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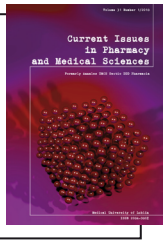

Assessment of noggin level in pulmonary arterial hypertension patients

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ABSTRACT

Noggin (NOG) is a protein that is involved in the development of many body tissues, including nerve tissue, muscles, and bones. The NOG protein plays a role in germ layer-specific derivation of specialized cells. Via NOG, the formation of neural tissues, the notochord, hair follicles, and eye structures arise from the ectoderm germ layer, while noggin activity in the mesoderm gives way to the formation of cartilage, bone and muscle growth. In the endoderm, NOG is involved in the development of the lungs.

NOG dimerizes by a core body, while two pairs of strands extend from it preceding by an N-terminal segment (called a clip segment) with approximately 20 amino acids. This clip twists around the BMP ligand and obstructs the growth factor surfaces from binding to both BMP receptors type I and type II. NOG binding to some BMPs inhibits these from combining and thus activating receptors of BMP, therefore, blocking non-Smad and Smad-dependent signaling.

The anti-proliferative noggin has particular effects in pulmonary arterial smooth muscle cells (PASMCs) that are exposed to specifically down regulated hypoxia. This occurs together with the BMP4 up-regulation levels of protein, and this imbalance between NOG and BMP4 consequence results in the activation and development of PAH disease. Our study consists of numerous examinations so as to explore new biomarkers in order to determine onset of PAH, and to discover the relationship between NOG serum level and gender, age, body mass index (BMI), waist circumferences (WC), smoking, types of PAH primaries and secondaries, as well as their grade.

Abbreviations

bFGF – basic fibroblast growth factor
BMI – body mass index
BMP – Bone Morphogenetic protein
CYP1B1 – Cytochrome P450 1B1
ERK/JAK – Extracellular signal Regulated kinase/Janus associated kinase
IPAH – idiopathic pulmonary arterial hypertension
NFAT – nuclear translocation of transcription factor
NOG – Noggin
p38 – protein 38
PAH – pulmonary arterial hypertension.
PASMCs – pulmonary arterial smooth muscle cells
Smad – Small mother against decapentaplegic
PPAR-γ – Peroxisome Proliferator-Activated Receptor--gamma
SOCE – operated calcium entry
STAT – signal transducer and activator of transcription and p38 MAP Kinase
SYM1 – symphalangism1
SYNS1 – synostosis syndrome 1
TRPC – transient receptor potential canonical

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is considered an infrequent and regularly lethal disease with progressive and sustained rising of mean pulmonary arterial pressure and pathological changes involving vascular remodeling, inflammation and vasoconstriction, leading to heart failure and

hypertrophy in the right ventricular [1-3]. In PAH, smooth muscle cells subsequently undergo excessive proliferation and endothelial dysfunction lead to plexiform lesions, as well as obliteration of the vascular lumen and medial hypertrophy [3,4]. The anti-proliferative NOG effects in PASMCs that are exposed to hypoxia, and the expression of the BMP antagonist NOG, in response to hypoxia, is in PASMC, specifically down regulated [5-7]. This occurs together with the BMP4 up-regulation levels of protein. This imbalance consequence is an activation of the ERK/JAK, STAT and p38 MAP Kinase[8]. Furthermore, due to increased expression of the main channels of store-operated calcium, transient receptor potential canonical (TRPC) 1 and 6 up-regulation elicits an elevation of Ca^{2+} influx that activates nuclear translocation of transcription factor (NFAT) to start the transcription process of promoting genes of PASMC proliferation, a basic pulmonary arterial hypertension feature[9,10].

NOG encoded by the NOG gene, is a homodimeric glycoprotein which is secreted with a 64 kDa molecular mass. NOG was identified with its capability of inducing subordinate formation of axis in Xenopus embryos. NOG release brings about the development of dorsal structures in UV-induced ventralized Xenopus embryos, and putative cDNA injection results in the excessive development of the head, hence the name 'noggin'[11]. The primary structure of NOG has a cysteine-rich carboxy-terminal and an acidic amino-terminal area. The carboxy-terminal region, by the forming of cysteine knots, is useful for classifying BMP antagonists into three different subfamilies: twisted gastrulation (nine-member ring), chordin and noggin (ten-member ring), and cysteine knots residue (eight-membered ring)[12]. NOG is recognized for regulating a metabologens major class, also known as 'bone morphogenetic proteins'.

