STUDY OF ATRIAL NATRIURETIC PEPTIDE IN PATIENTS WITH BRONCHIAL ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASES

By

Magdy M. Omar ; Magdy A. Abd El-Motaleb*

Awad M. El-Abd**; Sherif Abu El-Dahab***

Hasan G. Abdel-Salam***; and Eman A. El-Betar*

From

Departments of Chest; Cardiology*; Medical Biochemistry*

Internal Medicine***; and Public Health**'

Benha Faculty of Medicine, Zagazig University

ABSTRACT

The present study was carried out to study the changes in plasm arial natriuetic peptide (ANP) in asthmatic children and in cases witl chronic obstructive pulmonary disease (COPD) and to correlate thes changes with the ventilatory and cariac functions in the COAD group

About 105 subjects were selected. From pediatric and ches Departments, Menha university Hospital. They were calssified int 20 asthmatic children (Group A1), who were reinvestigated 4 week after treatment (Group A2). Their age ranged from 1.5 to 11 year old. This group of patients was cpmpared with 15 healthy childre matched for the same ages and sex. Another 60 patients with CoPI were subclassified into 3 subgroups. Subgroup I: included 20 case on hospital admission with acute infective exacerbation (subgrou Ia), those cases were reinvestigated 6 weeks after medical treatmen (subgroupIb). Subgroup II: included 20 patients with COPD cor pulmonale and right-sided heart

failure. Subgroup III: included 20 patients with COPD without cor-pulmonale. Their age ranged from 40 to 74 years old. This group of patients were compared with 10 healthy subjects matched for the same ages and sex.

For all groups, plasma ANP was done while ventilatory functions and echocardiography was done for COPD group only. The main results of this study, showed that plasma ANP was significantly elevated in asthmatic children compared with the control group but, it was significantly higher in subgroups AI in comparison to subgroup A_2.

In the COPD group, ANP was significantly elevated in all subgroups. The highest level in subgroup la, the lowest level was in subgroup III. ANP was significantly elevated in subgroup II in comparison to Ib and III subgroups.

Also, ANP was correlated with mean pulmonary artery pressure (PAP), surface area of right atrium (RAsa), right atrial diameter (RAD), right ventricu\ar diameter (RVD) and tight vetxttvculat anterior wall (RVAW) diameter. The decrease of ANP in subgroup Ib was associated with improvement in the ventilatory and cardiac functions,

We could conclude that; ANP was elevated and correlated with the severity of the disease in both asthmatic and COPD cases. Also, it was correlated with the degree of PAP, right atrial and right ventricular dimensions. So, ANP may give a new meaning in the future for the treatment of both asthmatic and COPD cases.

INTRODUCTION AND AIM OF THE WORK

Atrial natriuretic peptide (ANP) is a recently discovered polypeptide. Little has been written about this peptide in asthma or COPD although expanded researches were done in the different cardiac

conditions. It has an important hormonal regulator of salt and water and arterial blood pressure (Di-Nardo et al, 1992). It is synthesized mainly by the right atrium. In the lung, it is synthesized by type II alveolar cell and respiratory epithelial cell. It is also localized in the smooth muscle cell of the pulmonary vein (not the arteries) and the superior vena cava (Springall et al., 1988). It has been recovered in the pleural fluid in patients with congestive heart failure (Vesely et al., 1989).

The lung is the first and an important clearing organ for ANP through the neural endopeptidase enzyme (NEP) (Di-Nardo et al., 1996). In addition to natriuresis and arterial vasodilatation including pulmonary arteries, ANP produces a c-GMP mediated bronchorelaxation and protects against histamine induced bronchoconstriction (Kang et al., 1993). It may prevent pulmonary oedema by increasing c-GMP, decreasing intracellular Ca^{+2} and stabilizing tight junctions (Di-Nardo et al., 1996). It also stimulates surfactant production (Ishii, et al., 1989). These beneficial effects have led to the production of inhaled, oral and intravenous ANP to be used to modify bronchial tone and reactivity. However, as a peptide, ANP is not orally bioactive and inhalation studies demonstrate only mild effects (Hulk & Thomson, 1994). Many authors studied the effect of ANP inhibitors (e.g. thiorphan) by inhalation or infusion in asthmatic patients and the results were increase ANP and bronchodilatation, (Angus et al., 1990) (Angus etal., 1995).

