



Determination of Urinary Calcium and Magnesium Levels by Atomic Absorption Spectrophotometry in Hypertensive Patients

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Urinary magnesium and calcium excretion has been determined in 36 patients suffering from hypertension compared with 31 age and gender matched normotensive control subject living in Nigde. Overnight urine samples were collected. Magnesium and calcium were determined by atomic absorption spectroscopy. The differences in urinary calcium excretion between normotensive (171.3 ± 11.9 mg/day) and hypertensive (151.3 ± 13.0 mg/day) and urinary magnesium excretion between normotensive (40.1 ± 4.1 mg/day) and hypertensive (46.8 ± 3.2 mg/day) were not significant ($p > 0.05$).

Key Words: Hypertension, Urinary, Calcium, Magnesium, Systolic blood pressure, Diastolic blood pressure.

INTRODUCTION

Hypertension is a serious threatening and insidious disease for human health. Hypertension itself is not a disease but, it is an outcome of some of the diseases emerged in body. However, when hypertension is developed, it makes up the main cause for some of the diseases. High rates of tension is paralleled with life length cuts. An untreated hypertension patient has 20 years expected life length following the development of hypertension. However, individuals both provided treatment for hypertension and for major related disease may have normal life expectancy of a healthy individual¹⁻³. 90 % of the occlusions related to hypertension are constituted of primary hypertension¹. Contemporary medications have not achieved to manifest underlying causes for those cases. Primary hypertension cases are significantly important for the future of an individual's health. For the primary hypertension diagnosis secondary hypertension certainly must have been eliminated. That means, none of the diseases leading to hypertension must have been stated that are not possessed by that individual. For performing these checks urine plays an important role in the analysis carried out^{1,2-5}.

An adult individual carries *ca.* 1000-1200 mg calcium in the body. Normally 50 to 350 mg calcium is daily discharged from the body. In summer that rate is higher and varies among individuals^{6,7}. Calcium filtered by men through urine and other electrolyte quantities are 22 % higher than women⁸. Conflicting

results are provided by epidemiological studies investigating the relation between calcium intake and hypertension⁹. Many studies stated that calcium has reducing impact on tension in individuals with hypertension^{10,11}. According to previous report the rate of calcium turn-over is independent than the intake of diabetes¹². Antihypertensive impact of calcium is also proved by a study carried out on rats¹³.

An adult carries nearly 25 mg magnesium in the body. Half of that rate is supplied in bones and tooth, remaining is supplied in tissues (especially in liver and skeleton muscles) and little amount is supplied in liquids. Most of the magnesium in the body liquids is supplied in intra-cell liquid. 65 % of the magnesium in liquids is supplied related to ion and the remaining is supplied related to serum proteins¹⁴. Magnesium is supplied by most of the foods¹⁵⁻¹⁷. Increase of the magnesium eliminated by urine duct many diseases begin to emerge mainly in kidneys¹⁵. It is well known that parent-lateral magnesium salts in the body have lessening impact on blood pressure⁷. In the sustainable management of blood pressure, it has been stated that adequate evidence related to whether inclusion of magnesium salts on the diabetic of hypertension patients play important role or not have not been obtained⁹⁻¹⁷.

Urine factor as a crucial fact for the diagnosis of many of the diseases further to the fact that it is an extra-cellular liquid covering the body cells and since in that media cells meeting their survival function increase and decrease of the substance met in the urine, provide a significant marks for the diagnosis or for the monitoring a treatment applied in a disease^{2,5,6}.

Under normal conditions, together with no high level variation in the quantity of urine in various days, urine samples provided in various hours in a same day may exhibit considerable variations. Thus, quantitative experiments conducted in the urine samples detected are relied in 24 h urine collection¹⁸.

But, in the studies recently performed it has been found that urine amount collected in night represents the urine collected in 24 h¹⁹.

