Original Article

A Study of Plasma Aldosterone levels in Patients of Systemic Hypertension with Metabolic Syndrome

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ABSTRACT

Background : Human adipocytes produce an as-yet unidentified mineralocorticoid-releasing factor that stimulates adrenal aldosterone production. Nongenomic action of aldosterone leads to increased collagen synthesis in cardiac and vascular tissue leading to myocardial impairment, diastolic dysfunction and decreased arterial compliance. Therapy with mineralocorticoid receptor antagonist has a role in treating these patients.

Objective : To study and compare the plasma aldosterone level, LV mass index and diastolic dysfunction in patients of Essential hypertension with and without Metabolic Syndrome

Methods : This cross sectional, hospital based, observational study, included 100 newly diagnosed hypertension naive subjects, from GMC, Nagpur. The Plasma Aldosterone, Renin & Insulin levels were measured by Beckman Coulter Radioimmunoassay kit. The Plasma Aldosterone to Renin Ratio (A/R ratio) & HOMA index were calculated. Patients were classified as having Primary Aldosteronism (PA), Secondary Hyperaldosteronism (SHA), Low-Renin Hypertension (LRHT), Miscellaneous Hyperaldosteronism (MHA) Essential Hypertension (EHT). Subjects with EHT (n=92) were further subdivided into those with or without Metabolic Syndrome.

Results : Clinical characteristics, laboratory variables and echo parameters of subject's in-group A were compared to that of group B. The plasma aldosterone {p=0.011S}& Insulin levels {p=0.0007S} were raised significantly in group A as compared to group B. Plasma renin level were raised in both groups {p=0.4NS}. Higher grades of diastolic dysfunction and LV mass index were found in group A as compared to group B {r=0.50, r=0.55 respectively}.

Conclusion : The elevated plasma aldosterone in patients of hypertension with metabolic syndrome increases their cardiovascular risk as compared to those without metabolic syndrome. These results can have an implication in choosing the management strategies of these patients.

Key Words : Metabolic syndrome, Plasma Aldosterone, Aldosterone Renin ratio, LV mass index.

Introduction :

A growing body of evidence suggests that hyperaldosteronism contributes significantly to the development and the severity of hypertension as well as to resistance to antihypertensive treatment[1]. Insulin resistance, metabolic dyslipidemia, central obesity, and hypertension commonly cluster to comprise the metabolic syndrome. In cross-sectional analysis, plasma aldosterone levels have been shown to relate to BP levels, particularly in obese individuals[2].

In these same individuals, BP was not related to plasma renin activity, suggesting an effect of aldosterone on BP independent of reninangiotensin II [3,4]. Studies, suggest that 10 to 15% of individuals with hypertension fulfill the biochemical criteria for primary aldosteronism[5]. Demonstration of such a high prevalence of primary aldosteronism in patients with presumed primary hypertension suggests that aldosterone excess is a common contributing cause and this subclass of patients benefit by aldosterone antagonists as add-on therapy.

Hypokalemia is late presentation of these patients of aldosterone excess and is long preceded by the development of hypertension.

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So an important question in terms of treatment and perhaps prevention is what is the cause of the aldosterone excess that is now being so widely reported?

Stimuli that underlie the increasing occurrence of hyperaldosteronism remain obscure; however, recent studies suggest that obesity may be an important contributing factor. Factors that have been isolated from human adipocytes have been shown to function as aldosterone secretagogues independent of reninan-giotensin II[6]. *epoxy keto derivative of linoleic acid* - a potent stimulator of aldosterone production from extraadrenal sites.

The metabolic syndrome (MS) is a multiplex risk factor for atherosclerotic cardiovascular disease (ASCVD). It consists of an atherogenic dyslipidemia (i.e., elevated triglycerides and apolipoprotein B (apo-B) and low high-density lipoprotein cholesterol (HDL-C)), elevation of blood pressure and glucose, prothrombotic and proinflammatory states. The risk of ASCVD accompanying MS is approximately double compared with an absence of the syndrome.