This study was designed to study changes in plasma ANP in cases of bronchial asthma and COPD and to correlate these changes with the ventilatory functions and with right atrial and ventricular functions.

SUBJECTS AND METHODS

This study was conducted on 105 subjects divided into 2 main groups. The first group included 20 asthmatic children, 12 cases were males and 8 were females. Their age ranged from 1.5 to 11.0 yean old with a mean of 8.3 ±2.1 years. They were admitted to Benha University Hospital with acute severe asthma (Group Ai). Those cases were reinvestigated after improvement, at least 4 weeks after treatment (Group A_2). The selection of cases of bronchial asthma was based on the criteria stated by The National Heart, Lung and Blood Institute (1991).

The second group included 60 COPD patients, 39 cases were males and 21 were females. Their age ranged from 40 to 74 years old with a mean of 52.1 ± 6.6 years. The selection of cases of COPD was based on the criteria stated by The American Thoracic Society(1995). They were divided into 3 subgroups:

- *Subgroup I:* Included 20 cases on admission to the hospital with acute infective exacerbation of COPD (Subgroup la). Those cases were reinvestigated after clinical improvement at least 6 weeks after treatment (Subgroup Ib).
- *Subgroup II:* Included 20 cases with cor-pulmonale and right sided heart failure when clinically stable at least 6 weeks after their last infective exacerbation.

  Subgroup III: Included 20 cases without cor-pulmonale when clinically stable at least 6 weeks after their last infective exacerbation.

Twenty five subjects were taken as a control group. 15 healthy children and 10 healthy adults with comparable age and sex were taken for the asthmatic group (Control A) and for the COPD group (Control C); respectively.

All subjects were submitted to:

1. Thorough history taking.
2. Complete clinical examination.
3. Urine and stool analysis.
4. Plain X-ray chest (PA & Lateral views).
5. Electrocardiogram.
6. Ventilatory function tests using Spirosift (Fukuda Denshi, Model 3000). The following data were measured, forced vital capacity (FVC), forced expiratory volume in the first second (FEVi), FEVi/FVC ratio, forced expiratory flow at 25 - 75% of the FVC ($FEF_25\_75\%$). These tests were done before and 5 minutes after giving 2 puffs of salbutamol inhalation (done in the COPD group only).
7. **Echocardiography**

Echocardiographic examination was done in the COPD cases only to measure RAD, RVD, PAP, RPAW, RVD, RAsa and RAD, using HEWLETT PACKARD ultrasonic imaging (Sonos 1000).

Exclusion criteria :

* Patients with underlying heart disease apart from right side affection as congenital, rheumatic valvular and ischemic heart disease. Also, patients with arrhythmias, pericardial effusion, pericarditis or systemic hypertension which may affect the right or left side of the heart.
* Patients with underlying chest disease apart from bronchial asthma and COPD with or without acute infective exacerbation. Also, COPD patients with reversibility in FEVj more than 20% were excluded.

- Patients with impaired liver or kidney functions and other causes of generalized edema as nutritional edema. »
- Patients with parasitic infestations that can affect the pulmonary artery pressure or produce asthma like picture,
- Patients with any other systemic affection that might affect ANP level as diabetes mellitus,

7. Sampling

About 5.0 c.c. venous blood sample was collected while the patient was fasting and divided into 2 parts. The first part (2.0 c.c.) was transferred into poiypropiene tube containing 2mg of EDTA and Aprotonine (2000 KlU/ml). Blood was cenirifuged in a cooling centrifuge at 1.600 Xg for 15 minutes at 0^{C}C. Plasma was transferred to fresh poiypropiene tube and stored at -70°C until assay of ANP. The second part was left to be clotted. centrifuged and serum separated was used for determination of:

a- Fasting serum glucose (Trinder, 1969).

b- Liver function tests:

- SGOT & SGPT (Reitman & Frankel, 1957),
- S. albumin (Grant & Kachmar, *1*970).
- S. total protein (Henry & Beters, 1968).

c- Renal function tests :

- S.urea (Palton & Crouch, 1977).
- S. creatinine (Henry, 1974).

