to clot in the gel tube .The serum was isolated after centrifugation at 3000 run per minute for 15 minutes and then serum was separated and transported into new disposable tubes Eppendorf tube and stored at -20^{O}c.

Body mass index (BMI)

A person weight with kilograms divided by the square of height with meters called BMI.

$$BMI = Weight\ (kg) / (Height\ m)^2 \quad \ldots(1)$$

The person who have BMI ranges from (18.5 – 24.9 kg/m²) is normal and (25 – 29.9 kg/m²) is overweight while larger than (30 kg/m²) is obese[7].

Waist circumferences (WC)

The normal measurement for men is 102cm (40inch) while for women is 88cm (35inch)[8]. The measuring of (WC) mast be from the top of the iliac crest and lower margin of the least palpable rib at the midpoint by tape (stretch-resistant).

Primary and Secondary types

Primary type is a disease with no underlying cause. It comes in two forms, one is called familial a disease that runs in families and the second form is called idiopathic. Secondary type is high pressure in the pulmonary vessels due to some other underlying disease, the most common causesvalvular disease, COPD, CHD, Left Heart Disease systolic dysfunction or diastolic dysfunction (LHD sys or dia) and pulmonary embolism.

Grades of PAH

The patients were divided into grades of disease (Mild, moderate and severe types) depended on echocardiographic, which used to assessment of mean PAH classified into mild 25–35mmHg, moderate 35–45mmHg and severe more than 45mmHg also by measuring pressure gradient on tricuspid valve regurgitation and pressure gradient on pulmonary valve regurgitation[9].

Biomarker measurement

Determination of serum Chordin like-1 level

Specific kit for measuring human Chordin like-1 level in serum was supplied by Elabscience Catalog No: E-EL-H2629/96T.

Statistical analysis

Graphpad prism v6 windows software packages are used to analyze data of the present study (Version 6.01, 2012) for windows 2007, data were ordered as Mean ± Standard deviation (SD), unpaired sample t-test was used for the comparison between the patients and healthy group and one way ANOVA test was used for the comparison among subdivided groups in the measured parameters, while the figures constructed using EXEL program of Microsoft Office 2007, P value < 0.05 was used as a level of statistically significant.

RESULTS

Comparison of serum chordin like-1 level between patients group with pulmonary arterial hypertension and healthy group.

The results exhibit significant decrease ($P<0.05$) in serum chordin like-1level of patients group withpulmonary arterial hypertension (0.2314 ± 0.02083 ng/ml) compared with healthy group (2.051 ± 0.1492 ng/ml) as showed in figure (1).

Comparison of serum chordin like-1level between male and female patients group with pulmonary arterial hypertension disease and healthy group.

The result in figure (2) exhibit significant decrease ($P<0.05$) in serum chordin like-1 level of female patients group withpulmonary arterial hypertension (0.1033 ± 0.06785 ng/ml) compared with male patients group (0.4661 ± 0.0489 ng/ml) and also compared with male and female healthy group (2.421 ± 0.4251 ng/ml, 1.914 ± 0.7152 ng /ml) respectively.

Comparison of serum chordin like-1level among different ages of patients group with pulmonary arterial hypertension disease and healthy.

The results of figure (3) indicate there are significant decrease ($P<0.05$) in serum chordin like-1level of different ages group in patients with pulmonary arterial hypertension disease (0.1238 ± 0.0141 ng/ml) compared with the healthy (1.786 ± 0.1306 ng/ml, 1.787 ±0.088 ng/ml, 1.788 ± 0.1015 ng/ml and 1.866 ± 0.1803 ng/ml) for the ages (60-69y) ,(50-59y) ,(40-49y) and (30-39y) respectively .The age (60-69y) shows lowest significant decrease ($P<0.05$) in serum chordin like-1 level (0.1238 ± 0.0141 ng/ml) compared with the other ages, while the results shows non-significant differences between healthy group in all ages (figure 3).

Comparison of serum chordin like-1level of patients group with pulmonary arterial hypertension according to body mass index classification (normal weight, over weight, obese weight).

The results indicate there are non-significant differences in serum chordin like-1 level and the body mass indexes (normal weight, over weight, obese weight) of patients group with pulmonary arterial hypertension (figure 4).

Comparison of serum chordin like-1level among different waist circumferences of patients group with the pulmonary arterial hypertension.

The results in figure (5) exhibit non-significant differences

Comparison of serum chordin like-1 level between smoker and nonsmoker patients groups with pulmonary arterial hypertension.

The results shows non-significant differences in serum chordin like-1 level between smoker and nonsmoker patients group with the pulmonary arterial hypertension (figure 6).

The results of the figure (7) reveals there are significant decrease ($P<0.05$) in serum chordin like-1 level of secondary patients groups (0.1906 ± 0.01971 ng/ml) compared with primary patients groups (0.356 ± 0.05662 ng/ml).

Comparison of serum chordin like-1level among different diseases of secondary patients group with the pulmonary arterial hypertension.

The results in figure (8) gives a significant decrease ($P<0.05$) between the patients group have chronic obstructive pulmonary disease (COPD) and Left Heart Disease systolic dysfunction or diastolic dysfunction (LHD sys or dia) (0.1054 ± 0.01568 ng/ml) and (0.1211 ± 0.02224 ng/ml) respectively compared with the Valvular, Congenital and pulmonary embolism that have (0.2991 ± 0.02809 ng/ml, 0.2811 ± 0.04582 ng/ml and 0.2731± 0.01205 ng/ml) respectively, while the results gives non-significant differences between both patients group have chronic obstructive pulmonary disease (COPD) and Left Heart Disease systolic dysfunction or diastolic dysfunction (LHD sys or dia) alone and also results gives non-significant differences among valvular, congenital and pulmonary embolism.

