

Evaluation of cytogenetic damage induced by antihypertensive drug Amlodipine: *in vitro*

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ABSTRACT:

Background: Amlodipine is a calcium channel blocker medication used to treat high blood pressure and coronary artery diseases for long term of use. Sufficient studies reporting the cytogenetic effects of this drug have not reporting yet.

Aim of study: This study aimed to assess the frequency of chromosomal aberrations of amlodipine on human blood lymphocytes *in vitro*.

Material and Methods: In this study, the *in vitro* genotoxic effects of amlodipine have been determined in human peripheral blood lymphocytes by using chromosomal aberrations: 0; 5; 10; 15; 25 and 50mg/mL concentrations of amlodipine was used. All samples cultured in 37°C incubation for 72 hours. Then treated with Colcemid.

Results: Amlodipine did not appear to have cytogenetic effects *in vitro*, as no significant increases in the frequency of chromosomal aberrations were found in cultures treated with different concentrations compared to cultures treated with the recommended concentration (10 mg/mL). The highest concentrations (50 mg/mL) of amlodipine increased chromosomal aberrations compared to negative control, but these increases were not statistically significant

Conclusion: Amlodipine is not seemed to be genotoxic active ingredients in human lymphocytes *in vitro*.

Keywords: Antihypertensive, Amlodipine, chromosomal aberrations, cytogenetics.

المُدخل: املوديبين هو عقار مثبط لقنوات الكالسيوم ، يستخدم لعلاج امراض ارتفاع الدم و الشريان التاجي لفترة طويلة من الاستخدام . لحد الان لم تبلغ الدراسات الكافية عن التأثيرات الوراثية الخلوية.

الهدف من البحث: تهدف هذه الدراسة الى تقييم وتيرة الانحرافات الصبغية الاميلوديبين على الخلايا للمفاوية على الدم البشري خارج جسم الكائن الحي.

المواد وطرائق العمل: في هذه الدراسة ، تم تحديد التأثيرات اسمية الوراثية للاميلوديبين في الخلايا للمفاوية في الدم المحيطي البشري باستخدام الانحرافات الصبغية : 0, 5, 10, 15, وكذلك تراكيز 50 , 25 ملغم/مل من اميلودبي .

زرعت هذه العينات في حضانه 37م° لمدة 72 ساعة , ثم عولج بعقار كولسيميد

النتائج: اظهرت الدراسة بان الاميلوديبين ليس له تأثيرات وراثية خلوية خارج جسم الكائن الحي ، حيث لم يحدث زيادات معنوية في تكرار الانحرافات الصبغية التي عولجت بالجرعه (10 ملغم/مل) . أدت اعلى تركيز 50 ملغم/مل من اميلوديبين الى زيادة الانحرافات الصبغية مقارنة بالسيطرة السالبة ، ولكن هذه التراكيز ليس لها دلالة احصائية.

الاستنتاج : لم يظهر ان الميلوديبين من المكونات السامه للجينات في الخلايا للمفاوية للانسان خارج جسم الكائن الحي.

الكلمات المفتاحية: خافض الضغط، اميلوديبين ، انحرافات الكروموسومات ، الوراثة الخلوية.

1. INTRODUCTION

Hypertension is one of the most important preventable contributors to disease and death, and is commonly treated with antihypertensive drugs combined with appropriate changes in life-style. Hypertension affects approximately 1 billion subjects worldwide and the prevalence in Europe has been estimated to be approximately (44%), in some countries reaching up to (55%) [1].

Uncontrolled hypertension is seen as a major health risk, increasing the probabilities of myocardial infarction, heart failure, stroke, kidney disease and other severe condition [2,3].

The vast majority of patients with essential hypertension requires long-term therapy. The duration of the pharmacological treatment of hypertension may last for decades and patients are exposed to prolonged contact with these drugs. Therefore, documentation of safety and efficiency is required, including sensitive indices of genotoxic damage [4].

