Homocysteine levels in patients with masked hypertension

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ABSTRACT

Objective: Masked hypertension is a clinical condition, the importance of which is agreed in recent years and which is characterized by increased cardiovascular mortality and morbidity and is thought to be important endothelial dysfunction in the pathophysiology. Plasma total homocysteine levels are accepted as a major independent biomarker for endothelial dysfunction and/or a contributor to hypertension and coronary artery disease. In this study, we aimed to measure the level of serum homocysteine and to evaluate the relationship between the parameters of ambulatory blood pressure monitoring in patients with masked hypertension.

Methods: This cross-sectional observational study included 37 subjects with normal blood pressure, 30 with masked-hypertension and 27 patients with obvious hypertension. Masked hypertension (MHT) was defined as office blood pressure <140/90 mm Hg and mean daytime ambulatory systolic blood pressure in 24 hours monitoring ≥135/85 mm Hg. Homocysteine levels of the subjects were measured by using HPLC system with fluorescent detector. Lipid parameters were measured by routine methods. Mann-Whitney U test was used for statistical analysis.

Results: In the analysis of homocysteine, it was observed that there was no difference between the control group and patients with MHT. Patients with high blood pressure showed higher homocysteine levels when compared to MHT (p=0.02). Homocysteine levels showed a weak positive correlation with average systolic blood pressure (r=0.335, p=0.043). Homocysteine levels were higher in smokers than non-smokers. compared with non-smokers group in all participants (p=0.036).

Conclusion: We have reached the opinion that in the individuals with no obvious health problems but with MHT, homocysteine levels may not have any significant effect upon high blood pressure levels. (Anadolu Kardiyl Derg 2014; 14: 357-62)

Key words: atherosclerosis, homocysteine, masked hypertension.

Introduction

The determination of having clinically normal measurement levels of office blood pressure monitoring, but having 135 mm Hg and over average systolic blood pressure with 24 hours ambulatory blood pressure and 85 mm Hg and over diastolic blood pressure is defined as MHT (1-4). In the literature, the frequency of cases of the MHT that gain importance in the recent years is declared as between 10-20% (5). With 24 hours blood pressure monitoring, both false negativities and false positivities have been significantly decreased (6, 7). In cross-sectional studies, when the patients with the MHT have been compared with patients with normal clinic and ambulatory blood pressure, it is shown that the target organ damage prevalence has been quite much higher and the mortality and morbidity ratios have increased (8, 9). Recent studies are goal to research the effect of MHT on the endothelial dysfunction and the relation of it with the rising inflammation biomarkers in patients (10, 11).

Homocysteine is an aminoacid, which doesn’t included into the structure of protein, includes sulfur and is synthesized from methionine (12). Homocysteine transforms to cysteine as a result of transsulfuration with cystathionine β synthase enzyme sourced from B6 vitamin, to methionine by means of methionine synthase and folic acid sourced from B12 vitamin or to methionine by taking methyl group with betaine homocysteine methyltransferase enzyme (13). It is shown that in males homocysteine is 10% more than in females and it increases in both genders with age (14). It is declared that the diet customs, cigarette use and life styles affect homocysteine levels, the genetic defects in enzyme metabolism in homocysteine metabolism, the increase in oxidative stress, renal failure, the decrease in serum albumin levels, the full or relative lack of B vitamins cause the increase in serum homocysteine levels (13, 15). High homocysteine level, the cardiovascular, atherosclerotic
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and thromboembolic diseases are accepted as widespread, independent and modifiable risk factor for venous thrombosis (16). It is considered that homocysteine causes damage in vascular endothelial cells and so affects anticoagulant effect of endothelium and leads to proliferation in smooth muscle cells (17). In the literature, even if findings concerning homocysteine levels are often shown as endothelial damage indication, it is seen that in the recent times these findings are not supported epidemiologically especially with meta-analyses. As a result of meta-analysis, it is shown that homocysteine levels decreased with B vitamin supplementation only play important role in the reduction of cerebrovascular cases, but haven’t got important effect in cardiovascular sourced morbidity and mortality rates (18).

The aim of this study is to determine the level of homocysteine levels, the togetherness and relation of which with the obvious hypertension has been shown in the literature, in the masked hypertensive cases and the relation of them with the parameters of blood pressure.

