The Benefit of Unattended Automated Office Blood Pressure Measurement on the White-coat Effect: A Cross-sectional Study

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ABSTRACT

Objective: To compare blood pressure (BP) and heart rate measured by attended and unattended automated office blood pressure measurement (AOBPM) versus home blood pressure measurement (HBPM) and the effect of unattended AOBPM on the classification of BP phenotypes.

Materials and Methods: The cross-sectional study was conducted at the outpatient department in Siriraj Hospital, Thailand. All participants measured their office BP using attended and unattended techniques in random order and recorded home BP twice a day for consecutive 7 days. The agreement between office BP from both AOBPM methods and that from HBPM was analyzed using the Bland-Altman plot. The change in the proportion of each BP phenotype was also analyzed.

Results: We included 114 participants. The mean age was 57.96 ± 15.07 years. The average BP from attended AOBPM, unattended AOBPM, and HBPM were 150.52 ± 16.12/81.77 ± 11.04, 139.68 ± 13.80/78.55 ± 11.71, and 126.91 ± 9.80/76.40 ± 8.37 mmHg, respectively. The BP and heart rate measured by these techniques were significantly different (p-value of <0.001). Bland-Altman analysis showed the biases of attended and unattended SBP versus home SBP were 23.61 and 12.77 mmHg, respectively. Unattended AOBPM significantly decreased the numbers of patients classified as white-coat and sustained hypertension regardless of BP thresholds (p-value of <0.001 for both groups).

Conclusion: Unattended AOBPM significantly minimizes the white-coat effect in real-life clinical practice and may help physicians avoid overdiagnosis of hypertension. Nevertheless, it does not replace HBPM.

Keywords: Attended automated blood pressure measurement; unattended automated blood pressure measurement; home blood pressure; white-coat hypertension; masked hypertension (Siriraj Med J 2023; 75: 454-465)

INTRODUCTION

Nowadays hypertension is still a major problem of public health worldwide, and it is one of the etiologies of premature cardiovascular morbidity and mortality.[1] The early diagnosis and treatment of hypertension are important to prevent *de novo* hypertension-mediated organ damage and reduce cardiovascular risk. Blood pressure (BP) measurement is an essential part of hypertension management. According to the growing pieces of evidence which have indicated the better cardiovascular predictive power of home blood pressure (HBPM) over office blood pressure measurement (OBPM)[2,3], the current hypertension guidelines have not only emphasized the standard technique of OBPM but have also recommended using out-of-office BP measurement, especially HBPM.[4-7] Because of numerous interfering factors in the process of OBPM, including patients' effect, observers' effect, and the hospital's environment, the office BP may be

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inaccurate.[8,9] The white-coat effect is an important cause of inaccurate BP from conventional, or attended, OBPM.[9] Unattended OBPM is a newly developed technique that can be used to measure patients' BP by using automated BP devices in a private area without observers. It can be called unattended automated office blood pressure measurement (unattended AOBPM). This technique which was adopted in the systolic blood pressure intervention trial (SPRINT), has been widely debated in regard to BP values.[10-13] Some recent evidence has shown that unattended OBPM gets rid of the white-coat effect and results in a lower BP reading than attended OBPM.[14-16] However, the application of unattended OBPM in real-world clinical practice remains limited and the BP values resulting from different techniques of BP measurement are controversial due to the heterogeneity of the protocol of BP measurement.[14,17] The primary objective of this study was to investigate the difference and agreement of BP parameters and heart rate between attended AOBPM, unattended AOBPM, and HBPM in real-world clinical practice in the outpatient department of a tertiary care hospital. Moreover, this study aimed to investigate the proportion of BP phenotypes that were classified by the different OBPM techniques and BP thresholds.

MATERIALS AND METHODS

Study population

The cross-sectional study was conducted from March 1, 2021 to October 31, 2022, at the hypertension clinic, which is a part of the outpatient department of Siriraj Hospital, Bangkok, Thailand. We included clinically stable patients with an age of at least 18 years who visited the hypertension clinic and were able to completely measure their BP on the arm following this study's protocol. All eligible participants gave their informed consent. The exclusion criteria were pregnancy, cardiac arrhythmia, and incomplete data from BP records. This study was approved by the Siriraj Institutional Review Board (SIRB) (COA no. Si 085/2021). The Thai Clinical Trials Registry number is TCTR20230122002 (https://www.thaiclinicaltrials.org/show/TCTR20230122002).