It is proposed that null mice display significant development abnormalities because of lack of NOG interference with regard to BMP production[13,14]. NOG-null-mice have an increased BMP process which triggers a developmental abnormality series, including neural tube formation failure, weakness of hair follicle, joint lesions and axial skeleton dysmorphogenesis [14]. Since NOG-null-mice are lethal to the embryo, the NOG role in the homeostasis of mature tissue is mostly undecided. However, it is known that increased NOG activity results in skeletal dysplasia, for example, as well as multiple synostosis syndrome 1 (SYNS1) and proximal symphalangism1 (SYM1) [15,16].

Combined with basic fibroblast growth factor (bFGF), NOG is sufficient to uphold human embryonic stem cells (hES) bringing about prolonged growth *in vitro* [17]. Beyond this, a new NOG role in osteolytic cancer cells of prostate has been discovered. Herein, it was indicated that expression of NOG is restricted to cell lines inducing osteolytic metastases of bone. However, prostate cancer cell re-expression of NOG engenders a less osteosclerotic capacity, normalizes the total bone environment structure and balances the remodeling of bone[18].

NOG is also a pleiotropic factor, expressed in early development and final stages. In early gastrulation, Spemann organizer produce NOG and the action of BMP-2, -4, -7 is antagonized, causing the BMP gradient to be directed towards the patterning of the dorsal-ventral with the follow-up formation of the germ layer[19]. The expression of NOG in derivatives of ectoderm, along with the presence of NOG, is vital in ectoderm structure development, such as the formation of neural tubes, teeth, hair follicles and eyes[20,21]. Although neural tube induction occurs in the absence of NOG, NOG was shown to be crucial for neurogenesis[22]. NOG is expressed in the notochord and is increased with exposure to noradrenalin in ectodermal derivatives [23]. Thereupon, over-expression of noggin counteracts BMP-4 activity on neural precursor cells, inducing over-proliferation of neural tissue [24]. NOG has a vital role in the development of the eye. NOG over-expression in the epidermis induces retardation of cell differentiation in the epithelium of the eyelid, as well as decreased apoptosis [21]. NOG-induced effects are also noticeable in the periocular mesenchyme, retina, and lens. Here, its presence releases epiblast ablation and eye defects that are cell induced [20]. The expression of NOG in mesoderm derivatives is also slightly evident in mesoderm-derived tissues and is obligatory for embryonic somite and skeletal patterning. In addition, NOG is crucial for osteogenesis, joint formation, and embryonic chrondrogenesis [25,14]. Finally, osteoblast NOG expression is augmented when BMP-2, -4, -5, -6, -7 are available [8].

MATERIALS AND METHODS

Healthy, patients group & samples collection of blood

The current study included 67 patients suffering from PAH disease, as well as 21 healthy individuals who served as control. The samples were collected from the echocardiography unit in the 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, BMI types, WC types, smoking, primary and secondary PAH, types of secondary PAH and grade. The healthy control group of twenty-one are divided into subgroups according to gender, age, BMI, WC and healthy nonsmoking. A full history of each test subject was also obtained. In the study, 5 ml of venous blood was withdrawn from the test subjects between the hours of 9-11 a.m. via ante cubital venipuncture, using plastic syringes and a disposable needle. Subsequently, the blood was left at room temperature for 10 min in the gel tube to clot. The serum was then isolated after centrifugation at 3000 rpm for 15 minutes. After this, the serum was separated and transported into new disposable tubes (Eppendorf tube) and stored at -20°C.

Exclusion criteria. The history of each individual in the healthy group should have no indication of the presence of PAH, nor heart disease, chronic liver disease, cancer, thyroid disorders, anemia, diabetes mellitus, renal disorders, acute infections and myocardial infarction (MI).

Body mass index (BMI) and Waist circumferences (WC)

BMI is assessed by dividing a person's weight with (kg) by the square of height (m):

$$BMI = \text{Weight (kg)}/(\text{Height m})^2$$

A BMI ranging between 18.5-24.9 kg/m^2 is normal, while that of 25-29.9 kg/m^2 is over-weight, and a BMI greater than (30 kg/m^2) is obese [26]. A normal WC for men is between 102 cm or 40 in, while that for women is 88 cm or 35 in [27]. The measuring of WC is from the top of the iliac crest and the lower margin of the least palpable rib at the midpoint, and is done by stretch-resistant tape.

