

The Plasma Concentration Levels of Adrenomedullin in Egyptian Patients Complaining of Mitral Stenosis, With and Without Pulmonary Hypertension

¹Safa Refaat, ²Hany Abdel Rahman Negm,
³Nagwa Abdel Ghaffar Mohamed, ⁴Khaled Younes and ⁵Azza Khalil

¹Departments of Internal Medicine, Research Institute of Ophthalmology,

²Cardiology, Research Institute of Ophthalmology,

³Clinical and Chemical Pathology, National Research Center.

⁴Internal Medicine, National Research Center and

⁵Clinical Pathology, Research Institute of Ophthalmology,

Abstract: Mitral stenosis is associated with increased pulmonary artery pressure and resistance. Adrenomedullin, discovered in human pheochromocytoma tissue in 1993, has shown to dilate the pulmonary vessels and so may participate in the control of pulmonary circulation. In this study, we assessed the plasma concentration of adrenomedullin in patients complaining of rheumatic mitral stenosis, with and without pulmonary hypertension. The study revealed a significant increase in the plasma concentration of adrenomedullin, in patients with rheumatic mitral stenosis without pulmonary hypertension and highly significant increase in the plasma adrenomedullin concentration in patients with rheumatic mitral stenosis and pulmonary hypertension. The study revealed also a significant positive correlation between the plasma level of adrenomedullin and mean heart rate, mitral valve area and peak pulmonary artery systolic pressure in all cases of mitral stenosis, with and without pulmonary hypertension. In spite of the increased production of adrenomedullin, supplement of adrenomedullin would be of beneficial effect in improving deteriorated conditions.

Key words: adrenomedullin, mitral stenosis and pulmonary hypertension

INTRODUCTION

Adrenomedullin (AMD), is a 52 amino acid peptide, was discovered in human pheochromocytoma tissue in 1993 by Kitamura and colleagues (Kitamura, *et al.*, 1993). The adrenomedullin gene is situated in a single locus on chromosome 11 in humans and genomic DNA for human adrenomedullin consists of four exons and three introns (Ishimitsu, *et al.*, 1994). Its structure has some similarities to calcitonin, calcitonin gene related peptide, and amylin (Muff, *et al.*, 1995).

It is possible that some of the biological actions of this peptide are manifested by stimulation of calcitonin gene related peptide receptors, but reports suggest that there is also a specific adrenomedullin receptor that activates adenylcyclase through a G protein coupled mechanism (Kapas, *et al.*, 1995).

Adrenomedullin bioactivity may be manifested through a number of second messenger systems especially cAMP, but also nitric oxide, intracellular calcium and tissue prostaglandin (Yang, *et al.*, 1996). Adrenomedullin mRNA is highly expressed in the adrenal gland, lung, kidney, heart and vascular walls (Sugo, *et al.*, 1994). A variety of substances including adrenocortical steroids, thyroid hormone, interleukin1, tumor necrosis factor and lipopolysaccharides promote messages on production of the peptide (Sugo, *et al.*, 1995).

Peptide levels in plasma are raised in patients with essential hypertension, impaired renal functions, primary aldosteronism, poorly controlled diabetes, after acute myocardial infarction, in congestive heart failure in proportion to the severity of cardiac dysfunction and in thyrotoxicosis (Ishimitsu, *et al.*, 1994).

Corresponding Author: Safa Refaat, Departments of Internal Medicine, Research Institute of Ophthalmology, Departments of Clinical and Chemical.

Markedly elevated levels have been recorded across hypothermic cardiopulmonary bypass, in patients with a variety of severe illnesses and particularly in sepsis (Ehlenz, *et al.*, 1999).

Nakamura *et al* 1997, reported that adrenomedullin had approximately 10-fold and 200-fold greater vasodilator potency than sodium nitroprusside and acetylcholine respectively, when infused into the brachial artery in humans (Nakamura, *et al.*, 1997).

A link between adrenomedullin and endothelin has been noted in experimental models in both *in vitro* and *in vivo* suggesting that this autacoid's may act as a local modulator of the effects of endothelin in the cardiovascular system and in the kidney (Shindo, *et al.*, 1998).

Circulating adrenomedullin is partially metabolized in the lungs, which suggests an abundance of adrenomedullin receptors in the lungs (Nishikimi, *et al.*, 1994). Mitral stenosis is associated with increases in pulmonary artery pressure and resistance. Vasoactive substances such as endothelin and catecholamines have been linked to this disorder (Yamamoto, *et al.*, 1989).