Determiaiation of plasma ANP by ELISA (Prostmaan *&* Ksessig, 1992):

The kit of ANP was purchased from Peninsula Laboratories Inc. ANP was extracted from plasma using $C_{]B}$ sep columns and eluted with a mixture of acetonitrile and trifluoroacetic acid. Measurement was done by specific and sensitive competitive enzyme immunoassay.

Data analysis :

Results were expressed as mean value + standard deviation, paired and unpaired t-test was used for comparison. P-value of <0.05 was considered significant (Budneck, 1987).

RESULTS

Table (1) showed that plasma ANP was significantly increased in asthmatic children before and after treatment (Pi<0.001) compared with the control group while plasma ANP was significantly decreased after treatment (P_2<0.001) compared with the same cases before treatment.

Table (2) showed that plasma ANP was significantly increased in all COPD cases (P,<0.001) compared with the control group. Group !b and group III showed a more significant decrease in plasma ANP (P2<0.001) compared with the group la while, group II showed a significant increase of plasma ANP {P_3<0.01), group III showed a non-significant decrease compared with group Ib. Furthermore, group (II showed a significant decrease of plasma ANP (P_4<O.OOI) compared with group II.

Tables (3 & 4) showed a statistical comparison between different ventillatory functions and echocardiographic data in different COPD cases compared with the control group.

Table (5 & 6) showed the correlation coefficient (r) between plsma ANP with ventiliatory and cardiac functions.

*Table (1)**: Meen**± S$f t-iesf (paired and unpaired) xnti p values of plasma ANP in asthmatic children before and after medical treatment compared with each other and with the control group (A).*

Biochemical parameter Groups	ANP (ng/ml)	t-test	P-value
Control (A) (n = 15)	1.45 ±0.1 2	-	-
GroupA_{f} (before treatment) (n = 20)	9.25 ±2.95	10.83	p,<0.00i
Group A_2 (after treatment) (n = 20)	6.13±2.12	6.95 3.48*	P,<0.001 P_2< 0.001

PI: Probabiiity versus control group.
P_2: Probability versus group Aj (before treatment).

*Table (2) : Mean ±S£>, **t-test** **(**, ANP in COPD **sit** control group (C).* *and unpaired) and p values of plasma compared with each other and with the*

Biochemical parameter j Groups	ANP (ng/ml)	t-test	r-vaiue
Control (C) (n = 10)	0.5 ±0.06	-	-
Subgroup la (before treatment) (n = 20)	7.9 ±3.2	4.1	P1<0.001
Subgroup Ib (after treatment) (n = 20)	4.0 ±1.2	13.6 6.1[s]	P1O.OOl P_2<0.001
Subgroup n (n =20)	7.4 ±3.9	4.1 0.4 2.9	P1<0.00! P_2: NS P_3<0.001
Subgroup III (n =20)	3.9 ± 2. i	4.1 4.7 0.1 2.1	P1<0.001 P_2<0.001 P_3:NS P_4<0.001

P,: Probability versus control group.
P_2: Probabiiity versus group la.
P_3: Probabiiity versus Ib.
P.4: Probability versus IL

NS = Non significant

Table (3) : *Mean ± SD and P values of ventilator? fa fictions in COPD cases compared with the control group.*

VentlHatory. functions ! Studied groups	FVC (% Pred.)	FEV, (% Pred.)	FEVj/FV €	FEFis-75% (% Pred.)
Control (C)(n= 10)	94.4 ±2.5	91.6±2.8	96. 6 ±1.9	94.5 ±3.02
Subgroup la (n= 20)	60.8 ±8. 8 P<0,00!	4S.S±6.8 P < 0.00!	54.7 ±6.3 P < 0.001	28.7 ±7.9 P< 0.001
Subgroup Ib (n= 20)	70.4 ±8.3 P< 0.001	55.4 ±5.9 P< 0.001	59.9 ±6.1 P< 0.001	38.4 ±8.8 P< 0.001
Subgraup 11 (n=20)	73.7 ±8.1 P< 0.001	50.9 ±8.0 P<0.00!	52.1 ±7.5 P< 0.001	36.1 ±9.7 P< 0.001
Subgroup III (n=20)	64.2 ±9.7 P< 0.001	45.2 ±5.7 P< 0.001	53.9 ±7.5 P< 0.001	42.1 ±9.5 P< 0.001

P: Probability versus control group.