MS is a complex web of metabolic factors that are associated with a 2-fold risk of CVD and a 5-fold risk of diabetes. Individuals with MS have a 30%40% probability of developing diabetes and/or CVD within 20 years, depending on the number of components present[7].

Last, preliminary data link aldosterone levels to severity of obstructive sleep apnea in individuals with resistant hypertension[8]. Although none is definitive, these studies are provocative in potentially relating the seeming increase in aldosteronism to concurrent increases in obesity.

Methods :

After taking approval from Institutional Ethics Committee this hospital based analytical observational study was undertaken in GMC, Nagpur, from December 2011 to November 2013. 100 newly diagnosed treatment naïve hypertensive patients were included in the study after taking informed consent. Patients with Type II DM, secondary hypertension, Serum creatinine > 2.2 mg/dl, Myocardial infarction or Stroke within 6 months and pregnant ladies were excluded from the study. All patients were subjected to detail Clinical Examination, biochemical investigations, ECG; 2-D-ECHO studies for LV Mass, & LV Diastolic functions. Fasting venous blood sample was collected and plasma Aldosterone, Renin Activity, Insulin level were measured through Beckmann Coulter Radioimmunoassay kit by Gamma Counter Analyzer Sampling Procedure at ISO 9001:2008 CERTIFIED Laboratory. The Aldosterone to renin ratio (ARR) & Homeostasis Model Assessment (HOMA) Index were calculated. Patients were classified as

TYPE	CRITERIA
Primary Aldosteronism (PA)	*Aldosterone > 500, A/R ratio > 1,000*
Secondary Hyperaldosteronism (SHA)	*Aldosterone > 1,000, A/R < 400*
Low-Renin Hypertension (LRHT)	*Aldosterone < 500, A/R > 1,000*
Miscellaneous Hyperaldosteronism (MHA)	*Aldosterone > 1,000, A/R 400-1000*
Essential Hypertension (EHT)	*Aldosterone < 1,000, A/R < 1000)*

PLASMAALDOSTERONE :

Values were obtained in pg/ml which were converted to pmol/L by multiplying by 2.774.

Normal range : Supine : 27.74-291.27; Upright: 94.31-757.30.

PLASMA RENIN ACTIVITY (PRA) Radioimmunoassay (RIA) Of Angiotensin I PRA is measured indirectly by the measurement of in vitro generation of angiotensin I (A-I) per hour. Background A-I, determined on plasma samples incubated at 4°C, is subtracted from the A-II generated at 37°C for the calculation of PRA using following equation :

PRA ng/mL/hr = {A- II (37°C) A-I (4°C)} x 2
Enzymatic incubation time (hrs)

Normal range : *Supine* : 0.5 - 1.9 ng/mL/hr; *Upright* : 1.9 - 6.0 ng/mL/hr

Plasma Insulin : Normal Range : Fasting state 2.1-22μIU/ml

Aldosterone Renin Ratio (ARR) :

ARR values were determined by dividing aldosterone expressed in pmol/L by renin expressed in ng/ml/hr.

Insulin resistance was calculated by Homeostasis Model Assessment (HOMA) Index

Subjects with **EHT (n=92)** were further subdivided into those with - Metabolic Syndrome (Group A; *n=45*) or Without Metabolic Syndrome(Group B; *n=47*) according to NECP/ATP III criteria[9] and central obesity cut off points for South Asian were based on IDF criteria[10]:

STATISTICAL ANALYSIS : All demographic, laboratory values and echo parameters were presented as mean ± SD. Categorical variables were expressed in actual number & percentage. Age, sex, clinical characteristics, laboratory variables, hormonal analysis & all echo parameters were compared between patients of hypertension with MS and hypertension without MS by performing unpaired t-test for normalized data. Categorical variables were compared by chi square test. Pearson rank correlation coefficient was assessed to find significant correlation between study parameters & aldosterone levels. Clinical characteristics, laboratory variables and glucose metabolism parameters were compared with different tertiles of plasma aldosterone using one way ANOVA. All the tests were two sided; p-value < 0.05 was considered as statistically significant. Statistical software S TATA 10.0 was used for statistical analysis.