Adrenomedullin has been shown to dilate the pulmonary vessels and increase pulmonary blood flow. These findings suggest that adrenomedullin may participate in the control of the pulmonary circulation (Heaton, *et al.*, 1995).

MATERIALS AND METHODS

Fifty subjects participated in this study, which was approved by the local ethical comity, 20 of them were complaining of rheumatic mitral stenosis without pulmonary hypertension(group A), 20 of them were complaining of rheumatic mitral stenosis and pulmonary hypertension(group B) and 10 subjects were apparently healthy and matched for age and sex as a control group(group C).

All patients were selected from the outpatient clinics of cardiology and internal medicine of the research institute of ophthalmology and the national research center. A written consent was taken from each subject in the study.

Patients had been subjected to the following diagnostic work:

- Medical history with special concentration on the symptoms suggestive of pulmonary hypertension including dyspnea on exertion, chest pain, syncope or presyncope hoarseness of voice.
- Clinical examination to elicit signs of pulmonary hypertension including clinical findings of mitral stenosis in addition to loud pulmonary components of second heart sound, s3 gallop murmur of pulmonary origin and tricuspid insufficiency, elevated jugular venous pulsations and right ventricular hypertrophy.
- Plain chest x-ray.
- Transthoracic echo Doppler study to:

Diagnose mitral stenosis and determine its severity through measuring mitral valve area by planimetry of mitral valve area by two dimensional echo cardio graphic examination from short axis left parasternal view at mitral valve level.

This was followed by calculating mitral valve area by pressure half time method through the formula:

- Mitral valve area = $220/\text{pressure half time}$.

Pressure half time is measured by examining the left ventricular inflow tract at mitral valve level by combined two dimensional and pulsed wave Doppler using 3.5 mega Hertz transducer from two chamber apical view.

- Both readings of mitral valve areas had been averaged

Right ventricular inflow was examined by combined two dimensional and continuous wave Doppler from four chamber apical view, peak pulmonary artery systolic pressure (PPASP) was estimated by measuring tricuspid regurgitation maximum velocity (Vmax) and using the principles of modified Brenolli equation formula PPASP (predicted pulmonary artery systolic pressure) = $4V_{\text{max}}^2$: (4 multiplied by Vmax square).

- 10 mmHg had been added to all calculated values.

Antecubital venous blood samples (about 5ml) were taken from each subject participating in the study in the early morning on a tube containing EDTA (1mg/ml) and aprotinin (500U/ml). The plasma was separated by centrifugation at 4°C and stored at -80°C for analysis of human adrenomedullin by radioimmunoassay after extraction and purification (Kitamura, *et al.*, 1994). The kit was supplied from Peninsula Laboratories (Peninsula Laboratories Inc. 601 Taylor Way, San Carlos, CA 94070, California, USA).

Statistical Analysis: Data were expressed as mean ± standard deviation (M ± SD). Student’s unpaired t test was used to evaluate differences between normal subjects and patients with mitral stenosis only and patients with mitral stenosis and pulmonary hypertension. Plasma adrenomedullin was also evaluated by the student paired t test. Correlation coefficients were calculated by linear regressive analysis

RESULTS AND DISCUSSION

The study revealed increased plasma concentrations of adrenomedullin in all cases with mitral stenosis, especially those with pulmonary hypertension (table 1).

A significant positive correlation between the plasma levels of adrenomedullin and mean heart rate, peak pulmonary artery systolic pressure and maximum tricuspid regurge velocity was evaluated in patients with mitral stenosis only (p<0.05 in all) and more markedly in patients with mitral stenosis and pulmonary hypertension (p< 0.05, < 0.001 and <0.001 respectively) (table 3).

The study revealed also a negative significant correlation between mitral valve area and plasma adrenomedullin in patients of mitral stenosis, either with (p<0.001) or without (p<0.05) pulmonary hypertension (table 3).

Table 1: The clinical and biochemical characteristics of all groups participating in the study

Groups	Group (A)	Group (B)	Group(C)	P value
Parameters				
-Age (years)	38.1±5.2	41.2± 3.3	39.3± 4.1	<0.05
-Sex males	11	8	5	>0.05
females	9	12	5	
Plasma ADM (pmol/L)	8.3 ±1.2	11.5± 2.8	4.8 ± 0.8	<0.001
-MHR/m	83± 6	92± 3	73 ±1	<0.05
-MSBP (mmHg)	122 ±11	119 ±12	120 ±13	>0.05
-MDBP (mmHg)	78± 3	76± 6	81± 4	>0.05

-Group (A): patients with mitral stenosis only.