Table (4) : Mean ± SD and P-values of echocardiographic data in COPD cases compared with the control group.

Studied groups	Echocardiographic data						
	EF	PAP	RAD (cm)		R/Vsa	• RVO	RVAW
	<%}	(mmHg)	(LA)	<SA)	(cm^1)	(cm)	(cm)
Control (C) (n=10)	48.5 ± 3.5	16.3 ± 2.7	3.7 ± 0.5	3.5 ± 0.4	133± 0.8	IJ± 0.3	0.56 ± 0.1
Group la (a -20) {before treatment)	42.9 ± 6.5 P<0.05	34.4 ± 15.4 P<0.001	4.4 ± 0.9 NS	4.1± 0.9 P<0.05	3,9 ± 1.2 p<0.00i	0.9 ± 0.2 P<0.001	17.3 ± 2.3 P<0.001
Group Ib (n = 20) (after treatment)	48.2 ± *1.1* NS	32.4 ± 13.8 P<000i	4.2 ± 0.8 NS	3,9 ± 0.S NS	17.5 ± *2.6* P<0.001	3.4 ± (.5 PO.001	0.9 ± 0.2 P<O.C0l
Sugroup II {n =20)	47.5 ± 9.S NS	41. 9 ± 8.6 P<0.001	7.8 ± 0.8 P<0.00!	4.5 ± 0.7 P<0.001	18.5± 0.8 P<O.OOI	4.1 ± 0.6 P<0.001	U ± 02 P<0.001
Subgroup III (n=20)	45.1 ± 5.1 NS	15.2 ± 3.4 NS	3.2 ± 0.7 P<0.05	2.9 ± 0.7 P<0.05	13,2 ± 0.7 NS	¡!"> ≠ 0.3 NS	0,96 ± 0.2 P<0.001

P: Probability versus control group.

NS = Non significant.

Table (5) : Correlation coefficient (r) between the veittilatary functions and plasma atrtal natriuretic peptide (ANP) level in ihe different studied groups.

VeatiHatory Function	FVC (% Pred.)	FEV, *(% Pred.)	I"EV,/I'VC (%)	FEFis.75% (% PreiJ.)
ANP in control group	r = 0.086 P>0.05	r=0.3!6 P>0.05	r= 0.393 P>0.05	i=0.389 P>0.05
ANP in COPD with acute exacerbation	r= 0.146 P>0.05	r= 0.144 P>0.05	r= 0.317 P>0.05	r= 0.159 P>0.05
ANP in COPD 6 weeks after treatment	r= 0.465 P<0.05°	r= 0.152 P>0.05	r= 0.405 P>0.05	r= 0.108 P>0.05
ANP in COPD with cor-pulmonale	r= 0.349 P>0.05	r= 0.020 P>0.05	r= 0.260 P>0.05	r= 0.090 P>0.05
ANP in COPD without cor-pulmonale	r= 0.159 P>0.05	r=0.31Q P>0.05	r= 0.424 P>0.05	r= 0.023 P>0.05

*Significant

Table (6) : Correlation coefficient (r) between the echocardiognlphic data and plasma atrial natriuretic peptide (ANF) level in the different studied groups.