Methods

Study design and sample size

This study is a cross-sectional study and included total 94 participants as 37 normotensive, 30 MHT and 27 hypertensive. All participants applied to the cardiology outpatient clinic of Selçuk University Medical Faculty between 2010-2012 and met criteria of being taken into the study. The informed consent document was taken from participants, and those not accepting to attend to the study and those not having good general situation were not taken into the study. The inclusion criteria for the study group were: age ≥18 years, patient’s informed consent granted, absence of any acute disease, no administration of B-group vitamins or vitamin preparations within 6 months before the study and normontensive in physical treatment. Criteria of exclusion are those, with obvious coronary artery disease in routine examinations, who used vitamin B tablets, had chronic obstructive lung disease, had hyperthyroid and use thyroid drugs, had acute coronary syndrome in their past, had chronic renal failure, malignancy and acute infection were not included in the study.

Determination of masked hypertension patients group

Determination protocol is summarized in Figure 1. Briefly, after the individuals were informed and their informed consent was received, demographic properties (age, gender, height, body weight, taken medical treatment) of each one were recorded. Later, in the morning the venous blood samples were taken following 8 hours of fasting and serums seperated from these samples were kept at -80°C until biochemical analyses. In order to the determination of the control and masked hypertension patient groups, 24 hours ambulatory blood pressure monitoring (ABPM) method was used. In this study ABPM was attached to 98 participants. Since the validity of ambulatory results of 8 out of these was below 80% and the blood values monitoring of 23 couldn’t be made after the blood pressure monitoring, the masked hypertension was detected in 30 out of 67 participants. 37 participants, whose blood pressure monitoring results validity were close to 100% and age group was close to the masked hypertensive group, were determined as the control group as well. Ambulatory Holter was not attached to 27 hypertensive patients, who were diagnosed with hypertension and whose blood parameters were got thoroughly.

Clinic blood pressure measurement

In the clinic, systolic and diastolic blood pressures at rest were measured from brachial artery through the mercury sphygmomanometer (ERKA D-83646 Bad Tölz, Kallmeyer Medizintechnic GmbH & Co KG, Germany) and by supporting the forearm pit at heart level. After 5 minutes rest at least, blood pressure was measured twice giving 1 minute break. The evaluation was made by taking the average of results. A re-measurement was made in 3 patients, on whom the difference between reads was more than 5 mm Hg, and the average of these 3 measurements was taken.

Ambulatory blood pressure measurement

Twenty four hours ambulatory blood pressure measurement was performed with a non-invasive automatic device (Tracker NIBP2, Del Mar Reynolds Ltd, Hertford, England, UK) and the muzz of the device was placed on the rarely used branch. Participants were asked to continue their daily routine activities and sleeps and to stop their muscle activities (especially athletic activity) and to keep their arms absolutely motionless during the blood pressure measurement. The blood pressure measurement was programmed to measure for every 30 minutes over at least 24 hours. Every blood pressure measurement was recorded into a computer and the results, in which systolic blood pressure was less than 80 mm Hg or higher than 250 mm Hg and in which diastolic blood pressure measurement was less than 40 mm Hg or higher than 140 mm

Figure 1. Flowchart of the study.

ABPM - ambulatory blood pressure measurement
Hg were removed. Every recorded result was accepted when their validity was higher than about 80%. The average values were calculated in 2 periods. The first period was measured between 1 and 6 am during the night and the second period was measured between 9 am and 9 pm. Within patients having <140/90 mm Hg office blood pressure, those having ≥135/85 average blood pressure values in the day period of 24 hours ambulatory blood pressure monitoring records were evaluated as the masked hypertensive.

Biochemical tests

Serum homocysteine levels were measured with HPLC method. Serum homocysteine levels were analyzed by dissociating with 4.6X125 mm reverse phase colon (Munchen, Germany) including modified silicon dioxide cartilage. The total homocysteine levels were measured by using Chromsystems kits (Munchen, Germany) and fluorescent detector (excitation, 385 nm; emission, 515 nm) in HP Agilent 1200 device (HP Agilent 1200, Agilent Technologies, Palo Alto, CA, USA). The reference interval of the method used in our laboratory for the total plasma homocysteine level is stated as 5-12 μmol/L.

