

Effect of Amlodipine drug on male sex hormones of hypertensive patients in Al-Najaf province

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الخلاصة

أجريت الدراسة الحالية في مستشفى الصدر التعليمي محافظة النجف للفترة من 2011/6/5 إلى 2011/12/2. منته الدراسة تقويم لتأثير املوديبين وديبين المستخدم لعلاج ارتفاع ضغط الدم على الهرمونات الجنسية الذكرية (الهرمون اللبوتيني ون المحفز للجريبات وهرمون الشحمون الخفد) لدى 45 ذكرًا مريضًا ممن يعالجوا بهذا العقار بأعنتسارافوتجيد م يتقاسم 3-60مهم الـ 15 (اميع 15) ذكر/مجموعه ادا على الجرعة المختلفة من عقار املوديبين 2.5 ، 5 ، 10 ، 15 ملغم كما استخدمت 15 بحالة صدحية جيدة كمجموعة بطرة بأعم لتتواوح بين 30-63 سنة.

أظهرت نتائج الدراسة وجود انخفاض معنوي في فعالية هرمون LH و FSH و Testosterone في المرضى المعالجين في الجرعتين الأعلى من الاملوديبين مقارنة مع اميع السيطر والجرعة القليلة يتلحق بتأثير الأعمار المختلفة لمرضى ارتفاع ضغط الدم الذكور المعالجين بعقار املوديبين على هذه الهرمونات إذ أوضحت النتائج وجود انخفاض معنوي لمسدتوى هذه الهرمونات في الأعمار الأعلى خصوصاً عند 15 و 30 ملغم. بآخر أوضحت النتائج ان هذا التأثير للداخلين الجرعة وديبين وأعمار المرضى بارتفاع ضغط الدم المعالجين به ذا العقار على مستوى الهرمونات الجنسية الذكرية وبشكل خاص عند الجرعة والأعمار الأعلى وهذات التأثير يتمثل بانخفاض مستوى هذه الهرمونات.

تقترح هذه الدراسة بان العلاج بالاملوديبين ممكن ان يسبب خلافاً في الجهاز التناسلي للذكور يتبين من خلال تثبيط مسدتوى الهرمونات الجنسية الذكرية وهذات التأثير يعتمد على جرعة الاملوديبين وأعمار المرضى.

Abstract

The current study was carried out in Al-Sader teaching Hospital in Al-Najaf province during the period 2011/6/5 till 2011/12/2. The study was undertaken to evaluate the effect of Amlodipine drug that's uses to treatment of hypertension on male sex hormones (LH ,FSH and testosterone) in a number of males hypertensive patients .

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Forty five males patients who are took this drug to lower their high blood pressure participated in the study , their ages ranged from (31-60) years divided into three groups (15 male/group) according to amlodipine doses 2.5, 5 and 10 mg once daily .Also contribute fifteen health males as a control subjects, their ages ranged from (30-63) years.

Results of the study revealed a significant decreased of LH, FSH and testosterone activities in a patients who are treated with two highly doses of amlodipine when compared with control group, also when compared high dose group with low dose group. Regarding the effect of different ages of male hypertensive patients who are treated with amlodipine on these hormones , the findings were indicated a significant decreased of these hormones levels at the highest ages especially at 51-60 years old. On the other hand, the results obtained there were an effect of interaction between different doses of amlodipine and ages of hypertensive patients that treated with it on male sex hormones levels particularly at the higher doses and ages , and this effect was represented by decreased of these hormones levels. The study was suggested that the therapy with amlodipine may be causes defect in male reproductive system that indicated by inhibition of male sex hormones levels and this effect was amlodipine doses and patients' ages dependent.

Introduction

Hypertension is one of the most common diseases afflicting humans throughout the world because of the associated morbidity and mortality and the cost to the society (1,2). Hypertension has been an important public-health challenge over the past several decades. It is the most important modifiable risk factor for coronary heart disease (3).