Echocardiographic data Studied groups		EF	PAP	RAD (cm)		RAsa	RVD	liVAW
		(%)	(mmHg)	(LA)	(SA)	(cm^2)	(cm)	(cm)
ANP in control group	P	0.424 >0.05	0.010 >0.05	0.343 >0.05	0.459 >0.05	0.101 >0.05	0.494 >0.05	0.009 >0.05
ANP in COPD with acute exacerbation	r= P	0.353 >0.05	0.679 <0.01*	0.546^ <0.05*	0.493^ <0.05*	0.493 <0.05*	0.546, <0.05*	0.677 <0.01*
AN Pin COPD 6 weeks after treatment	r = P	0.026 >0.05	0.411 >0.08	0.193 >0.05	0.111 >0.05	0.370 >0.05	0.139 >U.05	0.281 >0.05
AN Pin COPD with cor-puimonale	V	0.217 >0,05	0.054 >0.05	0,241 >0.05	0.223 >0.05	0.164 >0.05	0.277 >0.05	0.305 >0.05
ANP in COPD Without COT' puimonale	r= P	0.164 >0.05	0.208 >0.05	0.434 >0.05	0.436 >0.05	0.238 >0.05	0.314 XJ.05	0.173 >0.05

*Significant

DISCUSSION

In this study, there was a highly significant increase of the serum ANP in the asthmatic children (P;<0.001) in comparison with control

group especially during acute attack (Table 1). This was in consistent with the work done by Di-Nardo et al. (1992) and De-Gouw et al, (1996), Almirall and Hedenstierna (1991), reported that ANP has a bronchodilator effect in asthmatic patients and it is c-GMP mediated and it may be considered a protective mechanism. Scharf et al.(1989), also postulated that acute asthma cause a fall in inspiratory pleural pressure with bronchoconstriction eliciting a marked increase in functional residual capacity (FRC). This results in a higher negative force surrounding the atria at inspiration, augmenting right atrial distension and contributing to ANP release. Another mechanism including increase in airway resistance which increase alveolar pressure and rise pulmonary vascular resistance, (Adnot et al., 1987), as well as activation of sympathetic nervous system and increase in heart rate which occur in acute severe asthma, (Scliiebinger & Linden, 1986) with a corresponding increase of atrial contraction that stimulate specific granules for ANP release. Di-Nardo et al. (1996), confirmed the enhancement of ANP receptor gene expression and localization in the respiratory system induced by hypoxia during the acute state.

Plasma ANP in our asthmatic children 4 weeks after the acute attack although was significantly higher (P_2<0.001) than the control group but still significantly lower than that during the acute attack (Table 1). A similar results were obtained by Skwarski et al. (1993).

In the COPD group, it was found that the highest level of serum ANP was in the subgroup la and it was statistically significant (P_2<0.001) when compared with subgroups (Ib & III) and with the control group (Pi<O.Q01). This was in consistent with the work done by Skwarski et al. (1993). The lowest level of ANP was in subgroup III (Table 2).

Several mechanisms could explain the very high level of serum ANP in COPD patients: (a) Hypoxia: which acts either directly on the right

atrium or indirectly through hypoxic pulmonary vasoconstriction which increase ANP and accordingly causes hypertrophy of the right ventricle and right atrium which are the main sites for ANP secretion (Winter et al., 1989). (b) Hypercapnoea: which acts through several mechanisms. All of them causes $Na^{1"}$ retention and expansion of the extracellular fluid volume with subsequent stimulation of ANP secretion. First, is renai retention of bicarbonate in the form of $NaHCO_3$ (Winter et al., 1989), Second, is directly stimulation of tT secretion, which is electronically balanced by reabsorption of Na^T (Koehny, 1986). Third, is renal afferent arteriolar constriction as a result of reduction of renal plasma flow (Faber et al. ,1986). Fourth, is increasing vasopressin secretion, which is mediated through activation of rennin-angiotensin-aldosteron system (Faber et al. , 3986).

It has been reported that very high level of ANP in COPD cases with exacerbation has a protective role against the development of oedema due to its beneficial hemodynamic effects and inhibition of refnin-angiotensin-aldosteron system (Espiner, 1994). However, Na^+ and water retention stiil occur in those patients inspite of the very high level of plasma ANP. This could be explained by the blunted renal response to ANP or the possibility of suppressive effect of ANP on the renin-angiotensin axis is outweighed by the stimulatory effect of inadequate renal perfusion on this axis (Shenker et al., 1985).