Primary and Secondary types and Grades of PAH

Primary PAH is a disease with no underlying cause. It comes in two forms, one is called familial, and is a disease that runs in families. The second form is called idiopathic PAH. Secondary PAH is indicated by high pressure being evidenced in the pulmonary vessels due to some other underlying disease, the most common being valvular disease, chronic obstructive pulmonary disease (COPD), congenital heart disease (CHD), left heart disease (LHD) systolic dysfunction or diastolic dysfunction (LHD sys or dia) and pulmonary embolism [28]. In our study, patients were divided into grades of disease (mild, moderate and severe types) depending on echocardiographic results. Herein, mean PAH was classified as mild at 25-35 mmHg, moderate at 35-45 mmHg and severe at more than 45 mmHg, by measuring the pressure gradient on tricuspid valve regurgitation and the pressure gradient on pulmonary valve regurgitation [29,30].

Biomarker measurement

The specific kit for measuring human NOG level in serum was supplied by Elabscience Catalog No: E-EL-H1945/96T.

Test principle of NOG

This ELISA kit uses Sandwich-ELISA as the applied method. The micro ELISA plate provided in this kit was pre-coated with an antibody specific to Human NOG. Standards or samples were then added to the appropriate micro ELISA plate wells and combined with the specific antibody. Subsequently, biotinylated detection antibodies specific for Human NOG and Avidin- Horseradish Peroxidase (HRP) conjugate were added to each micro plate well successively and incubated. After incubation, free components were then washed away. After this, each well was augmented with the Substrate Reagent. Herein, only those wells that contain Human NOG, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by adding Stop Solution and the plate well appears yellow in color. The optical density (OD) is then measured via spectrophotometry at a wavelength of 450 nm ± 2 nm. As the OD value is proportional to the concentration of Human NOG, the concentration of Human NOG in samples can be calculated by comparing the OD of the samples with the standard curve.

Components of Human NOG

Item	Specifications	Storage
Micro ELISA Plate	8 wells ×12 strips	4°C/-20°C
Reference Standard	2 vials	4°C/-20°C
Reference Standard & Sample Diluent	1 vial 20 mL	4°C
Concentrated Biotinylated Detection Ab	1 vial 120 µL	4°C/-20°C
Biotinylated Detection Ab Diluent	1 vial 10 mL	4°C
Concentrated HRP Conjugate	1 vial 120 µL	4°C(shading light)
HRP Conjugate Diluent	1 vial 10 mL	4°C
Concentrated Wash Buffer (25×)	1 vial 30 mL	4°C
Substrate Reagent	1 vial 10 mL	4°C(shading light)
Stop Solution	1 vial 10 mL	4°C
Plate Sealer	5 pieces	
Manual	1 copy	
Certificate of Analysis	1 copy	

Assay procedure

1. Add 100 µL standard or sample to each well. Incubate for 90 min at 37°C.
2. Remove the liquid. Add 100 µL Biotinylated Detection Ab. Incubate for 1 hour at 37°C.
3. Aspirate and wash 3 times.
4. Add 100 µL HRP Conjugate. Incubate for 30 min at 37°C.
5. Aspirate and wash 5 times.
6. Add 90 µL Substrate Reagent. Incubate for 15 min at 37°C.
7. Add 50 µL Stop Solution. Read at 450 nm immediately.
8. Calculation of results.

Statistical analysis

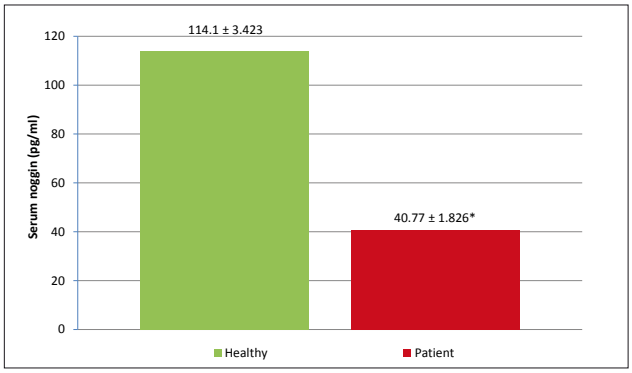
Graphpad crystal v.6 bundles for Windows were utilized to break down the information of the present investigation (Version 6.01, 2012 for Windows 2010); information was directed as Mean ± Standard deviation (SD), In addition, t-testing for unpaired examples was performed for assessing the situation in group comparisons, while one way ANOVA test was utilized so as to obtain the correlation among subgroups in the deliberate parameters.

All figures are worked by utilizing the EXEL program of Microsoft Office 2010. A P value < 0.05 was utilized as the level of statistical significance.

RESULTS

Comparison of NOG serum level between the PAH patients group and the healthy group

A significant decrease (p > 0.05) in NOG serum level was seen in the PAH patients group (40.77 ± 1.826 pg/ml) when compared with the healthy group (114.1 ± 3.423 pg/ml) as showed in Figure 1.