-Group (B): patients with mitral stenosis and pulmonary hypertension.

-Group(C): control group.

-ADM: adrenomedullin.

-P mol: picomol per liter.

-MHR: mean heart rate.

-MSBP: mean systolic blood pressure.

-MDBP: mean diastolic blood pressure.

- P>0.05: Non significant. P<0.05: significant. P<0.01: highly significant.

Adrenomedullin (ADM) is a pleiotropic polypeptide with potent vasorelaxant properties that is now considered as a paracrine or autocrine factor in the regulation of cardiovascular homeostasis (Kitamura and Eto, 1997).

ADM has a slight homology with calcitonin gene related peptide (CGRP) and its hypotensive activity is comparable with that of CGRP (Sakota, *et al.*, 1994). The plasma level of ADM would be expected to be the sum of synthesis and secretion from the various compartments in which it originates and its degradation in the serum (Palanisuramy, *et al.*, 1998).

Table 2: Echo-cardiographic findings of all subjects participating in the study

parameters	Group(A)	Group(B)	Group(C)	P value
MVA (cm ²)	1.5±0.35	1.23±0.37	4.95 ± 0.93	<0.001
MTRV(m/second)	1.3±0.42	3.27±0.94	0.51±0.23	<0.001
PPASP(mmHg)	17.45±4.32	56.08±24.06	11.26±0.99	<0.001

-MVA: mitral valve area.

-MTRV: maximum tricuspid regurge velocity.

-PPASP: peak pulmonary artery systolic pressure.

- P>0.05: Non significant. P<0.05: significant. P<0.01: highly significant.

Table 3: The correlation of plasma adrenomedullin to other parameters for all subjects participating in the study

parameters	Group(A)		Group(B)		Group(C)	
	r	p	r	p	r	p
-Age	0.52	<0.05	0.61	<0.05	0.21	>0.05
-Sex	0.24	>0.05	0.19	>0.05	0.23	>0.05
-MHR/min.	0.61	<0.05	0.58	<0.05	0.18	>0.05
-MSBP	0.31	>0.05	0.28	>0.05	0.22	>0.05
-MDBP	0.29	>0.05	0.27	>0.05	0.25	>0.05
-MVA	-0.62	<0.05	-0.74	<0.001	0.19	>0.05
-MTRV	0.59	<0.05	0.78	<0.001	0.22	>0.05
-PPASP	0.75	<0.001	0.82	<0.001	0.26	>0.05

-MHR: mean heart rate.

-MSBP: mean systolic blood pressure.

-MDBP: mean diastolic blood pressure. -MVA: mitral valve area.

-MTRV: maximum tricuspid regurge velocity.

-PPASP: peak pulmonary artery systolic pressure.

- P>0.05: Non significant. P<0.05: significant. P<0.01: highly significant.

ADM released from endothelial cells may have endocrine effects throughout the systemic and pulmonary circulations. It may also have local or paracrine effects on the neighboring smooth muscle cells (Shimokuko, *et al.*, 1995).

ADM secreted into the interstitium by endothelial cells may activate GS-proteins present in smooth muscle cells and thereby increase adenylyl cyclase activity. The resultant decrease in calcium sensitivity will result in vasorelaxation (Shimokuko, *et al.*, 1995).

In this study we assessed the plasma concentrations of adrenomedullin and the echo findings of all subjects participating in the study who were classified into 3 groups. One of them was complaining of rheumatic mitral stenosis without pulmonary hypertension. The second group was complaining of rheumatic mitral stenosis complicated by pulmonary hypertension and the third group was free of any cardiovascular or pulmonary diseases as proved by echocardiography, ECG and plain chest X-ray.

The study revealed an elevated plasma level of adrenomedullin in patients of the first and second groups and this elevation is significant in patients complaining of rheumatic mitral stenosis only and highly significant in patients complaining of rheumatic mitral stenosis complicated by pulmonary hypertension. The study revealed also a significant positive correlation between the level of adrenomedullin and the peak pulmonary artery systolic pressure.