A recent review about genotoxicity and carcinogenicity studies of antihypertensive agents suggests that for many antihypertensive drugs, the published data may not allow the evaluation of the genotoxic and carcinogenic risks to humans. The review reported the toxicological data available for each of 11 classes of antihypertensive agents. Only in

99 of the 164 marketed antihypertensive drugs, there was at least one result from genotoxicity or carcinogenicity assays and in only 34 of these 164 drugs, there was at least one genotoxicity result in human cells [5,6].

Among the most used antihypertensive drug, the group of chemicals known as beta adrenergic blockers has been found to have markedly beneficial therapeutic value in treating many cardiovascular symptoms such as hypertension, angina and arrhythmias [7].

The antihypertensive drug amlodipine is approved for the treatment of hypertension and angina, and in some countries for angiographically documented coronary artery disease and is available in doses of 5 and 10 mg. In some countries, amlodipine is used once daily in 2.5 mg doses for special patient populations [8].

Nowadays, as a result of the rapid increase in the use of antihypertensive drug, it become extremely important to determine whether these drugs have negative effects on the genetic structure [9].

In the present study, we aimed to update our knowledge about the genotoxic effects of antihypertensive drug, and to determine if amlodipine induces genotoxic damage in cultured human lymphocytes by using the in vitro cytogenetic assay.

2. MATERIAL AND METHODS

2.1. Ethical Approval

This study was approved by the Scientific Research Committee of the College of Medicine/ University of Duhok and the Ethics Committee of the Directorate of Health. Oral and written consent forms were obtained from all participants.

2.2. Preparation of different concentrations

The test substances amlodipine tablet was obtained from local pharmacy. amlodipine was dissolved in distilled water and different concentrations under and above the recommended concentration were prepared. The recommended concentration

was (10 µg/mL) and (0; 5; 15; 25; and 50 µg/mL) concentrations were prepared and tested on the culture containing human blood lymphocyte. with three replicates.

2.3. Sample

The study was carried out under sterile conditions using Human peripheral venous blood samples. from 20 participant dividing into two main groups:

Group One: 10 Treated culture of peripheral blood lymphocytes from healthy participants in vitro by adding different concentration of amlodipine.

Group Two: 10 Untreated culture of peripheral blood lymphocytes as negative control from healthy participants in vitro without adding different concentration of amlodipine.

All participants were non-smokers, non-drinkers, non-taking drug therapy at least for last six months and with no recent history of exposure to mutagenic agents. The ages of enrolled participants were ranged 23–31 years old (13 male and 7 female) and they selected randomly from rural area of Duhok province. Informed consent was obtained from all donors and the study was carried out according to the local ethics committee.

2.4. Chromosomal aberration Assay

Cytogenetics technique for the preparation of chromosome from the cultured lymphocytes of peripheral blood was carried out according to the method described by Rooney (2001) with some modification [11] like preparation of hypertonic solution and decreases amount of Colcemid treatment.

For each concentration, two chromosomal cultures were carried out by adding of 0.9 ml of peripheral blood in sodium heparinized tube, then added to culture tubes that containing culture media (10 ml of RPMI 1640\L-glutamine, 2 ml of fetal

bovine serum, 200µg of Phytohemagglutinin (10 µg/ml), and 200µg penicillin-streptomycin solution (10 µg/ml). After 24 hours of culturing, 200µg of each concentration of amlodipine added to culture tubes of first group and cultured for remaining 48 hours.

After 72 hours of culturing, 200µg of Colcemid added to each culture tube to stop the cell division in metaphase and incubated at 37c for one hours. Then, tubes were centrifuged at 1500/rpmi for 7 min. Then, 10 ml of 0.075M KCl solution was added, mixed, and incubated at 37°C for 30 min. After centrifugation at 1500/rpmi for 7 min, the cells treated with hypotonic solution (0.4% KCl). Then, 10 ml of cold, fresh fixative solution (3:1 Methanol: Glacial acetic acid) added drop by drop for the first 2 ml to the cell pellet. Centrifugation was done afterward, and the supernatant was removed, and the last step was repeated until a clear pellet was obtained.