Serum total cholesterol, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglyceride, urine and creatinine analyses were realized in routine laboratories by using auto-analyzers. Creatinine clearance was realized by using simple MDRD (Modification of Diet in Renal Disease) analysis (19).

Statistical analysis

The data got from patients were transferred into the computer medium through SPSS 15 (SPSS Inc, Chicago, IL, USA) package program for statistical analyses and the relevant analyses were made for each variant. If continuous variants showed normal distribution was determined by Kolmogorov-Smirnov test. While the difference between the groups' total blood pressure, total cholesterol, HDL-C, LDL-C, urine, creatinine, hemoglobin, hematocrit values was looked with Oneway ANOVA, the significant analysis of the difference between triglyceride and homocysteine values was made with Kruskall-Wallis H test. In analyses, in which there wasn't parametrical distribution and there was difference between groups, Mann-Whitney U test was used and Bonferroni arrangement was made for the analysis of differences of the group tests. The sub-group significant of ANOVA test was evaluated with posthoc Tukey test. By using Spearman correlation analysis, the correlation coefficient and significant values between blood pressure parameters and homocysteine were detected. The correlation results were controlled by applying logarithmic transformation additionally for homocysteine values. In our study, the parametric data results of groups were given as the mean-standard deviation and the data results not showing parametric distribution were given as median (minimum–maximum), and those having <0.05 p value within both two distributions were accepted as significant.

Results

Because ABPM was not used in detecting those having obvious hypertension within participants attending to the study, we couldn't get the detailed blood pressure values of our hypertensive patients. The daytime systolic blood pressure and mean systolic blood pressure were higher in masked hypertensive group when compared to the control (p=0.007, p=0.041 respectively) between the control and masked hypertension groups (Table 1).

The data of the control, MHT and hypertension patients concerning age, height, weight, body mass index and routine parameter results were given in Table 2. While there wasn’t any difference between groups in terms of age, height, weight, total cholesterol, HDL-c, LDL-c, creatinine, hemoglobin, hematocrit values, the difference in terms of urea (p=0.002) and homocysteine (p=0.001) was found significant. When urea and homocysteine analyses showing difference in the total analysis are examined between groups, it is seen that homocysteine values in hypertensive group were higher than both control and MHT group (ap=0.043; bp=0.014). There wasn’t any important statistical difference in homocysteine values of the control and MHT group (p=0.292). A significant less urea levels were detected in the MHT group according to hypertensive and control group (bp=0.004; cp=0.002). Nevertheless, it was observed that the average urea values stayed in normal reference interval in both 3 groups, urea values only in 4 hypertensive patients were slightly above the normal reference values, but creatinine levels and creatinine clearance were in normal interval. These 4 patients were included in the study because an apparent renal pathology was not detected in them.

In the correlation analysis, the relationship between the blood pressure parameters and homocysteine was made both for all participants, except hypertensive group, and also for the MHT group and normotensive group. Homocysteine didn’t show correlation with any parameter in the normotensive-MHT group. In the control group, a weak correlation was detected between average systolic blood pressure and homocysteine levels (r=0.335, p=0.043).

Table 1. ABPM values (mm Hg) of participants of the study

<table>
<thead>
<tr>
<th></th>
<th>Control (n=37)</th>
<th>Masked hypertension (n=30)</th>
<th>P</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime systolic BP, mm Hg</td>
<td>118.9±7.9</td>
<td>138.1±5.9</td>
<td>0.007*</td>
<td>7.675</td>
</tr>
<tr>
<td>Daytime diastolic BP, mm Hg</td>
<td>72.9±5.9</td>
<td>84.7±4.4</td>
<td>0.206</td>
<td>1.630</td>
</tr>
<tr>
<td>Daytime mean BP, mm Hg</td>
<td>82.6±5.9</td>
<td>96.1±4.5</td>
<td>0.091</td>
<td>2.937</td>
</tr>
<tr>
<td>Night systolic BP, mm Hg</td>
<td>110.2±10.9</td>
<td>126.6±11.5</td>
<td>0.5</td>
<td>0.421</td>
</tr>
<tr>
<td>Night diastolic BP, mm Hg</td>
<td>65.6±6.8</td>
<td>74.1±6.3</td>
<td>0.689</td>
<td>0.230</td>
</tr>
<tr>
<td>Night mean BP, mm Hg</td>
<td>75.1±7.4</td>
<td>85±7.3</td>
<td>0.953</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean systolic BP, mm Hg</td>
<td>116.3±8.2</td>
<td>135.1±6.3</td>
<td>0.041*</td>
<td>4.342</td>
</tr>
<tr>
<td>Mean diastolic BP, mm Hg</td>
<td>70.4±5.3</td>
<td>81.2±4.3</td>
<td>0.277</td>
<td>1.201</td>
</tr>
</tbody>
</table>