Toshio, *et al* 1997 showed in their study that venous concentrations of adrenomedullin were increased in patients with mitral stenosis compared with age matched normal controls, and that there was a significant reduction in ADM from the pulmonary artery to the left atrium. Venous concentrations of ADM were correlated with the pulmonary artery pressure, pulmonary vascular resistance and total pulmonary resistance.

The stimulation of ADM production had been attributed increased sympathetic nerve activity and body fluid volume (Sugo, *et al.*, 1994).

According to a report on the genomic structure of the human ADM gene, the 5'-flanking region contains multiple binding sites activator protein - 2 (AP-2) and cyclic adenosine monophosphate (cAMP) regulated

enhancer elements, suggesting that the expression of ADM gene may be the subject to the activity of protein kinase C and cAMP concentrations (Ishimitsu, *et al.*, 1994).

Because plasma noradrenalin is increased in mitral stenosis and noradrenalin is known to activate protein kinase C and phospholipase C through the α_1 receptor, increased noradrenalin concentrations in mitral stenosis may partially affect the gene expression of ADM through the α_1 receptor (Tsuchihashi, *et al.*, 1993).

Adrenomedullin has been shown to reduce pulmonary artery pressure by decreasing the pulmonary vascular resistance, partially mediated by nitric oxide (Nossaman, *et al.*, 1996).

Toshio *et al* 1997 observed significant relations between the plasma concentrations of ADM and the pulmonary artery pressure, pulmonary vascular resistance and the total pulmonary resistance which suggests that specific ADM binding sites in the pulmonary vasculature are present and that ADM may be involved in the defense mechanisms against further increase in pulmonary artery pressure in patients with mitral stenosis.

Hoepfer *et al* 2000 stated that endogenous ADM production is enhanced in a variety of cardiovascular diseases through a compensatory mechanism that the addition of supplementary ADM has beneficial effects in these diseases suggesting that endogenous ADM level is not sufficient enough to improve deteriorated conditions in spite of the increased ADM production.

Naritashi and Kenji, *et al* 2004 proved that intravenous infusion of ADM increased ADM level in patients with pulmonary hypertension and that this infusion significantly decreased pulmonary vascular resistance without inducing marked systemic hypotension and these results suggested that ADM has potent, relatively long lasting pulmonary vasodilator activity in patients with pulmonary hypertension as the administered ADM increases plasma cAMP, but not cGMP, in patients with pulmonary hypertension and the increase in cAMP in smooth muscle cells by ADM activates protein kinase A, resulting in the decrease in calcium content in smooth muscle cells and so, it is therefore possible that ADM may relax vascular smooth muscles through a cAMP protein kinase A dependant mechanism.

The inhalation of aerolized prostacyclin and its analogue, iloprost, has shown to cause pulmonary vasodilatation without systemic hypotension. In clinical settings, inhalation therapy may be more simple, noninvasive and comfortable than continuous infusion therapy (Hoepfer, *et al.*, 2000).

Conclusion: Plasma adrenomedullin levels increase in cases of mitral stenosis, especially those associated with pulmonary hypertension, as a compensatory mechanism to control the pulmonary circulation. In spite of this increase, adrenomedullin supplement either by intravenous infusion or more recently by inhalation would be of beneficial effect in improving deteriorated conditions, but more investigations are recommended concerning this subject.

REFERENCES

- Ehlenz, K, B Koch and P Preuss, 1999. High levels of circulating adrenomedullin in severe illness. *Exp. Clinical Endocrinology and Diabetes*, 105: 156-162.
- Heaton, J, B Lizi, JK Chang, S Steinberg, A Hyman and H Lippert, 1995. Pulmonary vasodilation to adrenomedullin: a novel peptide in humans. *American Journal of Physiology*, 268: 2211-2215.
- Hoepfer, MM, M Schwarze, S Ehlerding, A Adler-Schueurmeyer, E Speikerkoetter and J Niedermayer, 2000. Long term treatment of primary pulmonary hypertension with aerolized iloprost, a prostacyclin analogue. *New England Journal of Medicine*, 342: 1866-1870.
- Ishimitsu, T, T Nishikimi and Y Saito, 1994. Plasma Levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. *Journal of Clinical Investigation*, 94: 2158-2161.
- Ishimitsu, T, M Kojima and K Kangam, 1994. Genomic structure of human adrenomedullin gene. *Biochem. Biophys. Res. Commun.*, 203: 631-639.
- Kapas, S, KJ Catt and AJL Clark, 1995. Cloning and expression of cDNA encoding a rat adrenomedullin receptor. *Journal Biological Chemistry*, 270: 25344-25347.
- Kitamura K, Y Ichiki, M Tanaka, M Kawamoto, J Emura, S Sakakibara, K Kangawa, H Matsuo and T Eto, 1994. Immunoreactive adrenomedullin in human plasma. *FEBS (Fed.Eur.Biochem. Soc.) Lett.*, 341: 288-290.
- Kitamura, K and T Eto, 1997. Physiological regulator of the cardiovascular system or biochemical curiosity? *Current Opinion in Nephrology and Hypertension.*, 6: 80-87.