EXPERIMENTAL

Thirty six voluntary individuals among the primarily hypertensive patients from the various state hospitals and health units and another 31 voluntary individuals non-hypertensive residing in the same settlements with the normotensive individuals in Nigde city, Turkey participated to the study. Since it is known that the urine collected during the entire night represents the urine collected in 24 h, urine samples collected from hypertensive and normotensive groups during the entire night. While collecting urine samples, 2 L plastic pots were distributed among the patients. Those pots following conservation in 6 N HNO₃ 6 h previously, treated with tri-distilled water three times and dried in 40 °C in an oven. Following the collection of urine, its volume was measured and 100 mL of it separated in 100 mL dried plastic samples pots following treatment in acid and tri-distilled water respectively. Those samples were kept in deep freeze until the analysis and following the analysis. In order to provide relevant information for the individuals in both groups two separate forms were given to participants of the study. First form were compiled of the information belong to subjects. Second form was describing the ways for urine collection. That form was given to subjects. Those forms were provided by means of the tools supplied in the health care centers where the subjects were residing. Among those heights and weights measurements, following conducting twice by the 'height measurement device' and 'scale' supplied in health care centers body mass index (BMI) for each subject was provided on the basis of the following formula²⁰:

$$\text{Body mass index (BMI)} = \frac{\text{Body weight (kg)}}{[\text{Body height (m)}]^2}$$

Gaining from BMI separately based on body weights from men and women, considering the standards indicated in World Health Organization they were classified²⁰. For all subjects, blood pressure mean values based on measurements performed in morning, evening and night three times a day provided and

recorded in the forms. Calcium and magnesium analysis in urine samples were carried out by atomic absorption spectrometric method. For that treatment, Shimadzu AA-6501 model atomic absorption spectrometry was used. Taking urine samples from deep freeze were left in room temperature and heated in 37 °C in water bath. 0.1 mL urine collected and kept in 0.25 % Lantanium diluent solvent and prepared for the analysis, analysis was carried out based on 'standard adding' method¹⁰. In both hypertensive and normotensive groups evaluation was done by intra-group regression analysis. In normotensive subjects body mass index; diastolic blood pressure, systolic blood pressure, for primary hypertensive subjects, t-test for finding out the variation between calcium and magnesium was implemented²¹.

RESULTS AND DISCUSSION

Personal information related to hypertensive and normotensive groups and daily purification of calcium and magnesium were statistically evaluated and depicted down. Personal information fro both groups are given in Table-1.

As seen in Table-1, hypertensive group is older than normotensive group ($t = 2.82, p < 0.05$). Mean age of the men in primary hypertensive group were not found statistically higher than normotensives ($t = 1.78, p > 0.05$). Mean body mass index (BMI) values in primary hypertensive group were not statistically found different than mean BMI values of normotensive group ($t = 0.26, p > 0.05$). In the same way, in the evaluation between primary hypertensive men and normotensive men and primary hypertensive women and normotensive women no statistical variation was met between BMI values ($t = 0.80, t = 0.57, p > 0.05$).

The statistical evaluation of the calcium and magnesium quantities discharged in 24 h through urine duct is given in Table-2.

In normotensive men while daily 183 (± 70) mg calcium is discharged, in hypertensive men 163 (± 63) mg calcium was discharged. Discharged calcium quantity in normotensive group was not found over hypertensive group statistically ($t = 0.85, p > 0.05$). Similar situation was also observed among women ($t = 0.95, p > 0.05$). In the evaluation carried out considering any differentiation between men and women calcium discharged urine duct in hypertensive group in 24 h was not found different than normotensive group ($t = 1.15, p > 0.05$). In hypertensive group average calcium level daily discharged by men was 163 mg by women it was corresponding to 137

TABLE-1
COMPARISON OF THE INFORMATION RELATED TO SUBJECTS IN HYPERTENSIVE AND NORMOTENSIVE GROUPS

	Group	Man					Woman					Total				
		n	X ¹	SD ¹	t	p	n	X ¹	SD ¹	t	p	n	X ¹	SD ¹	t	p
Age (year)	HT	17	54.5	1.5	1.78	> 0.05	19	56.2	1.9	2.22	< 0.05	36	55.4	1.2	2.82	< 0.05
	NT	14	49.4	2.5			17	50.1	2.0			31	49.8	1.6		
DBP	HT	17	102.9	1.6	9.41	< 0.01	19	101.8	1.3	9.02	< 0.01	36	102.4	1.0	13.15	< 0.05
	NT	14	80.7	1.8			17	81.5	1.8			31	81.1	1.3		
SBP	HT	17	143.2	4.7	7.00	< 0.01	19	151.1	3.7	9.85	< 0.01	36	147.2	3.0	11.68	< 0.05
	NT	14	105.7	2.6			17	112.1	1.4			31	108.9	1.4		
BMI (w/hm ²)	HT	17	24.6	0.8	0.80	> 0.05	19	24.4	0.7	0.57	> 0.05	36	24.5	0.5	0.26	> 0.05
	NT	14	25.5	0.8			17	23.8	0.8			31	24.7	0.6		

X¹ = Arithmetic mean; SD¹ = Standard deviation; HT = Hypertensive; NT = Normotensive.