Blood pressure measurement protocol

The techniques of BP measurement consisted of attended OBPM, unattended OBPM, and HBPM. Well-trained nurses informed all participants about avoiding caffeine intake and exercise at least 30 minutes before the BP measurement and emptying the urinary bladder before BP measurement as well as all steps of the BP measurement protocols. All these methods were performed in a sitting position on a chair with back support and both feet were placed on the floor. An appropriate-sized cuff was placed on the non-dominant arm at the same level as the heart. A period of 5 minutes of resting was required before taking a BP measurement. In both office BP measurement techniques, an automated Omron HEM-907 (Omron Healthcare Co. Ltd.) device was used for three repeated BP measurements with a 1-minute of interval between each one. Attended AOBPM was performed by a nurse, who started the program of the automated BP device and then stayed in the same place as the patients until the measurement was finished. On the other hand, the only patients who performed unattended AOBPM were in a private room. There, the patients activated the automated BP device by themselves for three serial BP measurements one minute apart. There were no nurses or staff in the room until the BP measurement was completed. All records of office BP parameters and heart rates from each method were considered when calculating the average value from each one. The nurses and the investigators were independent. The sequence of attended and unattended AOBPM was randomized by the nurses in a 1:1 ratio.

The HBPM method was begun in a manner to the aforementioned OBPM method on the day after the OPD visit. Validated oscillometric BP devices including Omron HEM-7130 and HEM-7211 (Omron Healthcare Co. Ltd.) were recommended for use in HBPM. The patients were asked to perform two repeated BP measurements each morning and evening for consecutive 7 days. The data of the HBPM records were sent to the investigators at the next follow-up visit. All numbers of BP and heart rate were used to calculate to the average home BP. In addition, the average BP measurements, namely, the average morning and evening BP, were separately calculated.

Definition of BP phenotypes

We identified four BP phenotypes based on the type of BP measurement (office and home BP), with two different thresholds according to the 2017 American and 2018 European hypertension guidelines[4,5]; these phenotypes were as follows; normotension (both office and home BPs were normal), white-coat hypertension (office BP was abnormally high while home BP was normal), masked hypertension (office BP was normal but home BP was abnormally high), and sustained hypertension (both office and home BPs were abnormally high). Abnormal office and home BPs following the 2017 American hypertension guideline were defined as a systolic BP (SBP) which was at least 130 mmHg and/or a diastolic BP (DBP) which was at least 80 mmHg.[4] On the contrary, the 2018 European

hypertension guidelines defined an abnormal office SBP as an office SBP which was at least 140 mmHg and/or an office DBP was at least 90 mmHg.[5] An abnormal home BP was defined as a home SBP which was at least 135 mmHg and/or a home DBP which was at least 85 mmHg.[5]

Data collection

The demographic data were collected from the participants and their electronic medical records at Siriraj Hospital. They consisted of age, gender, body mass index, comorbidities (i.e., diabetes mellitus, dyslipidemia, coronary artery disease, cerebrovascular disease, and chronic kidney disease), current smoking status, use of antihypertensive medications and their types, and laboratory results (i.e., fasting plasma glucose, glycated hemoglobin, serum creatinine, estimated glomerular filtration rate (eGFR), and lipid profiles).

Statistical analysis

Based on a previous study that investigated the association between unattended office BP and home BP[17], we planned to enroll at least 55 participants to provide 90% statistical power for the detection of an approximately 10 mmHg difference in BP between unattended AOBPM and HBPM at a two-sided alpha level of 0.05.

Continuous variables of demographic data were recorded as mean ± standard deviation and median and interquartile range depending on the data' distribution. The Kolmogorov-Smirnov equality-of-distributions test was analyzed for identifying the normal distributed continuous variables. Categorical variables were recorded as number and percentage. The analysis of the difference and agreement of BP parameters and heart rates between the three different techniques of BP measurement was performed by using Bland-Altman plots. One-way analysis of variance (ANOVA) with the Bonferroni test was used for the comparison of the BP and heart rate parameters of the three techniques. A Pearson correlation was performed to analyze the relationship between the results of the three BP measurement techniques. The chi-square test or Fisher's exact test was used for the analysis of comparing the proportions of the BP phenotypes that were classified by using attended and unattended AOBPM at each BP threshold. The statistical significance was designated as a p-value of <0.05. Stata Statistical Software version 17 (StataCorp LLC, College Station, TX) was used for all analyses.