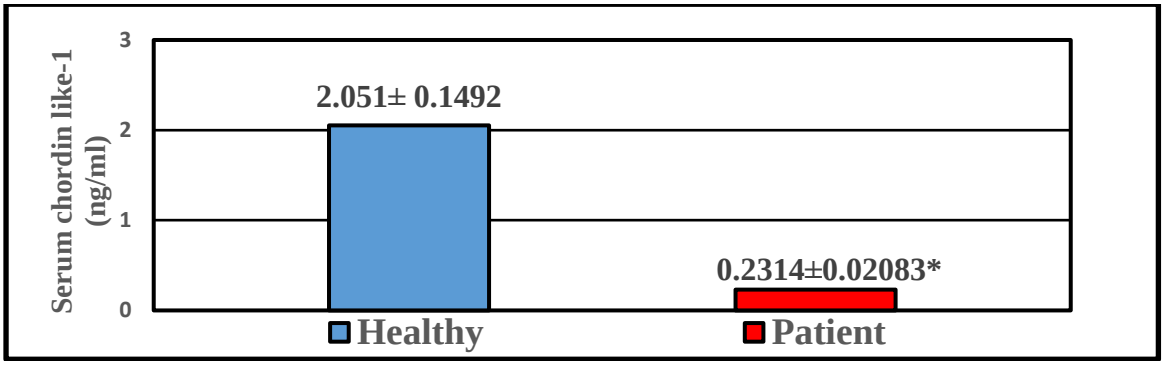
Comparison of serum chordin like-1level among different grades of secondary and primary patients group with the pulmonary arterial hypertension.

The results in figure (9) shows a significant decrease ($P<0.05$) among different grades severe (0.03316 ± 0.01468 ng/ml), moderate (0.1525 ± 0.0289 ng/ml), mild (0.2521 ± 0.02908 ng/ml) respectively of the secondary and primary patients group with the pulmonary arterial hypertension.

DISCUSSION

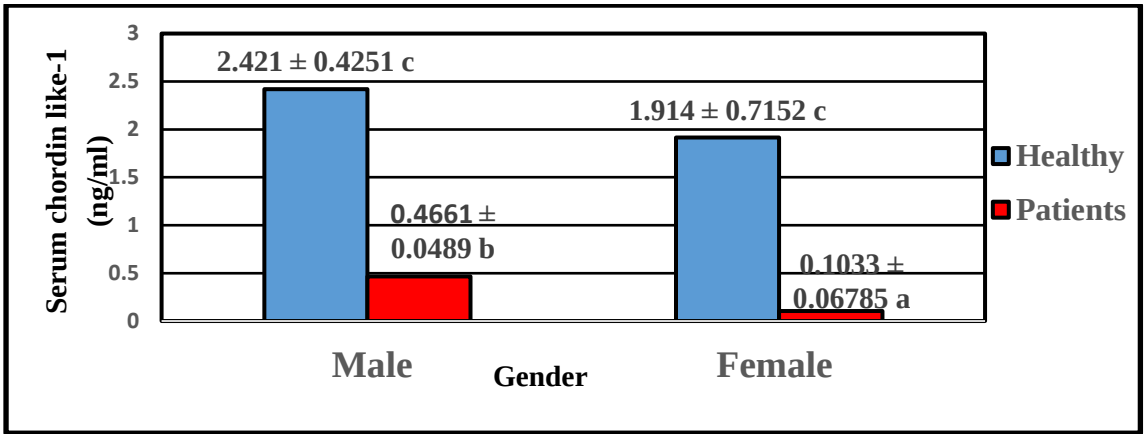
The results exhibit significant decrease ($P<0.05$) in serum chordin- like1level of patients group withpulmonary arterial hypertension compared with healthy group as showed in figure (1).The present study agreement with study of[10] which said that chordin-like 1 acts as an suppress of bone morphogenic protein-4 (BMP4) caused invasion and migration, it expression also play as a good outcomes and prognostic factor in patients .BMP antagonists such as chordin-like 1have been shown to be negatively correlated with BMP pathway , expression and activity by secreted proteins that block the cell surface receptors by interactions and bind with BMP ligands, therefore the inhibition of chordin-like1 lead to increase expression of BMP and activity in PAH[11]. The study of[12] have been suggested the balance between BMP and BMP antagonist activity and the disruption between this balance lead to numerous and progressive of several diseases such as PAH and cancer.Former study have been reported that Chordin-like 1 secreted by Smad pathway a antagonist of BMP mediated signaling in which inhibit BMP4 mediated signaling[13], therefore in the present study the decrement in Chordin-like 1 may enhance the expression and activity of BMP4 and lead to increase the pathogenesis of PAH.Another studies agreement with our present study which revealed chordin-like 1 encodes a bone morphogenic protein antagonist that regulation BMP signaling called ventroptin and suggested to play an important role in control and development of diseases by extracellular and intracellular mechanisms as well as absence or loss and reduced the functional ventroptin lead to dysregulation of BMP signaling and overactivity of BMP by inhibition of ventroptin and chordin-like[1,12,14,15], this is one of the mechanism in current study caused by decrement of chordin-like 1.The study of [16] have been reported that Chordinlik -1and Noggin are considered as structurally unrelated proteins tightly bind in the extracellular space with BMPs to prevent BMP receptors activation, therefore any decrement in both antagonists may lead to increase the activity of BMP and the activity of diseases may be developed .The study of[16,17] have been shown that the expression of Chordin-like 1is regulated by hypoxia inducible factor-1α (HIF-1α) and the down expression or down regulated of Chordin-like 1may be play important roles in the angiogenesis and pathogenesis of PAH through modulation of BMP4 actions on endothelial cells.The result in figure (2) exhibit significant decrease ($P<0.05$) in serum chordin like-1 level of female patients group infected with pulmonary arterial hypertension compared with male patients group and also compared with male and female healthy group.Recently the research of[18] have been showed that developed in female than male in PAH and suggested that overexpression of serotonin transporter (SERT), and overexpression of calcium binding proteins[19]. The SERT and calcium binding protein may be induced proliferation of human pulmonary artery smooth muscle cells (hPASMCs) by down expression of chordin like-1 in female than male.In ovariectomized mice SERT and 17-β estradiol level decreased can increased pulmonary pressure arteries and vascular remolding[18,20] The effects of estradiol on BMP signaling in endothelial cells of PAH have been shown to shift from augmentation to inhibition when studied under normoxic versus hypoxic conditions[21,22].