Finally, microscope slides were prepared by dropping cell samples, air drying and staining with 5% Giemsa solution at pH 6.8 for 5 min. They were finally washed in distilled water, and dried at room temperature. For each concentration and control, chromosomal abnormalities of 25 cells at metaphase stage were counted, analyzed and compared with control.

2.5. Statistical Analysis

All the statistical analyses were performed and the mean frequencies of chromosomal aberrations of each group were compared using Student's t-test. ($P \leq 0.05$) was considered statistically significant. A total of 25 cells were scored per participant to determine the frequency of various chromosomal aberrations.

3. RESULTS

The age of the enrolled participants ranged from 21 year to 44 years with mean (29.10±24) years. From these participants, 13 (65%) were males and 7 (35%) were

females. The ratio of male to female was 1.8:1. The demographic characteristics of all participants shown in Table 1

Table 1: Demographic characteristics of participants

Variable	Treated cultures	Untreated cultures
Number of Participants	10	10
Age (Mean±St)	28.20±65	31.30±25
Taking other medication	0	0
Alcohol consumption (Person)	0	0
No. of Cigarette per day	0	0

The frequency of chromosomal abnormalities was determined in human lymphocytes of both treated cultures with various doses of amlodipine (5; 10; 15; 25; and 50 µg/mL) and treated cultures

negative controls (0.0 µg/mL). The effect of amlodipine on chromosomes of the human peripheral lymphocytes for the two groups are represented in Table (2).

Table 2: Mean number of chromosomal aberrations in human peripheral blood lymphocytes treated with different doses of Amlodipine for 24 hours.

Type of abnormality	Treated cultures					Untreated cultures 0 µg/mL	Total
	5 µg/mL	10 µg/mL	15 µg/mL	25 µg/mL	50 µg/mL		
Ring chromosome	0	0	3	5	9	0	17
Chromatid break	2	5	5	6	11	1	30
Chromatid gap	0	0	0	3	2	1	6
Dicentric	0	0	0	1	0	0	1
Acentric	1	0	1	3	5	0	10
Interchange	1	5	6	10	10	0	32

For each concentration, the data of 25 metaphases per cell were scored in three replicates. The results revealed significant differences (P<0.05) in the means of chromosomes aberrations in treated groups

compare with control group. The effects increased constantly with increased of amlodipine concentrations.

In the present study, it has been shown that amlodipine dose not produce any no numerical chromosomal aberrations in blood lymphocytes in any treated concentrations even in the high dose of (50 µg/mL). In the same time, the results revealed that amlodipine caused six types

of structural chromosomal aberrations in treated cultures which are: Ring chromosome, chromatid break, chromatid gap, dicentric chromosome, acentric chromosome and interchange chromosomes as shown in Figure 1.