TABLE-2
STATISTICAL EVALUATION OF CALCIUM AND MAGNESIUM AMOUNTS FILTERED URINE DUCT IN 24 h

Group		Man					Woman					Total				
		n	X ¹	SD ¹	t	p	n	X ¹	SD ¹	t	p	n	X ¹	SD ¹	t	p
Calcium (mg/day)	HT	17	162.8	15.4	0.85 > 0.05	19	136.7	21.0	0.95 > 0.05	36	151.1	13.0	1.15 > 0.05			
	NT	14	183.3	18.8		17	161.4	15.6		31	171.3	11.9				
Magnesium (mg/day)	HT	17	43.1	4.8	0.28 > 0.05	19	49.8	4.2	1.33 > 0.05	36	46.8	3.2	1.28 > 0.05			
	NT	14	40.8	5.2		17	39.5	6.4		31	40.1	4.1				

X¹ = Arithmetic mean; SD¹ = Standard deviation; HT = Hypertensive; NT = Normotensive.

(± 91) mg. but, variation between those two group was not statistically significant ($t = 1.00$, $p > 0.05$). Similar situation was also met in normotensive group ($t = 0.91$, $p > 0.05$). Finally, both age factor and disease situation over calcium discharge were not having statistically important impact. In primary hypertensive group daily average 46.8 (± 19.2) mg magnesium is being discharged. In normotensive group that value corresponds to 40.1 (± 23.1). Average magnesium quantity discharged in primary hypertensive group although was higher compare to normotensive group, variation between both mean values was not statistically significant ($t = 1.28$, $p > 0.05$). Hypertension has not statistically effect urine duct calcium discharge. Daily magnesium discharged by women in hypertensive group (49.8 ± 18.2 mg) was higher than men (43.1 ± 19.7 mg). But, that excess ratio is not statistically significant ($t = 1.10$, $p > 0.05$). Any crucial impact caused by gender was not found on urinary magnesium discharge.

Calcium and magnesium quantities discharged urinary duct and correlations between BKI, DKB and SKB: No statistically significant positive correlation between BMI of men in hypertensive group related to urine magnesium levels ($r = 0.61$, $t = 3.05$, $n = 17$, $p < 0.01$). 37.2 % of urine duct magnesium intake was found correlated with BMI ($R^2 = 0.372$). In the same group, between BMI and urine calcium ratios and DKB and SKB rates negligible positive correlations were found ($t = 1.20$; $t = 1.12$; $t = 0.80$, $p > 0.05$). Among the hypertensive women a weak positive correlation was met between BMI and urine calcium amounts ($t = 1.77$, $p < 0.10$). In that group, infinitesimal positive correlation between BMI and urine calcium amounts, DKB, SKB are not statistically significant. In the evaluation carried out without considering variation between genders a significant positive correlation was seen between calcium rates discharged by urine and BMI ($r = 0.47$, $t = 3.10$, $n = 36$, $p < 0.01$). Urine duct calcium discharged was found related to BMI in 22.1 % ($R^2 = 0.221$). Among men hypertensive, between DKB and SKB, DKB and magnesium rates positive correlations were found ($r = 0.66$; $r = 0.48$, $t = 3.40$; $t = 2.12$; $p < 0.05$). In the evaluation carried out without gender variation positive correlation found between DKB and SKB was significant ($r = 0.47$, $t = 3.10$; $t = 2.12$, $p < 0.01$).

When SKP lead to an impact over calcium and magnesium discharged by urine was tested, only in the evaluation conducted by the entire hypertensive women and complete hypertensive group significant positive correlations were seen between SKB and urinary calcium ($r = 0.48$; $r = 0.35$; $t = 2.18$; $p < 0.05$). Positive correlations found between urinary calcium and SKB were not statistically significant ($r = 0.25$ and $r = 0.16$, $p > 0.05$). Also between urinary calcium and magnesium negligible

positive correlation was found ($r = 0.19$; $n = 36$; $p > 0.05$). In normotensive women, between BKI and DKB a significant correlation was found ($r = 0.58$, $n = 17$, $p < 0.05$). Positive correlation between DKB and urinary magnesium is also significant ($r = 0.45$, $t = 1.95$, $p < 0.01$). In normotensive women at the same time, as long as BMI rise up increase on DKB was met ($r = 0.36$, $n = 31$, $t = 2.08$, $p < 0.05$).