The present study may conclude that a link between 17-β estradiol with biomarkers such as chordin like-1 in female than males.The results of figure (3) indicate there are significant decrease ($P<0.05$) in serum chordin like-1level of different ages group in patients with pulmonary arterial hypertension disease compared with the healthy for the ages (60-69y) ,(50-59y) ,(40-49y) and (30-39y) .The age (60-69y) shows lowest significant decrease ($P<0.05$) in serum chordin like-1 level compared with the other ages, while the results shows non-significant differences between healthy group in all ages (figure 3).The results agree with a several results have been indicated a relation between heart and pulmonary disease



(*): represent the significant differences at (P<0.05) between means

Fig 1: Comparison of serum chordin like-1 level between patients group with pulmonary arterial hypertension and healthy group.



The dissimilar letters represent significant differences (P<0.05) between different groups.

The similar letters represent non-significant difference.

Fig 2: Comparison of serum chordin like-1level between male and female patients group with pulmonary arterial hypertension disease and healthy group according to the gender.

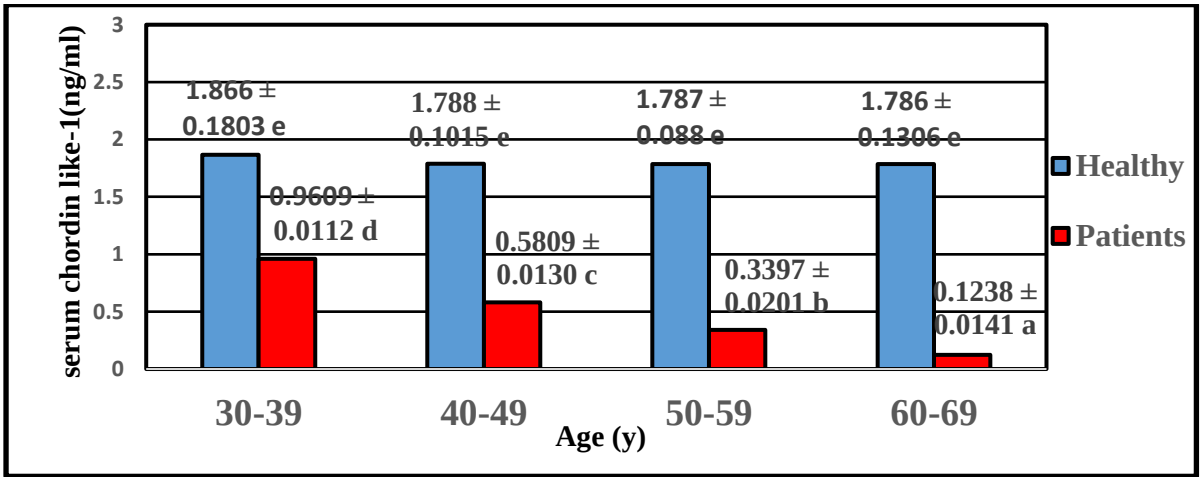
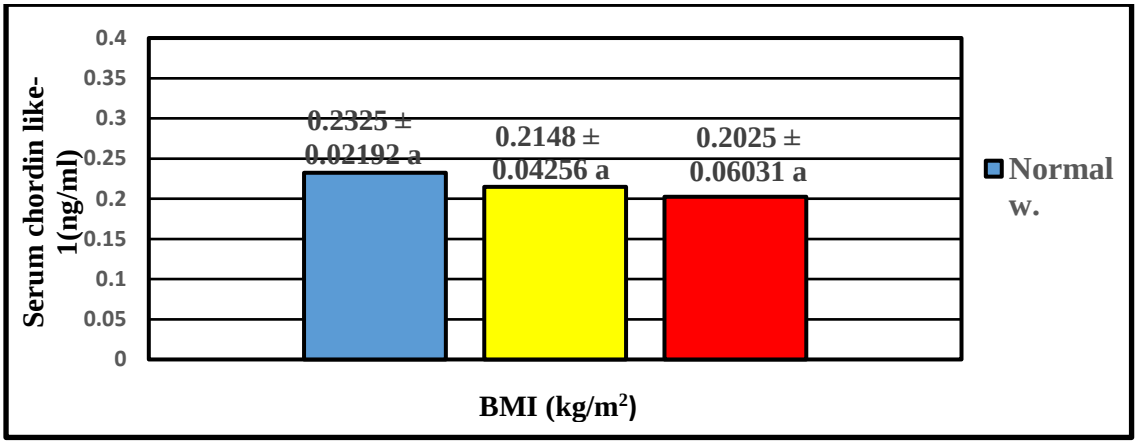


Fig 3: Comparison of serum chordinlike-1 level among different ages of patients with pulmonary arterial hypertension disease and healthy.