*significant in p<0.05 level. Data were given in mean±standard deviation. Differences between groups were analyzed with independent sample test. BP—blood pressure.
In this study, serum homocysteine level was researched in 30 masked hypertension patients, in 67 patients having normal tension and 27 hypertensive people who were monitored for ambulatory blood pressure. We didn’t meet any study-related with homocysteine levels in the MHT patients, in the literature. Various demographic, clinical, biochemical and life style properties of participants were researched in addition to homocysteine. While in participants any difference couldn’t be found in terms of age, height, weight, total-cholesterol, LDL-cholesterol, triglyceride, hemoglobin, hematocrit values, the difference in terms of urea and homocysteine was found significant. While homocysteine level came out similar in normotensive and MHT patients, it was found quite high according to the control and MHT in cases with the obvious hypertension.

Homocysteine is a molecule, which is synthesized from methionine and needs B12, B6 and folic acid as co-factor in its metabolism. The reference interval of the method used in our laboratory for the total plasma homocysteine level is stated as 5-12 μmol/L. Homocysteine blood levels can be affected by genetic enzyme diversity, vitamin intake by diet and life styles (20).

Homocysteine is a situation for increasing heart diseases and causing disorder in renal functions. It has been stated that hyperhomocysteinemia increases cardiovascular diseases, and that folate supplementation decreases homocysteine level and drops this risk (16). It is also stated that chronic hyperhomocysteinemia leads to coronary artery diseases, atherosclerosis and hypertension at some extent, and hyperhomocysteinemia has been detected in more than the half of the group people in a study made with hypertensive patient group (21). It was stated that every 5 μmol/L increase in the homocysteine concentration associated with increased atherosclerosis risk in 60-80% rate (22). In the literature, that in the last terms of the renal disease homocysteine values arrived to 25-50 μmol/L. In the literature, homocysteine blood levels can be affected by genetic enzyme diversity, vitamin intake by diet and life styles (20).

In the literature, homocysteine concentration of heavy smokers was found higher than non-smokers. The relation between cigarette and homocysteine was shown in lots of researches made before. Kim et al. (24) showed that even in passive smokers the cigarette increased homocysteine concentration. Although how cigarette elevates homocysteine level is not fully understood, it is considered that it essentially affects vitamin level negatively and leads to increase in homocysteine. This highness increases more especially in those smoking for more

**Table 2. Age, height, weight, body mass index and routine laboratory parameter results of the participants of the study**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=37)</th>
<th>MHT (n=30)</th>
<th>Hypertension (n=27)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46.2±12.2</td>
<td>50.1±8.7</td>
<td>57.7±14.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.6±5.7</td>
<td>29.7±3.4</td>
<td>28.7±3.1</td>
<td>0.578</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.4±8</td>
<td>164.5±10.1</td>
<td>165.3±8.7</td>
<td>0.172</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.3±17.8</td>
<td>80.2±10.4</td>
<td>77.3±6.5</td>
<td>0.422</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>199.3±40.4</td>
<td>205.9±37.9</td>
<td>199±40.2</td>
<td>0.742</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>44.7±9.2</td>
<td>40.8±7.7</td>
<td>42.9±9.6</td>
<td>0.297</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>119.6±26.2</td>
<td>125.23.4</td>
<td>125.9±37.7</td>
<td>0.728</td>
</tr>
<tr>
<td>Triglyceride, mg/dL median (min-max)</td>
<td>187.8 (72-545)</td>
<td>191.6 (69-705)</td>
<td>140.2 (32-273)</td>
<td>0.257</td>
</tr>
<tr>
<td>Urea, mg/dL</td>
<td>29.9±6.8c</td>
<td>23.8±8.6</td>
<td>31.7±11.3b</td>
<td>0.002*</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.86±0.15</td>
<td>0.76±0.18</td>
<td>0.79±0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>Clearance (MDRD)</td>
<td>87.9±14.6</td>
<td>97.2±17.3</td>
<td>90.8±11.54</td>
<td>0.51</td>
</tr>
<tr>
<td>Hgb, g/dL</td>
<td>14.4±1.2</td>
<td>13.5±1.7</td>
<td>13.8±2.1</td>
<td>0.115</td>
</tr>
<tr>
<td>Htc, %</td>
<td>43.9±3.3</td>
<td>41.7±5</td>
<td>41.4±6.3</td>
<td>0.148</td>
</tr>
<tr>
<td>Homocysteine, μmol/L median (min-max)</td>
<td>9.8 (4.23-15.57)</td>
<td>10.01 (3.66-30.8)</td>
<td>16.01 (6.05-67.52)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*significant in p<0.05 level.
+Statistical significant between hypertension and control, #between hypertension and MHT
and *between MHT and control. Chi-square value for triglyceride was calculated as 2.718, for homocysteine as 6.543; MHT - masked hypertension