Results :

Of the100 subjects evaluated, they were divided into five groups according to the aldosterone level and aldosterone to renin ratio (ARR)

Table No. 1

Essential Hypertension	82
Low Renin Hypertension (LRHT)	3
Primary Aldosteronism (PA)	2
Miscellaneous Hyperaldosteronism (MHA)	2
Secondary Hyperaldosteronism (SHA)	1

Table No. 2

Mean Plasma Aldosterone, Renin & Insulin level in group A & B

	Essential Hypertension (EHT) (n=92)		
	Group A With MS (n = 45) (30M/15F)	**Group B Without MS** (n = 47) (32M/15F)	**p Value**
Aldosterone (pmol/L) Mean±S.D.	200.31 ± 156.87	119.28 ± 124.87	0.0021
Renin (ng/ml/hr) Mean±S.D.	4.24 ± 6.01	2.71 ± 3.58	0.158
Insulin (uIU/ml)	33.61 ± 39.33	12.70 ± 19.88	0.0005
ARR pmol/L/ng/ml/hr	129.49 ± 154.69	114.61 ± 142.41	0.3967

Plasma aldosterone levels and Insulin levels were significantly high in group A as compared to group B.

Table No. 3

Shows no. of subjects with raised aldosterone (>291pmol/L) in group A & B		
Aldosterone	**Group A - HTN with MS, (n = 45)**	**Group B - HTN without MS, (n = 47)**
27.74 - 291.27	32	43
> 291	13	4

Hyperaldosteronism was seen in 13 subjects (28%) in group A as compared to 4 subjects (8%) in group B and the difference was statistically significant {Chi2 = 6.34, p=0.011S }

Table No. 4

Shows no. of subjects with raised renin (>1.9ng/ml/hr) in group A & B		
Renin	**Group A - HTN with MS, (n = 45)**	**Group B - HTN without MS, (n = 45)**
0.5 - 1.9	21	26
>1.9	24	21

Renin levels were raised in 24 subjects (53%) in group A & in 21 subjects (44%) in group B and the difference was not statistically significant {Chi 2 = 0.69, p =0.40 NS}

Table No. 5

Shows no of subjects with raised insulin (>22 μIU/ml) in group A & B		
Insulin	**Group A - HTN with MS, (n = 45)**	**Group B - HTN without MS, (n = 47)**
2.1 - 22	25	41
>22	20	6

Insulin levels were raised in 20 subjects (44%) in group A as compared to 6 subjects (12%) in group B and the difference was statistically significant

{Chi2 = 11.38, p=0.0007 HS}

Table No. 6

Showing correlation of Plasma Aldosterone level with various demographic, biochemical and hormonal parameters

	Pearson correlation coefficient	
	Group A with MS	**Group B without MS**
Abdominal circumference	0.63	0.14
BMI	0.64	0.32
Fasting Blood Glucose	0.66	-0.06
Plasma Insulin	0.51	0.01
HOMA index	0.55	-0.013
Plasma Renin	0.08	0.51
LV Mass Index	0.55	0.07
Diastolic Dysfunction E/E'>8	0.50	0.05