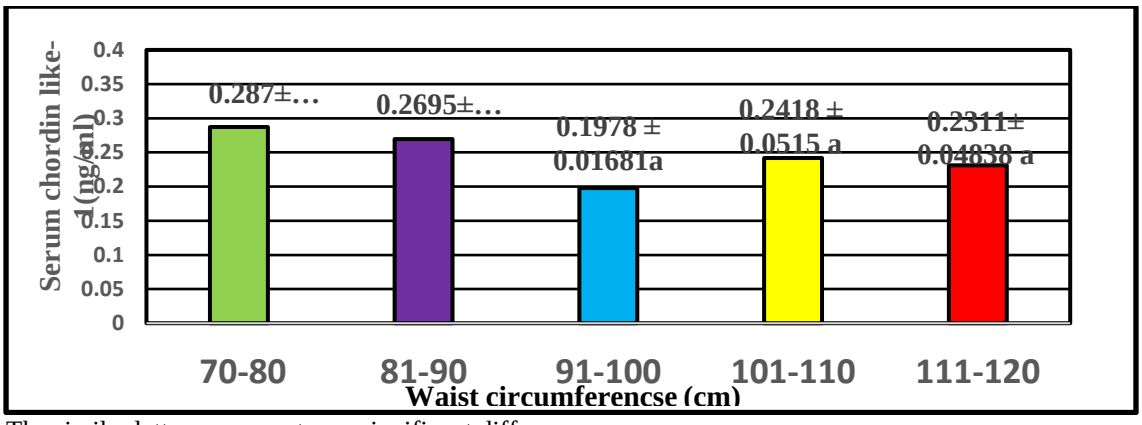
The dissimilar letters represent significant differences (P<0.05) among different ages.The similar letters represent non-significant difference.

with aging[23,24,25] have been concluded that an older age also several disease increased in number with aging[26]. According to several researches, the current study suggested that decreased of chordinlike-1 in older age may be like with increase of BMP4 and enhance a proliferation, migration and invasion of pulmonary arterial smooth muscle cells also increased pulmonary pressure in PAH. The results shows non-significant differences in serum chordin like-1 level between smoker and nonsmoker patients group with the pulmonary arterial



The similar letters represent non-significant difference.

Fig 4: Comparison of serum chordin like-1 level and body mass index (BMI) according to normal weight, over weight, obese weight of patients group with pulmonary arterial hypertension.



The similar letters represent non-significant difference.

Fig 5: Comparison of serum chordin like-1 level among different waist circumferences of patients group with the pulmonary arterial hypertension.

between all groups of waist circumferences of patients group with the pulmonary arterial hypertension.

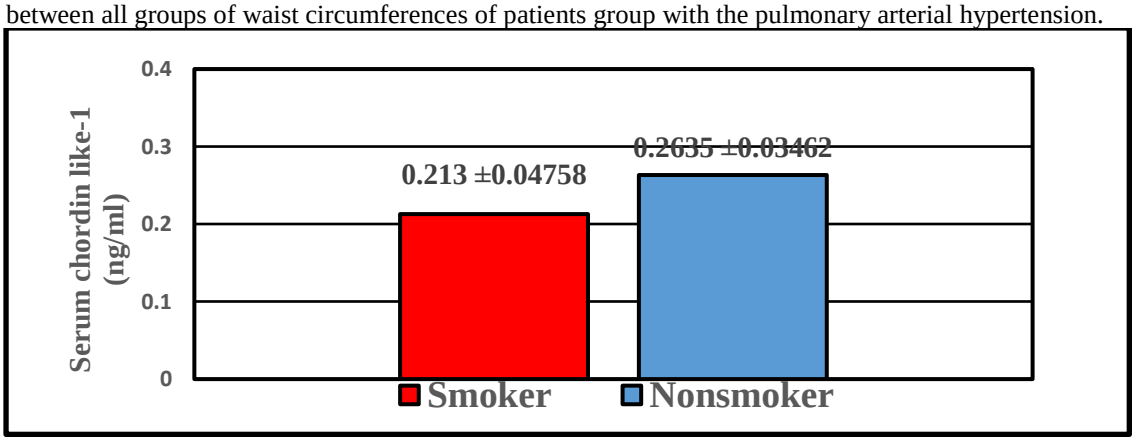
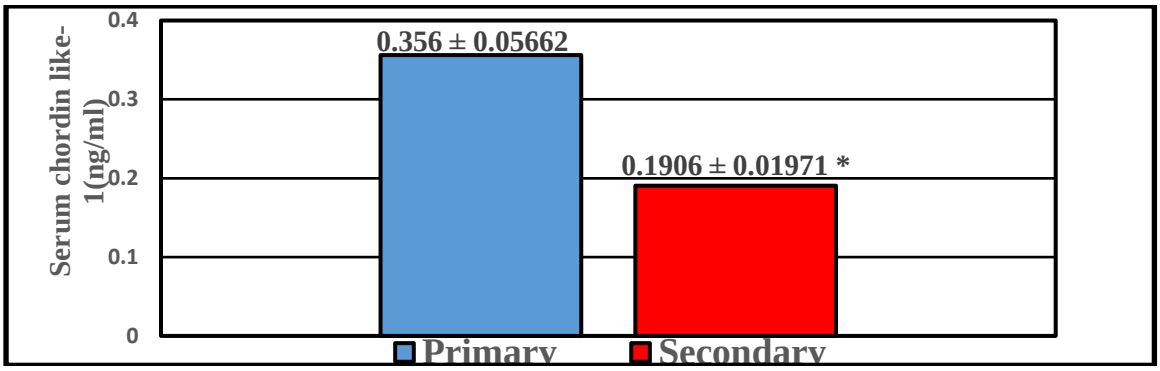