In this study, it was found that ANP in COPD cases 6 weeks after treatment (Subgroup Ib) although was significantly lower than that in subgroup la ($P_2<0.001$), it was still significantly higher than their control group ($P_t<0.001$) (Table 2). This was in consistent with the work done by Skwarski et al. (1993), who also reported that at least 6 weeks should be passed after the last exacerbation to detect accurately changes in ANP level.

The significant reduction *in* ANP level in subgroup to in comparison to subgroup la was also associated with significant improvement in the large arid small airway function parameter (a similar results were -also reported by Di-Nardo et a!. (1992), and also significant improvement in PAP, EF and RAD (LA)(Table 4). This improvement in ANP could be explained by a combination of factors including improvement in blood gases as a result of improvement of EF and PAP [a similar results were also obtained in this study (Table 4)] and hence perfusion is better and finally reduction in centra! venous pressure consequent on diuresis (Raine et al., 1986).

The mean plasma ANP level in subgroup II was significantly higher (P_1<G.001) as compared with its control group (Tabie 2). This was in agreement with work done by Habib et at (1994), and Skwarski et al. (1993), and could be explained by persistent elevation of the PAP with subsequent increase in RAD, RAsa, RVD and RVAW (Mac-Nee et a!., 1988), in addition to chronic hypoxia and hypercapnoea which are detected in such cases.

It was found that plasma ANP in subgroup ill was still significantly higher (P_t<0.001) as compared with their control group (Table 2). Habib et al. (1994) and Skwarski et al. (1993) abo obtained similar results. No significant difference in plasma ANP was detected between la and II subgroups. This could be explained by that both groups have comparable PAP (Table 2). Similar results were obtained by Skwarski et al. (1993). However, ANP was significantly higher (P_2<0.00l)in subgroup la in comparison to subgroup III (Table 2). This could be explained by the more hypoxia, hypercapnoea, increase PAP and chest infection in subgroup la (Skwarski et al., 1993).

There was a significant (P_3<0.01) decrease of plasma ANP in subgroup Ib in comparison with subgroup 11 (Table 2) which could be explained by the reduction in PAP and hence, right atrial pressure (Kwashima et at., 1989). Plasma AN? was significantly higher in subgroup II (P_4<0.001) in comparison to subgroup III (Table 2) which could be explained by the presence of chronic pulmonary hypertension in subgroup II but not in subgroup III (Mac-Nee et a!., 1988).

Plasma ANP in subgroup la significantly correlated with FVC (Table 5). This may be due to the effect of treatment that improves the ventillatory functions, blood gases, PAP and lead to reduction in ANP level (Di-Nardoetal., 1992).

Plasma ANP in subgroup la significantly correlated with PAP, RAsa (Table 6) which may indicate the role of ANP in modulating PAP through vasodilatation and blood volume regulation (Morice et al, 1987). This was in agreement with the work done by Adnot et al. (1987), Ibrahim et al. (1993) and Skwarski et al. (1993) but not in agreement with those done by Winter et al. (1989) and Habib et al. (3994). These conflicting results may be due to depletion of ANP stores due to long duration of the disease (Winter etai, 1989).

In this study, there was a significant positive correlation between plasma ANP and RAD, RVD and RVAW in subgroup la (Table 6). This may be due to pulmonary hypertension, which conflict on the right ventricle and right atriurn.

The highest value of PAP was found in subgroup II (Table 4). Weitzenblun and his collegue (1984), had reported that hypoxaemia was not significantly differ in those with or without cor-pulmonale but hypercapnoea and acidosis were higher in patients with cor-pulmonale and may have contributed to a higher increase in ANP.

From this study, it could be concluded that plasma ANP was elevated in asthmatic and COPD cases and it correlated with the severity of the disease and -iii COPD cases- with the degree of PAP, right atria! and ventricular dimensions. ANP has a beneficial effect in bronchial asthma and COPD patients. So, we recommend further studies to prolong its duration of action via blocking ANP degrading enzyme or its clearance receptor.