Abdominal circumference showed positive correlation with plasma aldosterone in group A {r=0.63}, while group B showed very weak correlation {r=0.25}. BMI was linear correlated with plasma aldosterone level in group A {r=0.64}, while in group B the correlation was very weak {r=0.32}. Fasting Blood Glucose level showed positive correlation with plasma aldosterone in group A {r=0.55}, while in group B there was no correlation as the r value (0.08) was close to zero. Fasting Plasma Insulin also showed positive correlation with plasma aldosterone level in group A {r=0.51}, while in group B there was no correlation {r=-0.02}. HOMA index had showed positive with plasma aldosterone in group A {r=0.54}, while in group B there was no correlation {r=-0.05}. Plasma Renin level showed positive with plasma aldosterone level in group B {r=0.58}, while in group A showed very weak correlation {r=0.08}. The value of LV Mass Index were linearly & positively correlated with plasma Aldosterone levels in group A {r=0.52}, while in group it showed very weak correlation {r=0.07}. The extent of

diastolic dysfunction (psuedonormalistion i.e E/E'>8) showed positive correlation with plasma aldosterone level in group A{r=0.5}, while in group B it was not correlated with plasma aldosterone level {r=0.05}

Table No. 7

Clinical characterstics, laboratory variables and glucose metabolism parameters of hypertensive subjects with metabolic syndrome (Group A) according to plasma aldosterone tertiles

GROUPA (MS n=45)	**Aldosterone 27-291pmole/L (n=32, 23M/9F)**	**Aldosterone 291-477 pmol/L (n=9, 4M/5F)**	**Aldosterone 4771000pmol/L (n=4, 3M/1F)**
Age	51.93±11.85	52.77±14.47	61.25±8.53
SBP	142.5±13.91	151.11±12.69	160±8.16
DBP	90±4.39	95.55±7.26	97.5±5
Abdominal Circumference	M 92.34±2.18 F 94.2±3.63	F 88.22±3.07 M 111.6±3.21	M 110±2.06 F 96
BMI	M 26.85±1.45 F 28.24±1.10	F 26.57±2.53 M 29.60±0.77	M 28.85±1.02 F 30.40
FBG	105.31±6.24	118.5±4.33	124.75±10.68
Insulin	19.13±21.73	27.75±22.21	56.25±32.66
HOMA index	5.19±6.21	8.56±6.91	16.32±8.61
Renin	3.90±6.74	4.9±3.29	5.58±5.21
LV Mass index	98.08±14.59	101.66±23.21	127.75±17.93
E/E'	10.97±4.23	11.22±2.59	17.11±1.79

Systolic & Diastolic Blood Pressure was also higher among subjects with rising plasma aldosterone tertiles {p=0.002S}. Fasting Blood Glucose, Fasting Insulin level & HOMA Index values were significantly higher in groups according to rising plasma aldosterone tertiles {pvalue 0.001,0.01 & 0.007 respectively S}. Mean Renin values were more in group with rising plasma aldosterone tertiles however the difference was not statistically significant {p=0.19NS}. The value of LV Mass Index showed rising trend according to the plasma aldosterone tertiles and it was statistically significant {p=0.007HS}. The value of E/E' was progressively rising according to the plasma aldosterone tertile in patients with metabolic syndrome {p=0.015S}

Discussion :

Why only metabolic syndrome??? Could the elevated aldosterone in subjects of MS just be because of higher BP in obese patients??? There has been extensive research regarding these since past decade in the west, however data regarding the same is very limited in Southeast Asia. A new role of Plasma aldosterone is being evaluated causing metabolic syndrome because of recognition of its nongenomic effect on adipose tissue. The nongenomic MR receptors present on the extraadrenal sites are responsible for these effects.

Recently in 2012 **O'Seaghdha CM et al**[10] evaluated Framingham third generation study regarding visceral and subcutaneous adiposity with renin, angiotensin and aldosterone system.