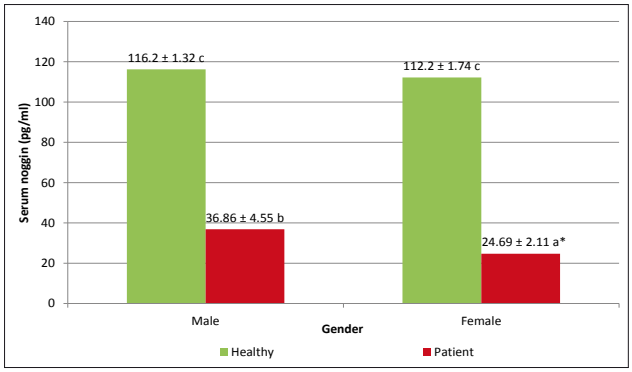


* - represents significant differences at (P > 0.05) between means

Figure 1. Comparison of NOG serum level between the PAH patients group and the healthy group

Comparison of NOG serum level between male and female PAH patients and also between these and their healthy counterparts

As shown in Figure 2, a significant decrease ($p > 0.05$) in the NOG serum level of the female PAH patients group (24.69 ± 2.11 pg/ml) was evidenced when compared with the male PAH patients group (36.86 ± 4.55 pg/ml), and also when compared with the male and female healthy group counterparts (116.2 ± 1.32 pg/ml, 112.2 ± 1.74 pg/ml), respectively.



The dissimilar letters represent significant differences (P > 0.05) between the different groups
The similar letters represent non-significant differences
*- represents significant differences at (P > 0.05) between means

Figure 2. Comparison of NOG serum level between males and females in the PAH patients group and between these and their healthy group counterparts according to the gender

Comparison of NOG serum level among different age subgroups of the PAH patients group and between these and their healthy group counterparts

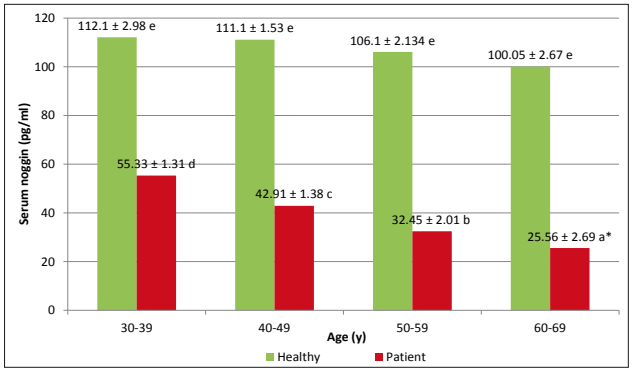


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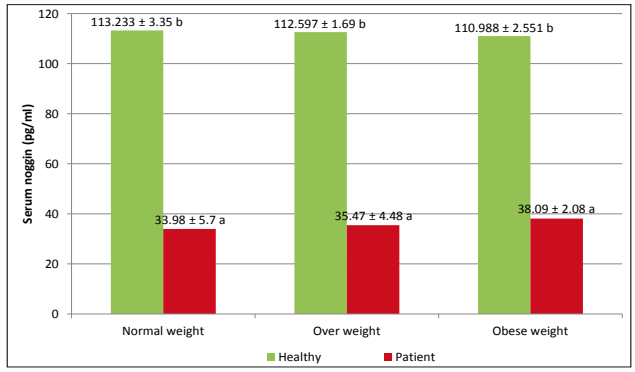
Figure 3. Comparison of NOG serum level among different age subgroups within the PAH patients group, and with their healthy group subgroup counterparts

Figure 3 reveals that there is a significant decrease (P > 0.05) in NOG serum level with respect to the age subgrouping within the PAH patients group (25.56 ± 2.69 pg/ml, 32.45 ± 2.01 pg/ml, 42.91 ± 1.38 pg/ml and 55.33 ± 1.3 pg/ml) when compared with their healthy counterparts (100.05 ± 2.67 pg/ml, 106.1 ± 2.134 pg/ml, 111.1 ± 1.53 pg/ml and 112.1 ± 2.98 pg/ml) for the ages 60-69y, 50-59y, 40-49y and 30-39y, respectively. The age group 60-69y shows the lowest significant decrease (P > 0.05) in NOG serum level (25.56 ± 2.69 pg/ml) compared with the other age subgroups, while the results shows non-significant differences between the healthy sub-groups in all ages (Figure 3).