- Adnot, S, ; Chabrier, P. ; Andrivet, C. ; and Brum, J. (1987): "Atriai natriuretic peptide concentration and pulmonary hemodynamic in patients with pulmonary hypertension". Am. Rev. Respir. Dis. ; 136 : 951-956.
- Almirali, J.; and Hedenstierna, K. (1991) : "Atriai natriuretic peptide in bronchial asthma", Med. Hypothes.; 36 (1): 73 - 74.
- American Thoracic Society (1995) : "Standards for the diagnosis and management of cr.ses of COPD[3]'. Am. J. Respir. Crit Care Med. ; 152 : 77-120.
- Angus, E. ; Millar, E. ; Chalmer, G. ; and Thomson, N. (1990) ; "Effect of inhaled thiorphan on the bronchodilator response to inhaled atriai natriuretic peptide". Thorax.; 51 (1): 71 - 74.
- Angus, E. ; Mc-Cammum, M. ; Nally, J. ; and Thomson, N. (1995): Effect of inhaled atriai natriuretic peptide and a neural endopeptidase inhibitor on histamine-induced broncho-constriction". Thorax.; 42 (8) :62-67,
- Budneck, L. (1987) : "Statistics". In : Cassem, B.J. : Preventive Medicine and Public Health, pp. 43 - 77. New York, John Wiley and sons.
- De-Gouw, I-L ; Oiaman, Z,; and Kuipers, E. (1996) : "Role of neural endopeptidase in exercise induced bronchoconstriction ;n asthmatics". J . APPL Physic!.; 81 (2): 673 -678.

- Di-Nardo, P.; Minieri, M.; Bartoli, M.; and Brum, J, (1992): "Atrial natriuretic peptide and pulmonary function". Ann. Ital. Med. Int. ; 7 (4) : 234 - 244.
- Di-Nardo, P. ; Minieri, M. ; and Sampadess, M. (1996) : "Atrial natr rare tic peptide and ANP receptor gene expression and localization and hemodynamic overload". Endocrinology ; 137 (10): 4334 - 4350.
- Espiner, E. (1994): "Physiology of atrial natriuretic peptide", J. Inter. Med.; 235: 527-541,
- Faber, M. ; Robert, O. ; and Wienberger, H. (1986): "Abnormalities of sodium and water handling in COPD". Arch. Intern. Med. ; 142 : 1326-1330.
- Grant, H.C. ; and Kachmar, J.F. (1970): "The protein of the body fluids". In : Fundamental of Clinical Chemistry. Tietz, N.W., Caraway, W.T., Frier, E.F., Kachmer, J.F., and Rawuzley, H.M. (eds) W.B. Saunders, Co., Pheladelphia, London, Tornto, Mexico City, Rio de Janeiro, Sydney, Tokyo, 3:298.
- Habib, Y. ; Kira, L. ; El-Sherbiny, H. ; Ehab, M, ; El-Shamy, E. ; Shaaia, Y. ; and El-Nouean, M. (1994) : "Hormonal regulation of fluid hemostasis in COPD". Bull. Alex. Fac. ^4ed, ; 30 (5) : 1095 – 1104.
- Henry, R.J. (1974) : "Determination of creatinine by kinetic method".
- In: Clinical Chemistry, Principles and Technics, 2nd edition. Harper &.
- Rowe., 525,
- Henry, Y, ; and Beters, T. (1968) : "Determination, of plasma total protein". Clin. Chem.; 14 : 1147.
- Huik, G.; and Thomson, Y. (1994) : "Inhaled atrial natriuretic peptide and asthmatic airways", Br. Med. 1.; 304 : 1156-1161.
- Ibrahim, M.; Ahab, A.; Saniia, F.; Nader, M.; and Maha, S. (1993): "The role **of** atrial natriuretic peptide in pulmonary liernodynamic regulation in COPD". J. Med. Researchs.; 1 (4): 23 - 37.