Calcium Channel Blockers (CCBs), the most frequently prescribed drugs for the cure of cardiovascular diseases, block transmembrane calcium influx through calcium channel (4,5). Calcium ions are vital in many biological processes including excitation-contraction coupling, excitation-secretion coupling,

mitosis, fertilization and regulation of gene expression, but the large effects of CCBs by remain confined to heart and vascular smooth muscles (6). Thus, the most widespread clinical usage of calcium channel blockers is to decrease blood pressure in patients with hypertension and angina pectoris with particular efficacy in treating elderly patients (7). The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels (8). Amlodipine is a new calcium antagonist of the 1,4-dihydropyridine group for the treatment of hypertension and angina pectoris, it is chemically described as 3-Ethyl-5-methyl-2- [(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate monobenzenesulphonate, Its chemical formula is $C_{20}H_{25}C_1N_2O_5$ (9). The presence of calcium ions is essential for the release of a variety of hormones, with regard to anterior pituitary and testes hormones secretion, in vivo studies have suggested a role for calcium ions in the release of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) from anterior pituitary gland cells and release of testosterone hormone from Leydig and Sertoli cells (10). Other in vivo studies have demonstrated that Ca^{2+} plays an essential role in gonadotrophin-releasing hormone (GnRH), when amlodipine administered, this leading to inhibition of LH and FSH release (11). In vivo, we shown that amlodipine a substance inhibit calcium entry into anterior pituitary cells as well as inhibition of LH and FSH release, also this study established this drug inhibition of testosterone release for the same reason (12). Thus, because of CCBs especially amlodipine inhibition of calcium ion influx into sexual cells through the slow calcium channels in cell membrane (13). And the sexual dysfunction has high prevalence among hypertensive men which receive antihypertensive drug (14). So, the present study was designed and carried out to observe the effect of amlodipine on serum testosterone, LH and FSH in male hypertensive patients.

Material and Methods

Patients

This current study was undertaken over a period of six months from 2011/6/5 till 2011/12/2 in Al-Sader Hospital in Al-Najaf province . Forty five male hypertensive patients who took amlodipine therapy (BRISTOL Laboratories ,UK) to lower their high blood pressure participated in the study , Their ages ranged from (31-60) years divided into three groups according to the ages (31-40, 41-50, 51-60) years old .The forty five patients divided to three groups (15/group) according to amlodipine doses 2.5, 5 or 10 mg once daily. Patients with any disease which may cause alteration of sex hormones were excluded from the study like sexual diseases and renal failure, also patients on continuous administration of other drugs were excluded from the study, these may be aided by taking history, physical and clinical examination of the patients.

Controls :

Also contribute fifteen health males as control subjects, their ages ranged from (30-63) years , subjects with any disease or continuous administration of any drug were excluded from the study.

Collection of samples :

About 5 ml of venous blood were taken from patients and controls. Serum was separated by centrifugation and used for estimation of testosterone , LH and FSH .

Determination of hormonal activity:

It Was estimated hormone Testosterone , Follicle Stimulating Hormone and Luteinizing hormone (LH) in serum, using the method of Enzyme Linked Immunosorbent Assay (ELISA) described by both researchers Knobil (1980) and Utila *et al.*, (1981), in the estimation of these hormones in the serum, The absorbance was read at a wavelength (450 nm) nm.

Statistical analysis

All results were expressed as the mean \pm SD. Statistical analysis was performed with statistical package for the social science for windows (SPSS, version 12).One-way analysis of variance

(ANOVA) was used to find the effect of amlodipine according to the doses and ages of men hypertensive patients on sex hormones. Also one-way ANOVA used to find the interaction between the doses and ages. The P.value <0.05 was considered statistically significant (15).

Results

Effect of different doses of amlodipine on male sex hormones in hypertensive patients:

To determine the effect of different doses of this drug on the selected male sex hormones (LH, FSH and Testosterone), a one-way ANOVA was used to analyze the data. Table (1) shows that LH levels was decreased significantly ($P < 0.05$) in D2 (4.38 ± 0.57) and D3 (4.21 ± 0.58) when compared with both control group (C) (5.16 ± 0.50) and D1 (4.97 ± 0.61). This table also indicates that FSH level decreased significantly ($P < 0.05$) in D2 (5.84 ± 0.48) when compared with C (6.26 ± 0.57), In contrast, FSH hormone levels decreased in the same significant in D3 (5.58 ± 0.65) when compared with C (6.26 ± 0.57) and D1 (6.06 ± 0.46) respectively.

Whereas testosterone hormone levels elicited a significant decrease ($P < 0.05$) in D2 (3.60 ± 0.50) and D3 (3.41 ± 0.69) when compared with both C (4.34 ± 0.63) and D1 (4.15 ± 0.37) respectively.