Comparison of NOG serum level between the PAH patients group and the healthy group according to BMI (normal weight, over weight and obese weight)

The results indicated in Figure 4 show that there are non-significant differences in NOG serum level between the PAH patients sub-groups, while there are significant decreases (P > 0.05) in a comparison with the non-PAH (healthy) patients subgroups (33.98 ± 5.7 pg/ml, 35.47 ± 4.48 pg/ml and 38.09 ± 2.08 pg/ml, respectively) for the PAH subgroups compared with their healthy group counterparts (113.233 ± 3.35 pg/ml, 112.597 ± 1.69 pg/ml and 110.988 ± 2.251 pg/ml, respectively), according to BMI (normal weight, over weight and obese weight).

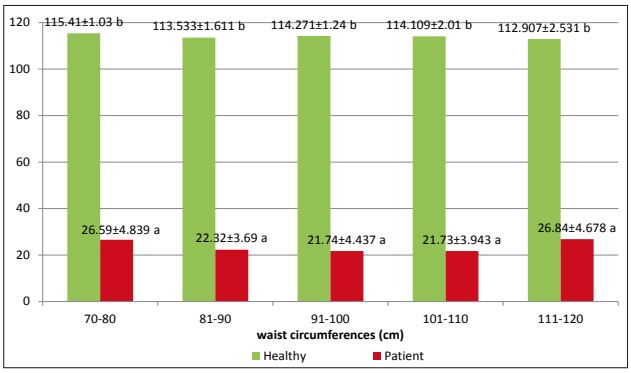


The dissimilar letters represent significant differences (P > 0.05) between different groups
The similar letters represent non-significant differences

Figure 4. Comparison of NOG serum level between the PAH patients group and the healthy group according to BMI (normal weight, over weight and obese weight)

Comparison of NOG serum level among the PAH patients subgroups and their healthy subgroup counterparts, according to waist circumferences

The results seen in Figure 5 reveal non-significant differences between all subgroups in the PAH patients group of waist circumferences, while there are significant decreases when compared with their non-PAH or healthy counterparts (26.59 ± 4.839 pg/ml, 22.32 ± 3.69 pg/ml, 21.74 ± 4.437 pg/ml, 21.73 ± 3.943 pg/ml and 26.84 ± 4.678 pg/ml, respectively) for the PAH subgroups, as compared with their healthy subgroup counterparts (115.41 ± 1.03 pg/ml, 113.533 ± 1.611 pg/ml, 114.271 ± 1.24 pg/ml, 114.109 ± 2.01 pg/ml and 112.907 ± 2.531 pg/ml, respectively), according to waist circumferences (70-80 cm, 81-90 cm, 91-100 cm, 101-110 cm and 111-120 cm).

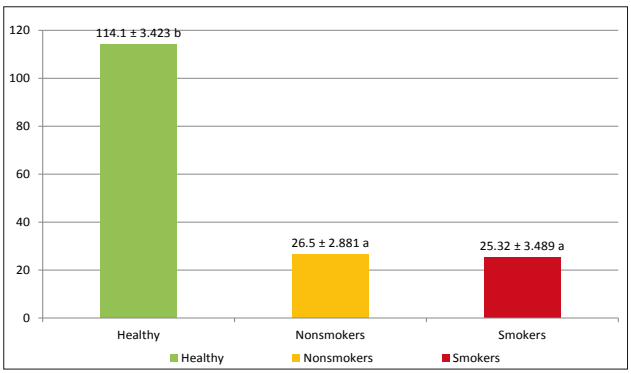


The dissimilar letters represent significant differences (P > 0.05) between different groups
The similar letters represent non-significant differences

Figure 5. Comparison of NOG serum level among PAH patient subgroups and their healthy subgroup counterparts, according to WC

Comparison of NOG serum level between PAH patient subgroups (nonsmokers and smokers) and between these and the nonsmoker healthy subgroup

The results revealed in Figure 6 shows non-significant differences in NOG serum level between PAH patient nonsmokers and smokers subgroups, while there is significant decreases ($p > 0.05$) in NOG serum levels between PAH patient nonsmokers and smokers subgroups (26.5 ± 2.881 pg/ml and 25.32 ± 3.489 pg/ml, respectively) compared to the healthy nonsmoker sub group (nonsmokers) 114.1 ± 3.423 pg/ml.