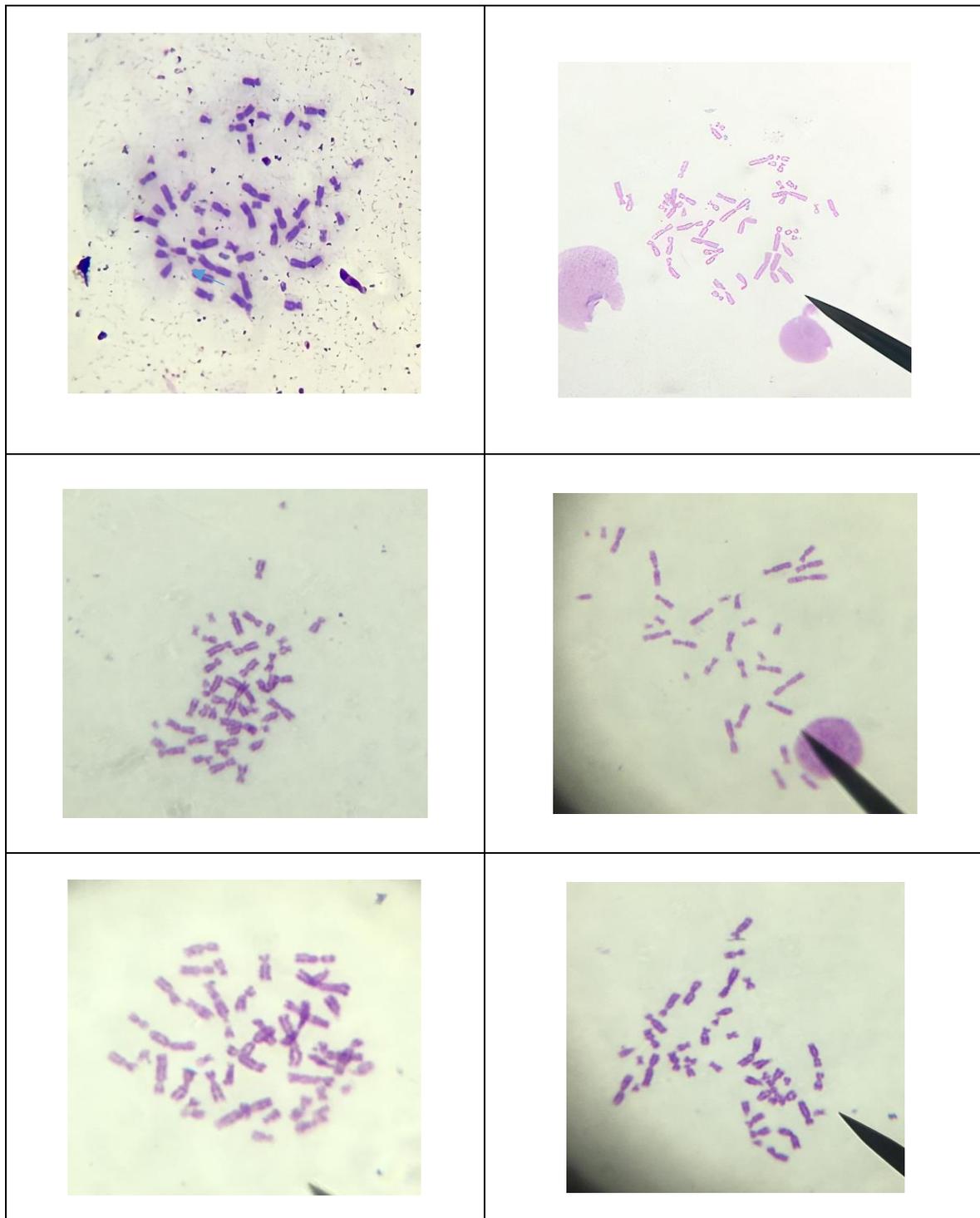


Figure 1: Structural chromosomal aberration induced by different concentration of Amlodipine in peripheral blood lymphocyte in Vitro. A: Chromatid break, B: Dicentric

chromosome, C: Ring chromosome, D: Interchange, E: Acentric chromosome, F: Chromatid gap.

In all these tested systems, data showed that amlodipine induce genotoxicity at almost all concentrations and induced a slightly significant increase in the frequency of chromosomal aberrations in all concentrations as compared to the negative control. For example, numbers of

chromatid breaks increased as the concentration of amlodipine increased: 0 µg/mL=1, 5 µg/mL=2, 10 µg/mL=5, 15 µg/mL=5, 25 µg/mL=6 and 50 µg/mL=11. Total chromosomal aberrations in each concentration shown in Figure 1.