In our study the difference between the two groups in the mean plasma aldosterone levels was statistically significant {p=0.0073 ***HS***} while the mean plasma renin levels were raised in both groups, similar finding were also found in studies conducted by **Bochud M et al**[11], **Colussiet GL al**[12], **Fallo F et al**[13] as shown in above table which further potentiates the hypothesis that the raised plasma

Study	Journal	Aldosterone (pmol/L)		Renin (ng/ml/hr)	
		Group A (with MS)	Group B (without MS)	Group A (with MS)	Group B (without MS)
Bochud M et al[11] {n=107}	Hypertension.2006; 48:239-245	163.66±74.98 {n=107}	135.92±55.48 {n=249}	0.44±0.49	0.33±0.41
Colussi GL et al[12] {n=356}	Diabetes Care September 2007	462.25±341.2 {n=356}	363.39±213.59 {n=102}	1.08±1.7	0.92±1.07
Fallo F et al[13] {n=466}	J Clin Endocrin Metab. 2006	527.06±257.9 {n=113}	571.44±230.24 {n=268}	3.32±2.4	3.03±3.1
Present study {N=92}		200.31±156.8 {n=45}	119.28± 124.8 {n=47}	4.24±6.01	2.71±3.58

aldosterone level in subjects of metabolic syndrome is independent of RAAS axis and could be accounted for the *epoxy keto derivative of linoleic acid* a potent stimulator of aldosterone production from extraadrenal sites.

In our study 13 patients (28%) from group A had Hyperaldosteronism (>291pmol/L) as compared to 4 patients (8%) in group B and further Abdominal circumference & BMI showed positive correlation with plasma aldosterone level {r=0.63 & 0.64 respectively} in group A as compared with group B which showed very weak correlation {r=0.25 & 0.32 respectively} and the values were also statistically significant with rising plasma aldosterone level tertiles. Hence this reconfirmed our hypothesis that abdominal obesity leads to increased aldosterone production through the secretion of aldosterone stimulating factor from the adipose tissue and this interaction of fat and the adrenal cortex is a positive servo regulatory relationship whereby fat increases aldosterone, and aldosterone, in turn, promote further adipogenesis and inflammation in fat tissue.

Fasting Blood Glucose, Fasting Insulin level & HOMA Index showed positive correlation with plasma aldosterone in group A {r=0.55,0.51 & 0.54 respectively}, while in group B there was no correlation as the r value {0.08,-0.02 & - 0.05 respectively} was close to zero and subjects with higher aldosterone levels had greater degree of insulin resistance which were evident with statistically significant result of FBG, insulin and HOMA values according to rising aldosterone tertiles . Hence we can say that aldosterone through its various proposed mechanisms (effects of hypokalemia on pancreatic beta-cells, direct effects of aldosterone on insulin receptors, activation of hepatic neoglucogenesis and effects on Na/glucose cotransporter) leads to a state of insulin resistance.

While, plasma Renin level showed positive with plasma aldosterone level in group B {r=0.58}, while in group A it showed very weak correlation {r=0.08}. this further confirmed our assumption regarding elevated aldoterone levels in metabolic syndrome which are not secondary to RAAS axis.

The subjects in group A had greater LV Mass Index and higher degree of Diastolic Dysfunction than subjects of group B who had essential hypertension without MS and the results correlated with rising plasma aldosterone levels tertiles. Hence we can propose that raised Aldosterone levels were responsible for mediating maladaptive remodeling in the heart in subjects with Metabolic Syndrome. Left ventricular hypertrophy, cardiac fibrosis and diastolic dysfunction were all associated with higher aldosterone. **Ana Azevedo et al[14] in 2007** found a positive association between the number of features of metabolic syndrome and parameters of cardiac structure and function.

Conclusions :

So to conclude we can say that aldosterone has a far more implication rather than being only a culprit to be sought for in cases of resistant hypertension. With our knowledge of nongenomic effects of aldosterone and a linoleic acid derivative from adipose tissue a potent stimulator for aldosterone secretion, aldosterone would play a far major role in obese individuals with MS causing further insulin

resistance and cardiac remodeling and emerge as a potential target for medical therapy. So agents acting as aldosterone antagonists could be studied as primary agent or as an add on therapy to be used in patients of Hypertension with Metabolic Syndrome having resistant hypertension and heart failure.

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