- Ishii, Y, ; Watanable, M. ; Watanabe, S. ; and Hasegawa, Y. (1989);
- "Effect of atrial natriuretic peptide on type I! alveolar epithelial cell of
- the iung. Autoradiographie and morphometric studies". J. Anat. ; 166
- :8S-95.
- Kang, K. ; Morrow, I. ; Robert, H. ; arid Newman, M. (1993) :
- "Airway and vascular effects of 8-epi-prosiaglandin F2 in isolated perfused rat lung'. *L* Appl. Physio!.; 74: 405 - 460.
- Kawashima, A. ; Kubok, R. ; Yoshikawa, S. ; and Koboyashi, T. (1989) : "Atria! natriuretic peptide under acute hypcxia in normal subjects". Resp. Physiol.; 76 (1): 79 - 91.
- Koehny, W. (1986) : "Pathogenesis and management of respiratory and mixed acid-base disorders". Boston, Toronto: Little Brown Company, 187-206.
- Mac-Nee, W. ; Wahen, C. ; Flenly, D. ; and Muiral, Y. (1988) : "Right ventricular function in patients with stable and decompensated pulmonary heart diseases and effect of controlled O_2 therapy". Am. Rev. Resp. Dis.; 137 : i289 - 1295.
- Morice, A. ; Jansen, T, ; and Broun, M. (1987): "The effect of atrial natriuretic peptide in vitro pulmonary artery pressure". Am. Rev. Resp. Dis.; 135:285-300.
- National Heart, Lung and Blood Institute (1991) : "National asthma education program. Expert panel report: Guidelines for the diagnosis of asthma'. J. Allergy. Clinic Immunology ; 88 : 425 - 534.
- Palton, C.J. ; and Crouch, S.K. (1977): "Enzymatic determination of S, urea by modified Berthelct reaction". Annal Chem.; 49 : 464.
- Prostmann, T. ; and Kiessig, T. (1992) : "Enzyme immunoassay techniques", I Immuno. Methods ; 150 : 23 - 32.
- Raine, A. ; Erne, P. ; Burgisser, E. ; Muller, F. ; and Burkart, F. (1986): "Atrial natriuretic peptide and atrial pressure in patients with CHF" N. Eng. J. Med.; 315 : 533 - 537.

- Reitman, S. ; and Frankel, S. (1957) : "Determination of glutamic-oxaloacetate and glutamic pynivate iransaminases". Am. J. Clin Pathol.; 28 : 56.
- Scharf, S. ; Brown, R. ; Saunder, N. ; and Green, L. (1989) : "Effect of normal and loaded spontaneous inspiration on cardiovascular function". J. Appl. Physio 1; 47 : 82 - 92.
- Sclliebinger, R. ; and Linden, L. (1986) : "Effect of contraction frequency OR atria! natriuretic peptide secretion". Am. J. Physiol. ; 250: 1095-1099,
- Shenker, Y.; Sider, S.; Ostafin, A.; and Grekin, R. (1985): "Plasma level of atria! natriuretic peptide in healthy subjects in patients with edema". J. Clin. Invest.; 76 : 1684 - 1687.
- Skwarski, K. ; Lee, M. ; Tunbull, L. ; and Mac-Nee, W. (1993) : "Atrial natriuretic peptide in stable and decompensated COPD". Thorax.; 48: 630-735.
- Springall, D. ; Bhatnagar, J. ; Whaton, Q. ; and Hamid, S. (1988) : "Expression of atrial natriuretic peptide gene in the cardiac muscle of rat extrapulmonary veins". Thorax; 43 : 44 - 52.
- Trinder. P. (1969): "Enzymatic determination of glucose". Ann. Clin. Biochem.; 6 : 24.
- Vesely, K. ; Winter, J. ; and Sallman, A. (1989) : "Atrial natriuretic peptide in congestive heart failure". Chest; 95 : 1107 - 1109.
- Weitzenblun, F,; Santegeaa, A.; Ehrhart, M.; Memmosser, M. ; and Roegel, E. (1984) : "Long term course of pulmonary artery in COPD". Am. Rev. Resp. Dis.; 130 : 993-998.
- - Winter, R. ; Davidson, D. ; Treacher, R. ; and Bloom, R. (1989) : "Atrial natriuretic peptide concentrations in hypoxic secondary pulmonary hypertension : relation to hemodynamic and blood gas variables and response to oxygen". Thorax; 44 : 58 - 62.