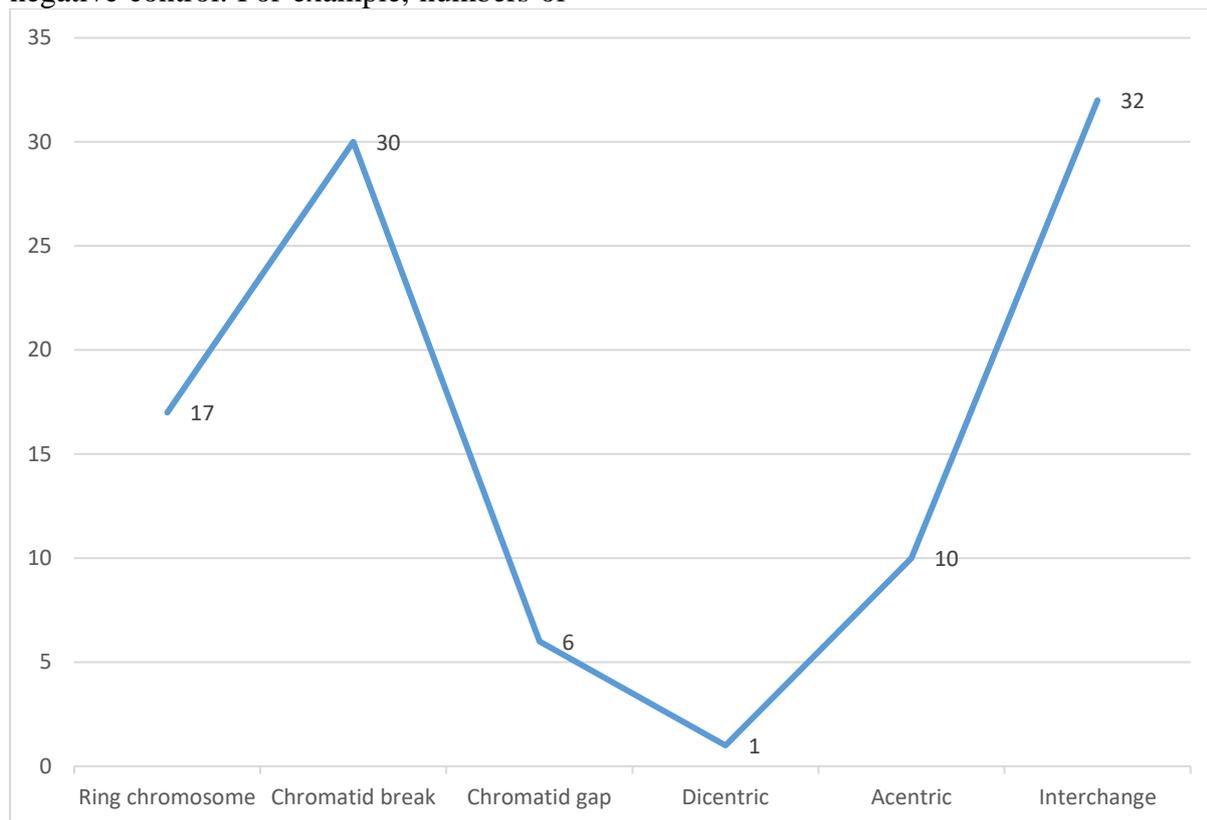


Figure 1: Total chromosomal aberrations in each concentration

Summarizing the results for all analyzed groups, we observed that the average chromosomal aberrations in high doses was statistically significantly higher ($P < 0.05$), in comparison with average chromosomal aberrations in the negative control group.

4. DISCUSSION

Antihypertensive medications are the most important factor in effectively regulating blood pressure. The primary goal of antihypertensive therapy is to reduce cardiovascular mortality and morbidity

caused by systemic arterial hypertension [12].

The vast majority of hypertension patients require long-term antihypertensive treatment because the duration of pharmacological treatment for hypertension might last for decades.