Fig 6 : Comparison of serum chordin like-1 level between smoker and nonsmoker patients group with the pulmonary arterial hypertension

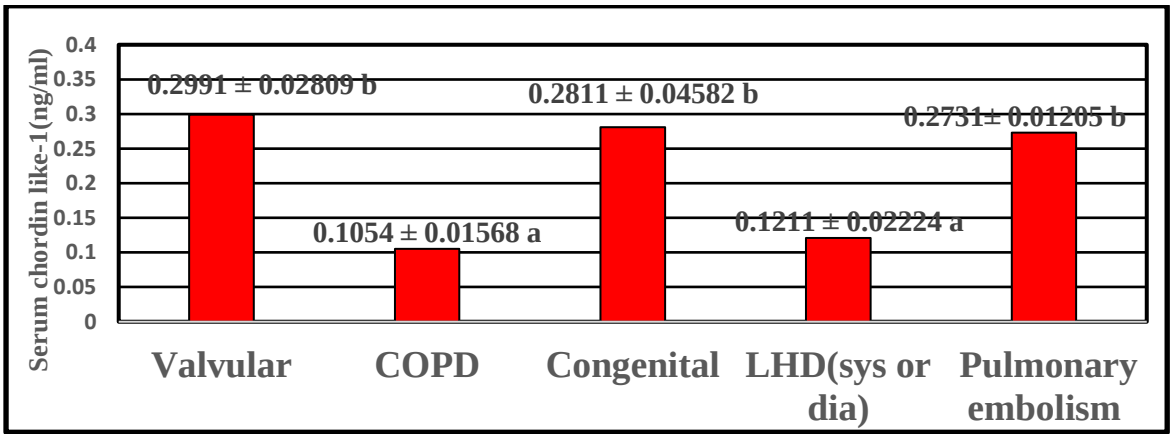
Comparison of serum chordin like-1 level between primary and secondary patients group with the pulmonary arterial hypertension.

hypertension (figure 6).The non-significant differences in smokers and nonsmokers in agreement with several studies that demonstrated 25% of smoking patient because up regulated with BMP4 and overexpression of BMPR2 then induced PAH[27,28].The non significant results in smoker patients in compare with non smoker may due to that the decrement in chordin like-1 levels in smokers patient were not sufficient to antagonist for BMP4 or increased the hypoxia inducible factor-Iα (HIF-Iα).The results of the figure (7) reveals there are significant decrease ($P<0.05$) in serum chordin like-1 level of secondary patients groups compared with primary



(*): represent the significant differences at (P<0.05) between means

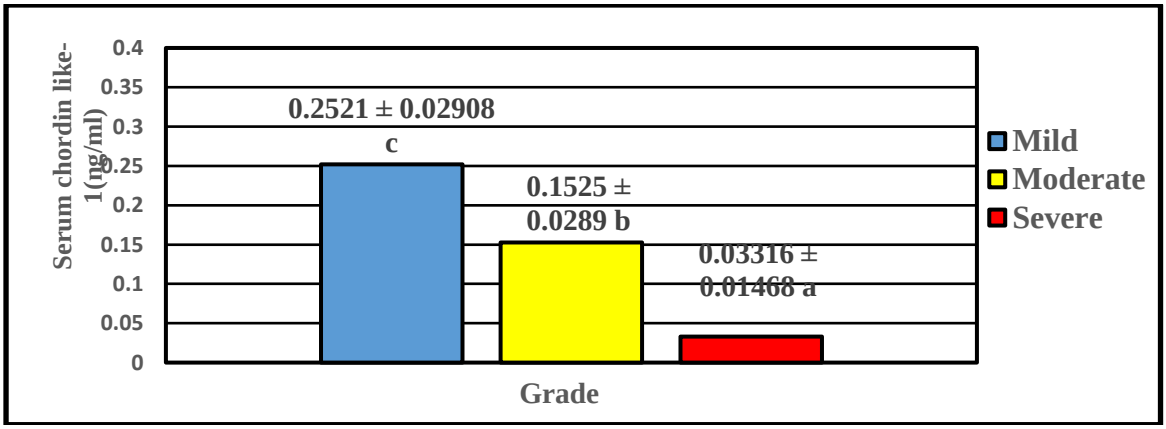
Fig 7: Comparison of serum chordin like-1 level between primary and secondary of pulmonary arterial hypertension patients groups.



The dissimilar letters represent a significant difference (P<0.05)

The similar letters represent non-significant difference.

Fig 8: Comparison of serum chordin like-1 level among different diseases of secondary patients group with the pulmonary arterial hypertension.