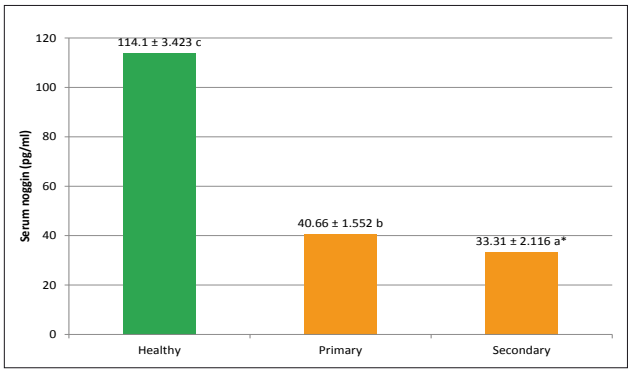


The dissimilar letters represent significant differences (P > 0.05) between different subgroups
The similar letters represent non-significant differences

Figure 6. Comparison of NOG serum level between PAH non-smokers and smokers subgroups and between these and the non-smoker healthy subgroup

Comparison of NOG serum level between PAH primary and secondary subgroups and between these and the healthy group.

The results indicated in Figure 7 reveal there are significant decreases ($p > 0.05$) in NOG serum level of the PAH secondary subgroup (33.31 ± 2.116 pg/ml) when compared with the primary subgroup and also when compared with the healthy group (40.66 ± 1.552 pg/ml and 114.1 ± 3.423 pg/ml, respectively).

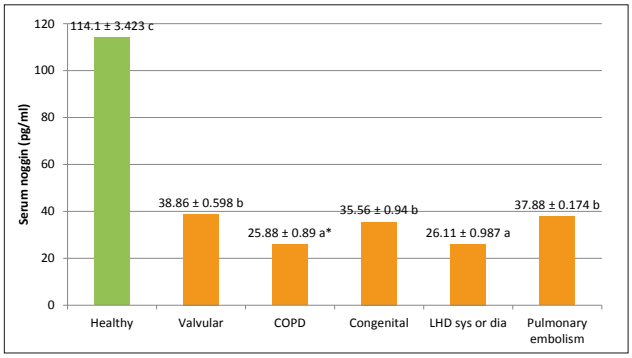


The dissimilar letters represent significant differences (P > 0.05) between different groups
* - represent significant differences at (p>0.05) between means

Figure 7. Comparison of NOG serum levels between PAH primary and secondary subgroups and between these and the healthy group

Comparison of NOG serum level between the PAH subgroups of different secondary diseases and between these and the healthy group

The results shown in Figure 8 reveals a significant decrease ($p > 0.05$) between the PAH COPD and LHD sys or dia subgroups (25.88 ± 0.89 pg/ml and 26.11 ± 0.987 pg/ml, respectively) and when compared with the healthy group. In the former, valvular, congenital and pulmonary embolism are at 114.1 ± 3.423 pg/ml, 38.86 ± 0.598 pg/ml, 35.56 ± 0.94 pg/ml and 37.88 ± 0.174 pg/ml respectively, while the results gives non-significant differences between COPD and (LHD sys or dia) subgroups alone. The figure also reveals non-significant differences among valvular, congenital and pulmonary embolism subgroups.

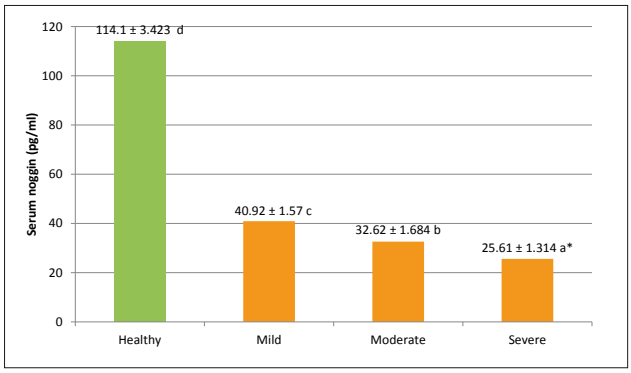


The dissimilar letters represent significant differences (P > 0.05) between different groups.
The similar letters represent non-significant differences.
* - represent significant differences at (p>0.05) between means.

Figure 8. Comparison of NOG serum level between PAH patients group of different secondary diseases and and between theses and the healthy group

Comparison of NOG serum level between PAH primary and secondary subgroups of different grades and between these and the healthy group

The results revealed in Figure 9 show a significant decrease ($p > 0.05$) among the different grades, with severe at 25.61 ± 1.314 pg/ml, moderate at 32.62 ± 1.684 pg/ml and mild at 40.92 ± 1.57 pg/ml, respectively, of PAH primary and secondary subgroups as compared with the healthy group (114.1 ± 3.423 pg/ml).