Kitamura, K, K Kangawa and M Kamamoto 1993. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res. Commun.*, 192: 533-560.

Muff, R, W Born and A Fischer, 1995. Calcitonin, calcitonin gene related peptide, adrenomedullin and amylin: homogenous peptides, separate receptors and overlapping biological actions. *Euro Journal Endocrinology*, 133: 17-20.

Nakamura, M, M Yoshida, N Arakura, S Makita, M Nunuma and K Hiramori, 1997. The vasodilator potency of adrenomedullin in human forearm vascular bed. *Circulation*, 95: 1214-1221.

Naritashi, N and K Kenji, 2004. Adrenomedullin in the treatment of pulmonary hypertension peptides, 25: 2013-2018.

Nishikimi, T, K Kitamura, Y Saito, K Shimada, T Ishimitsu and M Takamiya, 1994. Clinical studies for the sites of production and clearance of circulating adrenomedullin in human subjects. *Hypertension*, 24: 600-604.

Nossaman, BD, CJ Feng, AD Kaye, B Devitt, DM Cay and WA Murphy, 1996. Pulmonary vasodilator response to adrenomedullin is reduced by NoS inhibitors in rats but not in cats. *American Journal of Physiology*. 270: L 782-789.

Palanisuramy, V, S Laszlyo, G Thomas, M Andrew, B Ko and W John, 1998. Adrenomedullin in patients at high risk for pulmonary hypertension. *Annals of Thoracic Surgery.*, 66: 500-505.

Sakota, J, T Shimokuko, K Kitamura, S Nakamura, K Kangawa, H Matsuo and T Eto, 1994. *Felis. Lett.*, 352: 105-108.

Shimokuko, Y, K Nagata and S Ohta, 1995. Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca mobilization in bovine aortic endothelial cells. *Journal of Biological Chemistry.*, 270: 4412-4417.

Shindo, T, M Kurihara, Y Kurihara, M Morita and Y Yazaki, 1998. Up regulation of endothelin-1 and adrenomedullin gene expression in the mouse endotoxin shock model. *Journal of Cardiovascular Pharmacology*, (31 supplement 1): S541-S544.

Sugo, S, N Minamiro, K Kangawa, Myamoto, K Kitamura and J Sakata, 1994. Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem. Biophys. Res. Commun*, 201: 116-116.

Sugo, S, N Minamiro and M Shoji, 1995. Interleukin-1, tumor necrosis factor and lipopolysaccharide additively stimulate production of adrenomedullin in vascular smooth muscle cells. *Biochem. Biophys. Res. Commun*, 211: 686-693.

Toshio, N, N Seiki, S Tatsuya, T Shigehiro, M Hiroaki, T Shuichi, K Kazuo, M Atsuro, M Hisayuki and K Kenji, 1997. Plasma concentrations of adrenomedullin correlate with the extent of pulmonary hypertension in patients with mitral stenosis. *Heart*, 78: 390-395.

Tsuchihashi, K, N Saurai, H Takizawa, N Takahashi, T Ishiguro and N Hikita, 1993. Plasma noradrenalin as an indicator of functional state in hearts with mitral stenosis, the influence of acutely reduced left atrial pressure by balloon mitral commissurotomy. *Heart Vessels*, 8: 85-99.

Yamamoto, K, M Nagata, M Mito, M Fujikama, M Sekiguchi and K Shimada, 1989. Endothelin production in pulmonary circulation of patients with mitral stenosis. *Circulation.*, 79: 47-50.

Yang, BC, H Lippton and B Gumusel, 1996. Adrenomedullin dilates rat pulmonary artery during hypoxia: role of nitric oxide and vasodilator prostaglandin. *Journal of Cardiovascular Pharmacology*, 28: 458-462.