Effect of different ages of male hypertensive patients who are treated with amlodipine on sex hormones :

To determine the effect of different ages of male hypertensive patients on reproductive hormones, a one-way ANOVA was used to analyze the data, in this approach of analysis, the effect of dosing level is neglected and the data was collected taking in our consideration the result of patients' ages of the (A1), (A2) and (A3) that's mean 31-40, 41-50 and 51-60 years old respectively. Table (2) shows that LH (4.41 ± 0.66) and FSH (5.74 ± 0.74) levels was decreased significantly ($P < 0.05$) only in A3 when compared with LH level in A1 (4.86 ± 0.63) and FSH level in A1 (6.13 ± 0.44) respectively.

While testosterone levels decreased significantly ($P < 0.05$) in A2 (3.79 ± 0.51) and A3 (3.66 ± 0.79) respectively when compared with A1 (4.11 ± 0.63).

Effect of interaction between different doses of amlodipine and ages of male hypertensive patients who are treated with it on sex hormones:

ANOVA was used to analyze the interaction between the doses and ages on selected sex hormones, table (3) show the effect of this interaction on LH hormone and indicates there are a significant decrease ($P < 0.05$) in LH of patients treated with 5mg (4.07 ± 0.62) when compared with control (5.05 ± 0.12) at 51-60 years ages, also it founded these significant decrease in this hormone of patients treated with 10mg at 31-40 years old (4.44 ± 0.52), 41-50 years old (4.31 ± 0.52) and 51-60 years old (3.88 ± 0.46) when compared with controls at the same ages above respectively (5.22 ± 0.54), (5.21 ± 0.77) and (5.05 ± 0.12). Also ($P < 0.05$) in LH of patients treated with 10mg (3.88 ± 0.46) when compared with 2.5mg (4.65 ± 0.44) at 51-60 years old ages.

On the other hand, table (4) explain the effect of this interaction on FSH hormone and elicited there are only a significant decrease ($P < 0.05$) in FSH of patients treated with 10mg (4.93 ± 0.52) when compared with control (6.38 ± 0.61), 2.5 (5.96 ± 0.53) and 5mg (5.68 ± 0.52) respectively at 51-60 years old ages.

Whereas table (5) illustrate the effect of this interaction on Testosterone hormone and demonstrated there are a significant decrease ($P < 0.05$) in testosterone of patients treated with 5mg (3.45 ± 0.33) and 10mg (3.37 ± 0.38) when compared with both of control (4.39 ± 0.09) and 2.5mg (4.20 ± 0.05) respectively at 41-50 years old ages. Also there are same significant in this hormone of patients treated with 2.5mg (4.00 ± 0.45) when compared with control (4.62 ± 0.71) at 51-60 years old. Besides ($P < 0.05$) in the patients treated with 5mg (3.24 ± 0.17) when compared with both of control (4.62 ± 0.71) and 2.5mg (4.00 ± 0.45) respectively at 51-60 years old ages, while testosterone level in the patients treated with 10mg (2.77 ± 0.44) at the similar age above, it shown the same significant when compared with control (4.62 ± 0.71), 2.5 (4.00 ± 0.45) and 5mg (3.24 ± 0.17) at the same age.

Table (1) Effect of different doses of amlodipine on male reproductive hormones in hypertensive patients:

Hormones	Group	Amlodipine doses (mg/day)	SD	± Mean
LH (mIU/ml)	C	0	0.50	±5.16
	D1	2.5	0.61	±4.97
	D2	5	0.57	ab± 4.38
	D3	10	0.58	ab± 4.21
FSH (mIU/ml)	C	0	0.57	±6.26
	D1	2.5	0.46	±6.06
	D2	5	0.48	a± 5.84
	D3	10	0.65	ab ±5.58
Testosterone (ng/ml)	C	0	0.63	±4.34
	D1	2.5	0.37	±4.15
	D2	5	0.50	ab± 3.60
	D3	10	0.69	ab± 3.41

C: control, **D1.....►D3:** patients groups treated with different doses.
a: means there are a significant difference between (D2 ,D3) and control group.
b: means there are a significant differences between (D2 ,D3) and D1.
 number of participated males :15/group.