The dissimilar letters represent a significant difference (P<0.05)

Fig 9: Comparison of serum chordin like-1 level among different grades of secondary and primary patients group with the pulmonary arterial hypertension.

patients groups. With misregulation of TGF-β signaling and stimulating BMP4 which induce to proliferation and migration SMCs of vascular lead to secondary PAH[29]. The more expression of BMP4 in PAH mean low level of its antagonist chordin like-1 in secondary compared to primary patients groups.The results in figure (8) gives a significant decrease (P<0.05) between the patients group have (COPD) and Left Heart Disease systolic dysfunction or diastolic dysfunction (LHD sys or dia) compared with the Valvular, Congenital and pulmonary embolism while

the results gives non-significant differences between both patients group have (COPD) and Left Heart Disease systolic dysfunction or diastolic dysfunction (LHD sys or dia) alone and also results gives non-significant differences among valvular, congenital and pulmonary embolism.The significant decreased in secondary arterial hypertension and especially in COPD and ischemic heart disease accordance with study of[30] that showed an increase in right ventricular mass wall and hypertrophy associated with COPD in PAH patients[31]. The decrease in chordin like-1 may be related with overexpression of VEGF and BMP4 which lead to vascular remolding and luminal narrowing also arteriolar muscularization, intimal thickening and proliferation due to increase collagen and elastin deposition. The results in figure (9) shows a significant decrease ($P<0.05$) among different grades severe, moderate and mild of the secondary and primary patients group with the pulmonary arterial hypertension.The severity of disease may play important roles in down regulating of noggin and chordin and the current results agree with a results of[32] that showed down regulation of chrordin and noggin level in differentiated VSMCs make them more susceptible for BMP. The missregulation of TGFβ lead to vascular pathologies instances cardiovascular disease and PAH because the TGFβ signaling is essential for regulation vasculogenesis and angiogenesis[33,34,35]. The relation between TGFβ once secreted also regulation chordin in sever pulmonary artery down regulation of chordin lead to disregulation of TGFβ signaling and induce of BMP9 also BMPRII / ActRII / ALK1 and these were required for more Smad phosphorylation and in PAH patients[36,37]. The results indicate there are non-significant differences in serum chordin like-1 level and the body mass indexes (normal weight, over weight, obese weight) and waist circumferences of patients group with pulmonary arterial hypertension figure (4),(5) respectively. Studies of[38,39,40] in cardiometabolic risk factors in adults agreement with present study who arerecommend that the WC and BMI be adjusted to show that having a high WC even in the healthy range of BMI.The body mass index and waist circumference of the PAH patient vary may be depends on some things, heredity, physiological factors metabolism, type of food intake, type of physical activities performed by the individual on a daily basis and the time period of the disease, therefore we find that the patients of the PAH either obese weight, over weight , normal weight and in different waist circumferences large or small ,and there is no significant relation between all biomarkers level and the BMI , waist circumference criteria.The non-significant differences with biomarker a relation with BMI and waist circumferences chordin like-1 may be explained by[41] that demonstrated the absence of relation between obesity and BMI and the obesity is not to be considered a risk factor for PAH and there are no data of studies documented the relation between BMI and PAH.In the study of[42] have been showed there no significant differences in BMI between PAH patients, the obese percentage ≥ 30% and normal weight >20.8% also underweight <18.5%.In the study of[43,44] have been reported that IPAH with metabolic syndrome are highly frequency of obesity with lung function and documented that metabolic syndrome is an independent predictor for IPAH.The current study was excluded the factors that affected and only PAH patients without any related diseases such as metabolic syndrome ,diabetes, thyroid disorder ,chronic liver disease and renal disorder, therefore the current results documented there is no relationship between BMI and PAH.Former study[45] also showed an association between lower body mass index as factor for PAH in younger patients and prognosis with IPAH also with mortality.

CONCLUSION

Chordin- like 1 is the new biomarkers for detection and diagnosis of PAH.

REFERENCES

1. Allen, R.P., Schelegle, E.S., and Bennett, S.H.. Diverse forms of pulmonary hypertension remodel the arterial tree to a high shear phenotype. Am. J. Physiol. Heart Circ. Physiol. *307*, 2014 ;H405-17.
2. Montani, D., Günther, S., Dorfmüller, P., Perros, F., Girerd, B., Garcia, G., Jaïs, X., Savale, L., Artaud-Macari, E., Price, L.C., et al. Pulmonary Arterial Hypertension. Orphanet J. Rare Dis. *8*, 2014; 51–59.
3. Al-Najeem, H.T., and Al-dujaili, A.N.G. Assessment of Gremlin-1 Level in Pulmonary arterial hypertension disease. *10*, 2017; 1994–1997.
4. Al-Najeem, H.T., and Al-Dujaili, A.N.G.. Assessment of Bone Morphogentic protein receptor 2 Level in Pulmonary Arterial Hypertension Disease. Res. J. Pharm. Technol. *10*, 2017; 2614–2618.
5. Hsu, C.H., Ho, W.J., Huang, W.C., Chiu, Y.W., Hsu, T.S., Kuo, P.H., Hsu, H.H., Chang, J.K., Cheng, C.C., Lai, C.L., et al. guidelines of taiwan society of cardiology (TSOC) for the management of pulmonary arterial hypertension. Acta Cardiol. Sin. *30*, 2014; 401–444.
6. Davidson, A.E., Cheong, S.S., Hysi, P.G., Venturini, C., Plagnol, V., Ruddle, J.B., Ali, H., Carnt, N., Gardner, J.C., Hassan, H., et al. Association of CHRDL1 mutations and variants with X-linked megalocornea, Neuhäuser syndrome and central corneal thickness. 2014;PLoS One *9*,.
7. Freedman, D.S., Horlick, M., and Berenson, G.S. A comparison of the Slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular disease risk factor levels in children1-4. Am. J. Clin. Nutr. *98*, 2013; 1417–1424.
8. Rothberg, A.E., McEwen, L.N., Kraftson, A.T., Ajluni, N., Fowler, C.E., Nay, C.K., Miller, N.M., Burant, C.F., and Herman, W.H. Impact of weight loss on waist circumference and the components of the metabolic syndrome. BMJ Open Diabetes Res. Care *5*, 20171–7.
9. Physicians, A.C. of. MKSAP : medical knowledge self-assessment program VIII (Philadelphia, PA : American College of Physicians, 2015; c1988-c1989.).