The dissimilar letters represent significant differences (P > 0.05)
* - represent significant differences at (P > 0.05) between means

Figure 9. Comparison of NOG serum level between PAH primary and secondary subgroups and the Healthy group, as well as between the various secondary subgroups of different grades and the healthy group

DISCUSSION

The results exhibit significant decreases (p > 0.05) in NOG serum level in the PAH patients group when compared with the healthy group – as showed in Figure 1. Elevations in BMP4 level expression have been associated with down-regulation of NOG level in both pulmonary arterial smooth muscle cell (PASMC) and lung tissue that has been exposed to hypoxia, therefore, the loss of NOG in hypoxia might account for the triggered BMP4 signaling transduction [10]. Furthermore, the elevation in the BMP4 signaling pathway due to the decrease of expression in the naturally endogenous BMP4 antagonist NOG, as well as due to partly at least hypoxia and signaling transduction probably triggered BMP4 activity due to the lack of the protective role of NOG. This evoked BMP4 signaling which leads to up-regulation of the transient receptor potential cation channels (TRPC) store operated calcium entry (SOCE) axis. This event eventually resulted in excessive PASMC proliferation in PH development [31].

Pathological changes in high progressive mean pulmonary arterial pressure have been associated with low levels of NOG, and lead to inflammation, vasoconstriction, vascular remodeling, heart failure and right ventricular hypertrophy [3]. The decrement in NOG level in PAH with excessive proliferation of smooth muscle cells and endothelial dysfunction progresses towards the obliteration of the vascular lumen and to hypertrophy of the plexiform lesion [4].

Many factors have been shown to play important roles in PAH pathogenesis. These include NOG level decrement with vasoconstriction and persistent hypoxemia [3]. The study of Boucherat and Bonnet (2015) [9] confirms that NOG is a new therapeutic target for pulmonary hypertension as it inhibits hypoxia-induced proliferation by targeting store-operated calcium entry and transient receptor potential cation channels [9]. A study by Wang *et al*. (2015) [6] employed the NOG BMP4 antagonist to block BMP4 signaling so as to eliminate endogenous BMP4 expression. Here, hypoxic responses were completely blocked by pretreatment with the BMP antagonist NOG [6]. In addition, the study of La Rosa *et al*. (2011) [32] showed that high levels of BMP4 are implicated in increased cell proliferation, as does a low NOG level. Hence, the correct balance of BMP4 signaling and NOG presence is needed for proper tissue development [32].

The result seen in Figure 2 indicate a significant decrease (p > 0.05) in serum NOG level in the female PAH patients group, as compared with the male patients group and with the male and female healthy subgroups. The NOG level decreased in PAH females as compared with males may be explained by the role of estradiol on BMP signaling, as this has been shown to be important in homeostasis roles inside the lung [33]. That BMP inhibits proteins in females more than in males have been suggested to play important roles in modulating NOG protein, as the high expression of BMP leads to low expression of BMPR2 and NOG in serum. A study by White *et al*. (2012) [34] suggests that high expression of CYP1B1 (a cytochrome enzyme) catalyzes the conversion of estradiol to 4-hydroxyestrogen in pulmonary arterial hypertension patients, and induces polymorphism in the CYP1B1 genotype, hence enabling a high penetration in PAH patients, which, in turn, is associated with BMRP2 and NOG mutation, and leads to decreased NOG levels in serum [35].

The results revealed in Figure 3 demonstrate that there are significant decreases (P > 0.05) in serum NOG levels within the different PAH patient age subgroups, as compared with their healthy counterparts. This is particularly noticeable for the ages 60-69y, 50-59y, 40-49y and 30-39y. Herein, the 60-69y age group shows the lowest significant decrease (p > 0.05) in serum NOG level when compared with the other ages, while the results show non-significant differences between all healthy age subgroups (Figure 3). The low level of NOG in the elderly is probably due to the low expression of NOG that comes about because of the altered signaling of BMPR2 which develops with the progression of age. It has been demonstrated that age-related vascular stiffness contributes to systemic systolic hypertension in elderly] [36-38] and leads to pulmonary artery thickening [39-41]. These events may subsequently contribute to alterations in BMP signaling, along with low expression of NOG levels.

Our study demonstrates non-significant differences in NOG serum level between the smoker and nonsmoker PAH patients subgroups (Figure 6). The current results are in disagreement with the study of Zhao *et al*. (2014) [42]who showed that remolding of rats pulmonary artery is induced by chronic smoking exposure and up-regulation of BMP and TRPC expressions, which in turn, leads to altered NOG level [42].

The results evidenced in Figure 7 reveals there are significant decreases (p > 0.05) in NOG serum levels in the Secondary PAH patient subgroup, as compared with the primary PAH patient subgroup. Secondary PAH comes about with mis-regulation of TGF-β signaling and BMP4 stimulation which induces the proliferation and migration of vascular SMCs and a decrease in NOG [43].