**Table 3. The relation between participants’ homocysteine values and cigarette**

<table>
<thead>
<tr>
<th>Homocysteine, μmol/L median (min-max)</th>
<th>Smoker (n=29)</th>
<th>Non-smoker (n=65)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine, μmol/L median (min-max)</td>
<td>13.45 (55.56-4.23)</td>
<td>10.9 (67.52-3.66)</td>
<td>0.036*</td>
</tr>
</tbody>
</table>

* significant in p<0.05 level.
than 20 years (15, 25). Because while in smokers the fruit-vegetable consumption and therefore vitamin intake decrease, it is seen that vitamin need increases (24). In our study, homocysteine levels in smoker participants were found higher than homocysteine levels of non-smoker participants.

In this study, we observed that as a result of 24 hours ambulatory blood pressure monitoring homocysteine levels in 30 patients determined the MHT doesn’t have a significant difference when compared with normotive cases. This study shows that the increase in homocysteine levels is apparent only in obvious hypertension. While the relation between hypertension and homocysteine was apparently shown in the literature, the relation between MHT and homocysteine was not examined. So we couldn’t meet any literature finding to compare our MHT results. In spite of the limited number of patients, in the light of this study we see that homocysteine hasn’t got very important role in MHT development. However, our study indicates that homocysteine can possibly play role during the time of passing from the MHT situation to obvious hypertensive situation and contribute the increasing dysfunction of endothelium. However it is clear that a study having more participants and a long time clinical monitoring is necessary before arriving to this certain judgment. Our study indicates that, in the monitoring of patients individually, monitoring individuals with relatively high homocysteine more strictly will be appropriate no matter they are normotensive or in the masked hypertensive clinical situation. In determining the masked hypertensive transition process, homocysteine can be the target molecule. We can see that increasing levels of homocysteine come out only in obvious hypertension cases. Showing that high concentration of homocysteine can be a risk factor for cardiovascular disease makes important the determination of factors determining levels of this amino acid in the blood with respect to public health.

Study limitations

The most important limitation of our study is the number of cases. Due to relatively low prevalence of MHT and the time-consuming aspect of ambulatory blood pressure measurement for patients coming from periphery, the number of cases remained limited. However, the number of cases is adequate to give partial opinion for comparisons between groups. Even if patients taking vitamin supplementation like B12 and folic acid have been excluded from the study, that B12 and folic acid levels have not been measured is another limitation of the study. The patients were not questioned in detail for the diet properties. However, none of patients was in a special diet program, so that differences between groups could be explained with diet was not considered.

Conclusion

Although it is seen that homocysteine has role in the increase in the blood pressure, the effect mechanism of homocysteine is still uncertain. This study shows that homocysteine levels doesn’t change in the MHT and probably hasn’t got effect on the increased blood pressure. To determine the role of homocysteine during the passing of asymptomatic masked hypertensive cases to obvious hypertension, studies, in which more participants are available and more case controls are made, are needed.

Knowledge

The approval was received from the Ethics Committee (Ethics Committee no: 197, date: 05.29.2009) and ethical values have been abided during the study. Chemical substances, commercial kit and colons and various consumables that are necessary for the research were met by the Selçuk University Coordinatorship of Scientific Research Projects and this Project was supported with the Project number 09202067 by Coordinatorship of Scientific Research Projects.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.


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