10. Cyr-Depauw, C., Northey, J.J., Tabariès, S., Annis, M.G., Dong, Z., Cory, S., Hallett, M., Rennhack, J.P., Andrechek, E.R., and Siegel, P.M. Chordin-like 1 suppresses BMP4-induced breast cancer cell migration and invasion. Mol. Cell. Biol. *36*, 2016;1509–1525.
11. Brazil, D.P., Church, R.H., Surae, S., Godson, C., and Martin, F. BMP signalling: Agony and antagony in the family. Trends Cell Biol. *25*, 2014; 254–264.
12. Walsh, D.W., Godson, C., Brazil, D.P., and Martin, F. Extracellular BMP-antagonist regulation in development and disease: Tied up in knots. Trends Cell Biol. *20*, 2010; 244–256.
13. Nakayama, N., Han, C.E., Scully, S., Nishinakamura, R., He, C., Zeni, L., Yamane, H., Chang, D., Yu, D., Yokota, T., et al. A novel chordin-like protein inhibitor for bone morphogenetic proteins expressed preferentially in mesenchymal cell lineages. Dev. Biol. *232*, 2001; 372–387.
14. Reis, L.M., Tyler, R.C., Schilter, K.F., Abdul-Rahman, O., Innis, J.W., Kozel, B.A., Schneider, A.S., Bardakjian, T.M., Lose, E.J., Martin, D.M., et al. BMP4 loss-of-function mutations in developmental eye disorders including SHORT syndrome. Hum. Genet. *130*, 2011;495–504.
15. Webb, T.R., Matarin, M., Gardner, J.C., Kelberman, D., Hassan, H., Ang, W., Michaelides, M., Ruddle, J.B., Pennell, C.E., Yazar, S., et al. X-linked megalocornea caused by mutations in CHRDL1 identifies an essential role for ventroptin in anterior segment development. Am. J. Hum. Genet. *90*, 2014;247–259.
16. Stottmann, R.W., Anderson, R.M., and Klingensmith, J. The BMP antagonists Chordin and Noggin have essential but redundant roles in mouse mandibular outgrowth. Dev. Biol. *240*, 2001; 457–473.
17. Kane, R., Godson, C., and O'Brien, C. Chordin-like 1, a bone morphogenetic protein-4 antagonist, is upregulated by hypoxia in human retinal pericytes and plays a role in regulating angiogenesis. Mol. Vis. *14*, 2008;1138–1148.
18. White, K., Dempsie, Y., Nilsen, M., Wright, A.F., Loughlin, L., and MacLean, M.R. The serotonin transporter, gender, and 17beta oestradiol in the development of pulmonary arterial hypertension. Cardiovasc. Res. *90*, 2011; 373–382.
19. Dempsie, Y., and Maclean, M.R. The influence of gender on the development of pulmonary arterial hypertension. Exp. Physiol. *98*, 2013;1257–1261.
20. Dempsie, Y., Nilsen, M., White, K., Mair, K.M., Loughlin, L., Ambartsumian, N., Rabinovitch, M., and MacLean, M.R. Development of pulmonary arterial hypertension in mice over-expressing S100A4/Mts1 is specific to females. Respir. Res. *12*,. 2011
21. Ichimori, H., Kogaki, S., Takahashi, K., Ishida, H., Narita, J., Nawa, N., Baden, H., Uchikawa, T., Okada, Y., and Ozono, K. Drastic shift from positive to negative estrogen effect on bone morphogenetic protein signaling in pulmonary arterial endothelial cells under hypoxia. Circ. J. *77*, 2013; 2119–2126.
22. Singla, S., and Machado, R.F. The imitation game in pulmonary arterial hypertension: Sex, bone morphogenetic protein receptor, and the estrogen paradox. Am. J. Respir. Crit. Care Med. *191*, 2015;612–613.
23. Peacock, A.J., Murphy, N.F., McMurray, J.J., Caballero, L., and Stewart, S. An epidemiological study of pulmonary arterial hypertension. Eur. Respir. J. *30*, 2007 ;104–109.
24. Lam, C.S.P., Roger, V.L., Rodeheffer, R.J., Borlaug, B.A., Enders, F.T., and Redfield, M.M. Pulmonary Hypertension in Heart Failure With Preserved Ejection Fraction. A Community-Based Study. J. Am. Coll. Cardiol. *53*, 2009; 1119–1126.
25. Hoeper, M.M., Barberà, J.A., Channick, R.N., Hassoun, P.M., Lang, I.M., Manes, A., Martinez, F.J., Naeije, R., Olschewski, H., Pepke-Zaba, J., et al. Diagnosis, Assessment, and Treatment of Non-Pulmonary Arterial Hypertension Pulmonary Hypertension. J. Am. Coll. Cardiol. *54*, 2009.
26. Berra, G., Noble, S., Soccal, P.M., Beghetti, M., and Lador, F. Pulmonary hypertension in the elderly: a different disease? Breathe (Sheffield, England) *12*, 2016;43–49.
27. Wright, J.L., Tai, H., Wang, R., Wang, X., and Churg, a. Cigarette smoke upregulates pulmonary vascular matrix metalloproteinases via TNF-alpha signaling. Am. J. Physiol. Lung Cell. Mol. Physiol. *292*, 2007;L125–L133.
28. Zhao, L., Wang, J., Wang, L., Liang, Y.T., Chen, Y.Q., Lu, W.J., and Zhou, W.L. Remodeling of rat pulmonary artery induced by chronic smoking exposure. J. Thorac. Dis. *6*, 2014; 818–828.
29. Archer, A., Weir, E., and Wilkins, M. The Basic Science of pulmonary arterial hypertension for clinicians: new concepts and experiemntal therapies. Circulation *121*, 2006; 2045–2066.
30. Vonk-Noordegraaf, A., Marcus, J.T., Holverda, S., Roseboom, B., and Postmus, P.E. Early changes of cardiac structure and function in COPD patients with mild hypoxemia. Chest *127*, 2005;1898–1903.
31. Elwing, J., and Panos, R.J. Pulmonary hypertension associated with COPD. Int. J. Chron. Obstruct. Pulmon. Dis. *3*, 2008;55–70.
32. Nakagawa, Y., Ikeda, K., Akakabe, Y., Koide, M., Uraoka, M., Yutaka, K.T., Kurimoto-Nakano, R., Takahashi, T., Matoba, S., Yamada, H., et al. Paracrine osteogenic signals via bone morphogenetic protein-2 accelerate the atherosclerotic intimal calcification in vivo. Arterioscler. Thromb. Vasc. Biol. *30*, 2010;1908–1915.
33. ten Dijke, P., and Arthur, H.M. Extracellular control of TGFbeta signalling in vascular development and disease. Nat. Rev. Mol. Cell Biol. *8*, 2007; 857–869.
34. Pardali, E., Goumans, M.J., and ten Dijke, P. Signaling by members of the TGF-β family in vascular morphogenesis and disease. Trends Cell Biol. *20*, 2010;556–567.
35. Doetschman, T., Barnett, J. V., Runyan, R.B., Camenisch, T.D., Heimark, R.L., Granzier, H.L.,