Figure 8 shows the presence of a significant decrease (p > 0.05) in NOG levels within COPD and LHD sys or dia PAH patient subgroups, as compared with the healthy group and those with valvular, congenital and pulmonary embolism. At the same time, non-significant differences are indicated between COPD and LHD sys or dia patient

subgroups, or with valvular, congenital and pulmonary embolism pAH subgroups and the healthy group. The significant increase in secondary pulmonary hypertension COPD may be the result of complications that occur, because COPD is an important risk factor for PAH and it has been demonstrated that 25% of all COPD patients have progressive increases in PAH [44,45].

The aforementioned results may have come about due to the roles of both PPAR-γ and BMP4 in the regulation of upstream Ca^{++}. PPAR-γ expression is found to be inhibited while BMP4 is up-regulated in both pulmonary arteries and lung. In such situations, Ca^{++} is induced as a vasoconstriction by BMP4 signaling in the pulmonary arterial smooth muscle cells [46,47]. What is more, an increase in the expression of BMPR2 occurs in the arteries alone and not in the lung, therefore, the signaling can be altered and the level of BMPR2 and NOG be reduced in serum, leading to vascular remolding in PAH.

A study by Boucherat and Bonnet (2015) [9] determines that in rat lungs, chronic hypoxia exposure leads to significantly diminished NOG levels and a reduction in the expression of NOG. This promotes pulmonary artery wall proliferation and apoptosis by the BMP4 inhibitor which induces ERK1/2, JAK2, STAT3 and p38 phosphorylation [9]. In addition, the hypoxial condition induces greater stored operated calcium entry by enhancing the expression of TRPC1 and TRPC6 which are considered to be the main calcium channels responsible for calcium influx. Boucherat and Bonnet also note that, more importantly, NOG expression leads to attenuating these channels [7]. The present results document that the diminished level of NOG may lead to the induction of more store operated calcium entry and the promotion of cell proliferation and resistance in pulmonary arteries in PAH scenarios.

The results displayed in Figure 9 reveal a significant decrease ($p > 0.05$) among different grade subgroups (severe, moderate and mild) of PAH secondary and primary patients. The low expression of NOG antagonist BMP signaling may be promoted by enhanced disease severity as a result of inducing more expression of TRPC1 and TRPC6, and greater up-regulation of BMP4. The inhibition of hypoxia-induced proliferation by NOG, in addition to targeting store-operated calcium entry and transient receptor potential cation channels [10], therefore, decreases the expression of NOG binding with the severity of PAH disease.

Our results indicate there are non-significant differences in serum NOG level and body mass indexes (normal weight, over weight, obese weight) and waist circumferences in PAH patients (as seen in Figures 4 and 5). The studies of Ashwell and Gibson (2016) [48], Hammod *et al.* (2016) [49] and Hammod *et al.* (2016) [50] of adult cardiometabolic risk factors are in agreement with the present study. We recommend that the WC and BMI be adjusted to show that a high WC is non-problematic in healthy BMI ranges [48-50]. This is because the BMI and WC of PAH patients may vary depending on heredity, physiological metabolism factors, type of food intake, type of physical activities performed by the individual on a daily basis and the period of the disease. Hence, we find in PAH patients who are either obese, overweight, of normal weight, and who hold large or small Wcs, no significant relation between NOG level and the BMI, WC criteria.

The non-significant differences with NOG and the relationship with BMI and WC may be explained by Taichman and Mandel (2007) [51] who demonstrated the absence of relation between obesity and BMI and that obesity is not to be considered a risk factor for PAH [51]. Furthermore, the study of (Burger *et al*. (2011) [52] also showed no significant differences in BMI between PAH patients. Therein, the obese percentage was ≥ 30%, normal weight was > 20.8% and underweight was < 18.5% of the entire study group [52]. However, the work of Leone *et al*. (2009) [53] and Taraseviciute and Voelkel (2006) [54] indicate that in patients with IPAH and metabolic syndrome, a high frequency of obesity coexists with impaired lung function. They also document that metabolic syndrome is an independent predictor for IPAH. The current study excluded other factors that affected PAH patients (metabolic syndrome, diabetes, thyroid disorder, chronic liver disease and renal disorder), therefore, our results indicate that there is no relationship between BMI and PAH [53,54]. Still, a former study by Zeng *et al*. (2012) [55]showed an association between lower BMI as factor for PAH in younger patients, as well as a prognosis for IPAH and enhanced mortality [55].

CONCLUSION

Noggin is a suitable biomarker for predicting and identifying PAH.

SIGNIFICANCE STATEMENTS

This examination is a primary clinical investigation that occurred in Iraq.

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