When both groups are evaluated together, increase in urinary duct magnesium discharge lead to increase in DKB ($r = 0.22$, $t = 1.82$, $n = 67$, $p < 0.10$). Moreover, positive correlation between DKB and SKB is found significant ($r = 0.30$, $t = 2.54$, $n = 67$, $p < 0.02$). Urinary magnesium increase also rises up DKB even though in weak ratios ($r = 0.26$) among normotensive subjects. Among primary hypertension subjects blood pressure decompressing impact of calcium and magnesium has been proved by many investigations conducted. However, studies conducted to find out correlation between calcium and magnesium quantity discharged by urine revealed contrary outcomes^{9,22-27}.

In the present study, 75 % of the hypertensive subjects found older than 50 years of age. As seen in Table-1 hypertensive group is older than normotensive group ($t = 2.82$, $p < 0.05$). At the end of the studies conducted frequency of hypertension is known to be increasing related to age^{1,9}. Our findings in that matter are in accordance with the other remaining studies.

In our research, mean BMI value of hypertensive group is not statistically different than normotensive group's BMI value ($t = 0.26$, $p > 0.05$, Table-1). In the same manner, in the evaluation held between primary hypertensive men and normotensive men and hypertensive women and normotensive women no statistically significant variation was seen in their pertaining BMI ratios ($t = 0.80$, $t = 0.57$, $P > 0.05$, Table-1). Obesity is shown as a cause for hypertension^{9,28}. Findings provided in that study completed with a limited number of subjects are contrary with the findings covered in literature.

Daily average amount of calcium discharged urine duct in hypertensive group was found as 151 (± 78) mg, in normotensive group was found as 171 (± 67) mg. Urinary calcium discharge varies considerably among different individuals. Also in the past studies similar findings were found^{24,25}. Calcium discharge varies in the range^{7,14} of 50-350 mg. Lowest value found was 41 mg highest value was 341 mg. those values are in accordance with the values given in the literature. Urinary calcium discharge in hypertensive group was found same with the urinary calcium discharge of normotensive group. All these findings provided in other studies related to whether hypertension has an impact leading to increase of urinary calcium

discharge are clashing with the findings provided in that study⁹. While its increasing impact was found in some studies, in some of the others it was not found^{22,24-27}.

In another study empirical findings of related 15 hypertensive subjects were compared with the normotensive subjects in a group constituted of the individuals *ca.* 16 years of age. In normotensive group ordinary nutrition is applied while daily 130 (\pm 14) mg is discharged urinary duct in hypertensive group 201 (\pm 37) mg calcium was discharged. No significant variations were found in those values²⁴. In another study calcium discharge speed rate in hypertensive group was found greater than normotensive group (199.0 \pm 44.7 and 152.3 \pm 33.6 mg Ca p < 0.001)²⁵. Average values provided in that study are in accordance with the other studies. In that study blood pressure rate below 90 mm Hg was selected as a criterion as normotensive. No variation was made as less than 85 mm Hg.

Urinary duct calcium discharge varies among the individuals. Average ratios provided are as follows; in hypertensive group 46.8 (\pm 19.2) mg magnesium, in normotensive group 40.1 (\pm 23.1) mg magnesium. Urinary magnesium quantity in hypertensive group although appeared higher in normotensive group it was found that statistically it was not significant. If study was possible to be conducted with higher number of subjects probably that difference would have been more remarkable. In the studies performed in that subject similar results were provided^{22,26,29,30}.

In one of the study, it was found that urinary duct magnesium discharged in a group with high diastolic blood pressure is higher than urinary duct magnesium discharge in normotensive²⁶. But in a different another study, urinary duct discharged magnesium quantity is higher than normotensive group³⁰. Data are present pertaining to the fact that as blood pressure rises up it raises in divalent cations²².

As conclusion mean urinary duct magnesium values and correlations of those values with the blood pressure are in accordance with the results reported in other studies.

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