Conway, S.J., and Azhar, M. Transforming growth factor beta signaling in adult cardiovascular diseases and repair. Cell Tissue Res. *347*, 2012;203–223.
36. Upton, P.D., Davies, R.J., Trembath, R.C., and Morrell, N.W. Bone morphogenetic protein (BMP)and activin type II receptors balance BMP9 signals mediated by activin receptor-like kinase-1 in human pulmonary artery endothelial cells. J. Biol. Chem. *284*, 2009;15794–15804.
37. Pardali, E., and Ten Dijke, P. TGFbeta signaling and cardiovascular diseases. Int J Biol Sci *8*, 2012;195–213.
38. Ashwell, M., and Gibson, S. Waist-to-height ratio as an indicator of early health risk: Simpler and more predictive than using a matrix based on BMI and waist circumference. BMJ Open *6*, 2016.
39. Hammod, H.J., Al-Dujaili, A.N., and Al-Dujaili, M.N. The Correlation between cardiovascular diseases in obese men with the inflammatory markers: Dyslipidemia, C-reactive protein and tumor necrosis factor-α. Res. J. Pharm. Biol. Chem. Sci. *7*, 2016;809–814.
40. Hammod, H.J., Al-Dujaili, A.N., and Al-Dujaili, M.N. Relationship between adipocyte fatty acid-binding protein in obese men with cardiovascular diseases. Res. J. Pharm. Biol. Chem. Sci. *7*, 2016; 804–808.
41. Taichman, D.B., and Mandel, J. Epidemiology of Pulmonary Arterial Hypertension. Clin. Chest Med. *28*, 2007;1–22.
42. Burger, C.D., Foreman, A.J., Miller, D.P., Safford, R.E., McGoon, M.D., and Badesch, D.B. Comparison of body habitus in patients with pulmonary arterial hypertension enrolled in the registry to evaluate early and long-term PAH disease management with normative values from the national health and nutrition examination survey. Mayo Clin. Proc. *86*, 2011;105–112.
43. Taraseviciute, A., and Voelkel, N.F. Severe pulmonary hypertension in postmenopausal obese women. Eur. J. Med. Res. *11*, 2006; 198–202.
44. Leone, N., Courbon, D., Thomas, F., Bean, K., Jego, B., Leynaert, B., Guize, L., and Zureik, M. Lung function impairment and metabolic syndrome: the critical role of abdominal obesity. Am. J. Respir. Crit. Care Med. *179*, 2009;509–516.
45. Zeng, W., Sun, Y., Gu, Q., Xiong, C., Li, J., and He, J. The impact of pulmonary arterial hypertension-targeted therapy on survival in Chinese patients with idiopathic pulmonary arterial hypertension. Pulm. Circ. *2*, 2012; 373–378.