Therefore, documentation of long-term safety and efficacy, particularly sensitive indicators of genotoxic damage, is required for long-term therapy. [13].

Amlodipine is a calcium channel blocker that is frequently administered. In the practice of cardiovascular medicine, its toxicity is the most common cause of medication overdose. It can cause severe hypotension and shock [14].

To our understanding, no data using cytogenetic assays in human or peripheral blood lymphocytes to detect chromosomal damage caused by amlodipine has been reported. Patients are exposed to frequent contact with these drugs because the therapy of amlodipine is prolonged and continuous for several years. As a result, it would be interesting to assess potential long-term genotoxic consequences of this antihypertensive medication utilizing the various in vitro and in vivo assays available in humans.

Cytogenetic assays including Karyotyping, Florescence In Situ Hybridization (FISH), sister chromatid exchange (SCE), and micronucleus (MN) formation have prospective use as indicators of genotoxicity and are used in many studies in past few decades to assess the formation and persistence of cytogenetic modifications in persons with medical, occupational, or accidental exposure [15].

The goal of this investigation was to see if long-term exposure to the antihypertensive medicine Amlodipine has any genotoxic effects on peripheral blood lymphocytes in vitro. The results of cytogenetic analysis of ten peripheral blood lymphocyte cultures treated with amlodipine at various doses and ten untreated cultures as a negative control are presented in this paper.

According to the findings in this study, prolonged amlodipine exposure did not appear to have cytogenetic effects in vitro, as no significant increases in the frequency of chromosomal aberrations were found in cultures treated with different concentrations compared to cultures treated with the recommended concentration (10 mg/mL). It also appears that amlodipine had no effects on the frequency of chromosomal aberrations in untreated negative control cultures. Even though slight increases were observed in vitro when compared to negative controls and recommended concentration, but these increases were beyond statistically significant. As a result, after in vitro exposure, amlodipine was unable to induce chromosomal aberrations in peripheral lymphocytes.

The current findings on the frequency of chromosomal aberrations in human peripheral blood lymphocytes are consistent with previous research conducted by Télez et al. (2000) in a study of the genotoxic potential of Atenolol, which was tested in vitro and in vivo for its potential to stimulate Sister chromatid exchange and Micronuclei formation in cultured human peripheral blood lymphocytes of treated patients and controls. They observed that the antihypertensive medicine Atenolol has no clastogenic effects in vivo and does not cause a significant increase in Sister chromatid exchange in patients when compared to controls in any therapy after long and short in vivo or in vitro exposure to the chemical. They also stated that Atenolol resulted that there is a statistically significant increase in the total frequency of MN when patients compared to controls, but there were no differences

when comparing the different treatments made among control individuals. This appears to imply that the effect of Atenolol is only observed *in vivo* and after continuous exposure.

In another study, Martinez et al. (2001) published results from a study of the genotoxic potential of long-term therapy with Nimodipine, which looked at the drug's ability to induce SCE and MN in cultured human peripheral blood lymphocytes of treated patients and *in vitro* exposed control individuals. They found that the mean frequency of SCE in patients was not significantly higher than

in controls. according to their findings in human lymphocytes Nimodipine also has limited effect in producing SCE *in vivo* and *in vitro*.

Through the analysis of statistical data from the assessment of genotoxicity of amlodipine on the peripheral blood lymphocyte *in vitro*, showed evidence of genotoxicity for this medication. Being the amlodipine of 50µg/mL is the most genotoxic. Additional studies are needed for further assessment of toxicity, cytotoxicity, and genotoxicity of amlodipine.

5. CONCLUSION

Concerning the results obtained in our *in vitro* study, chronic exposure to amlodipine is not associated with genotoxic effects because no significant increases in the frequency of chromosomal

aberrations were found in peripheral blood lymphocytes cultures treated with different concentrations of amlodipine. Therefore, further attempts are need to evaluate the genotoxic effects of this drug *in vivo*.

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