Table (2) Effect of different ages of male hypertensive patients who are treated with amlodipine on reproductive hormones :

Hormones	Group	Ages (years)	Mean±SD
LH (mIU/ml)	A1	31-40	4.86±0.63
	A2	41-50	4.78±0.70
	A3	51-60	4.41± 0.66 a
FSH (mIU/ml)	A1	31-40	6.13±0.44
	A2	41-50	5.94±0.51
	A3	51-60	5.74±0.74 a
Testosterone (ng/ml)	A1	31-40	4.11±0.63
	A2	41-50	3.79±0.51 a
	A3	51-60	3.66±0.79 a

A1.....►A3: different ages.
a: means there are a significant differences between (G2 ,G3) and G1.
 number of participated males :20/group

Table(3)Effect of interaction between different doses of amlodipine and ages of male hypertensive patients who are treated with it on LH hormone:

Ages (years) Doses (mg/day)	Mean \pm SD		
	31-40	41-50	51-60
Control	0.54 \pm 5.22	0.73 \pm 5.21	0.12 \pm 5.05
2.5	0.30 \pm 5.15	0.69 \pm 5.12	0.44 \pm 4.65
5	0.55 \pm 4.61	0.54 \pm 4.46	0.62a \pm 4.07
10	0.52a \pm 4.44	0.52a \pm 4.31	0.46 ab \pm 3.88

a: means there are a significant difference between (5 and 10mg) and control group.

b: means there are a significant differences between 10 mg and 2.5mg group .

Table(4) Effect of interaction between different doses of amlodipine and ages of male hypertensive patients who are treated with it on FSH hormone:

Ages (years) Doses (mg/day)	Mean \pm SD		
	31-40	41-50	51-60
Control	0.64 \pm 6.13	0.54 \pm 6.27	0.61 \pm 6.38
2.5	0.49 \pm 6.15	0.47 \pm 6.07	0.53 \pm 5.96
5	0.49 \pm 6.08	0.43 \pm 5.78	0.52 \pm 5.68
10	0.08 \pm 6.17	0.50 \pm 5.64	0.52 abc \pm 4.93

a: means there are a significant difference between 10mg and control group.

b: means there are a significant differences between 10 mg and 2.5mg group .

c: means there are a significant differences between 10 and 5mg group.

Table (5) Effect of interaction between different doses of amlodipine and ages of male hypertensive patients who are treated with it on Testosterone hormone :

Ages (years) Doses (mg/day)	Mean \pm SD		
	31-40	41-50	51-60
Control	1.05 \pm 4.02	0.09 \pm 4.39	0.17 \pm 4.62
2.5	0.50 \pm 4.25	0.05 \pm 4.20	0.45a \pm 4.00
5	0.48 \pm 4.11	0.33 ab \pm 3.45	0.17 ab \pm 3.24
10	0.63 \pm 4.08	0.38 ab \pm 3.37	0.44 abc \pm 2.77

a: means there are a significant difference between (2.5 , 5 and 10mg) and control group. **b:** means there are a significant differences between (5 ,10 mg) and 2.5mg group.

c: means there are a significant differences between 10 and 5mg group.

Discussion

Calcium-Channel Blockers (CCBs) are class of drugs used in treating hypertension, angina pectoris and certain arrhythmias, it prevents the calcium ions needed for muscle contraction from entering the cells of smooth and cardiac muscle, this causes blood vessel walls to relax and blood to flow more freely to the heart (16,17). However, despite exhibiting substantial cardiovascular selectivity, reports exist for infertility in males who are using CCBs (18). So, Amlodipine one of these drugs that's blocked calcium ions influx through calcium channels which have vital role in sex hormones release from anterior pituitary gland and testes, thus it's reduce activity of these hormones (19).

Regarding the effect of the dosing levels of amlodipine administration on male sex hormones, the present study revealed a significantly decreased in LH, FSH and Testosterone in patients groups who are treated with 5 and 10mg amlodipine/daily when compared with control group, also these parameters significantly decreased in these doses when compared with low dose 2.5mg except the effect of 5mg dose on FSH. These decrease showed when the dosing level elevated, so, the variation being significant in those groups of patients administered higher dose of amlodipine. The data from the present study can confirm with results obtained from a studies which cleared that the effect of amlodipine on sex hormones of men hypertensive patients are dose related, that's mean it is higher at high doses (20,21, 22,23,24). Similar findings were reported also by several studies as the researchers who are declared that animal studies have given signals of sexual toxicity of amlodipine indicated by decrease sex hormones activity in thirty male Sprague Dawley rats receiving 5,10mg amlodipine/daily for 50 days (25). Also the same result was reported when uses 28 human male with chronic stable angina and hypertension administrated 2.5,5mg amlodipine/day for 30 months (26). The decreased in serum sex hormones was showed in males group with hypertension who are administrated different doses of amlodipine for several months (27). Other researchers emphasized that testosterone level is decreased significantly in 67% of 54

hypertensive patients who are treated with amlodipine and transdermal for 4-months period (28). In contrast, the present findings were inconsistent by (11,29) that showed no significant effect on sex hormones in hypertensive patients who are treated with amlodipine.

Ages distribution of the measured male sex hormones in the age groups in the present study revealed an inhibition of these parameters according to age elevation, these results may demonstrate an opposite relation between ages and sex hormones, i.e. lower values are associated with high ages, suggesting that the effect of amlodipine on these hormones are age-dependent. These data were supported by previous studies (30,31,22,24). Similar results are also in agreement with this study such as the researchers who performed a study with amlodipine which is administered to 34 hypertensive patients their ages ranged 43-71 years old treated with amlodipine for 8 months, at the end of this study, they declared the Testosterone, LH and FSH was reduced by high ages of patients (32). Also eighty five patients with ischemic stroke and hypertension aged (≥ 61 years) treated with several drugs such as amlodipine and methyldopa, the decline of Testosterone, LH and FSH in patients older than 61 years old was significantly higher than in those younger than 65 (33). One hundred and ten hypertensive men, aged 30-59 years old were administered amlodipine for 39 weeks, reduction of testosterone value observed at different ages but the highest levels of it showed at high ages (34).

Recently, *in vitro* studies demonstrated that Ca^{2+} plays an essential role in gonadotropin-releasing hormone (GnRH)-stimulated LH and FSH release (35). Also a hormonal stimulation of testicular development and testosterone releasing by (LH) involves a Ca^{2+} influx possibly through calcium channels in Leydig cells membranes (36). Reduction of LH, FSH and Testosterone levels in the current study were observed. These reduction in serum testosterone levels by amlodipine may be indicates either a direct effect of drug at Leydig cell level or an indirect effect by disturbing the hormonal milieu at hypothalamo-

pituitary axis that's causes inhibition of LH and FSH releasing and decrease levels of it ,then reduce of testosterone level (37). The presence of calcium channel blockers, the liberation of GnRH from hypothalamic neurons was reduced that's lead to decrease of LH and FSH secretion so, decrease testosterone level (38). In vivo study shows the amlodipine is capable of inhibiting basal gonadotropin release as well as the release of LH and FSH decreased in response to an inhibition of GnRH, as result testosterone level also decrease (10). Also the decreasing of these hormones may be result from CCBs like amlodipine causes blocked of Ca^{2+} channels and inhibition Ca^{2+} entry to gonadotrophs that's causes repression of the $LH\beta$ and $FSH\beta$ genes expression which lead to inhibition of LH and FSH synthesis and decrease testosterone value because of the GnRH that's induced LH and FSH secretion from pituitary gonadotrophs is calcium dependent for the reason that the induction/repression of the $LH\beta$ and $FSH\beta$ genes are dependent on calcium influx (39).

A common belief is that the Ca^{2+} influx and outflux should be tightly regulated to maintain the intracellular Ca^{2+} homeostasis, and an alteration in the Ca^{2+} -transport across the cell membrane by CCBs could result in a drastic impact on spermatogenesis and steroidogenesis (40,41). So, the decline of testosterone may be attributed to the Ca^{2+} channels blockers are effective in causing a premature arrest of developing spermatids and diminishing Leydig cell abundance by abrogating StAR protein expression and thus testosterone production (42). StAR protein expression was abolished by Ca^{2+} channel blockers ,it is assumed that StAR proteins are at the late-limiting step of steroidogenesis in Leydig cells by transporting cholesterol into mitochondria, finally, the serum testosterone level was drastically lowered by the treatment, concomitant with a reduced expression of StAR proteins , so a blockade of Ca^{2+} channel by its respective blockers may lead to a impairment of normal spermatogenesis and steroidogenesis, in particular, during testicular maturation and lowered testosterone level (43).

Also the decline of testosterone level may be due to the effect of CCBs administration that's causes significant oxidative stress in the male reproductive milieu in term of decrease in catalase, superoxide dismutase and glutathione enzymes activities in the testes that's lead to produce free radicals which causes damage or death of Leydig cells and a drastic decline of serum testosterone level (44).

These results also pointed out there are interaction between the dosing levels and ages on these three hormones , that's indicate these parameters levels decreased when the doses and ages raised, these observing were conformity with preceding conclusions (22,24).

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