RESULTS

A total of 114 patients were enrolled in this study. Most of them were female patients (62.3%). Of all patients, 94% were taking at least one antihypertensive medication. Dyslipidemia was the most common comorbidity (81.6%). The mean age was 57.96 ± 15.07 years and the median body mass index was 24.59 kg/m^2. The details of the demographic data are shown in Table 1. The results of Kolmogorov-Smirnov equality-of-distributions test of the continuous variables (i.e., age, eGFR, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, SBP, DBP, and heart rate) indicated the normal distribution (p-value of >0.05).

Comparison of the BP and heart rate of the different BP measurement techniques

Table 2 shows the data of systolic and diastolic BP that were measured; they were calssified by attended OBPM, unattended OBPM, and HBPM. The average morning home BP was 127.19 ± 10.28/77.30 ± 8.39 mmHg and the average evening home BP was 126.63 ± 11.05/75.50 ± 9.27 mmHg. Overall analysis revealed that that SBP, DBP, and heart rate from the different methods of measurement were significantly different. Bonferroni's post-hoc analysis indicated that attended OBPM provided the significantly highest systolic blood pressure (SBP), with significance, among all BP measurement techniques. The average home SBP was significantly lower than office SBP as well. Only the diastolic blood pressure (DBP) from attended OBPM was significantly higher than the average home DBP. Moreover, the average heart rate from HBPM was significantly lower than the office heart rate that was measured during both attended and unattended OBPM. Even though the DBP and heart rates of both attended and unattended OBPMs were not significantly different, unattended OBPM provided a lower DBP and heart rate than attended OBPM.

Bland-Altman plot analysis is shown in Table 3 and Fig 1 – 3. The results revealed that the biases of attended versus unattended office SBP, DBP, and heart rate were 10.83 mmHg, 3.23 mmHg, and 3.42 beats/minute, respectively. Both OBPM techniques had higher biases of SBP, DBP, and heart rate than the HBPM technique. The BP and heart rate of all techniques of BP measurement were significantly associated, as shown in Table 4. The correlation between BP and heart rate was particularly high between attended and unattended OBPMs. Notwithstanding, the correlation coefficients of the SBP from both OBPM technique versus HBPM technique were quite low (r = 0.19, p-value of 0.041 for attended office SBP versus average home SBP; r = 0.28, p-value of 0.003 for unattended office SBP versus average home SBP).

TABLE 1. Demographic data.

Parameter	Results (N = 114)
Age (years)	57.96 ± 15.07
Male, number (%)	43 (37.72)
Body mass index (kg/m^2)	24.59 (22.40, 27.47)*
Comorbidity, number (%)	
Diabetes mellitus	32 (28.07)
Dyslipidemia	93 (81.58)
Coronary artery disease	1 (0.88)
Cerebrovascular disease	3 (2.63)
Chronic kidney disease	13 (11.40)
Current smoking, number (%)	1 (0.88)
Number of antihypertensive medications, number (%)	
0	7 (6.14)
1 – 2	74 (64.91)
≥ 3	33 (28.95)
Type of antihypertensive medication, number (%)	
Diuretics	16 (14.04)
Calcium channel blockers	81 (71.05)
Angiotensin converting enzyme inhibitors	18 (15.79)
Angiotensin-II receptor blockers	38 (33.33)
Beta blockers	34 (29.82)
Others	34 (29.82)
Laboratory result	
Fasting plasma glucose (mmol/l)	5.61 (5.16, 6.44)*
Hemoglobin A1C (%)	6.00 (5.60, 6.50)*
Serum creatinine (µmol/l)	75.14 (61.00, 101.66)*
Estimated glomerular filtration rate (ml/min/1.73m^2)	80.20 ± 26.40
Cholesterol (mmol/l)	4.74 ± 1.06
Triglycerides (mmol/l)	1.20 (0.87, 1.75)*
HDL-C (mmol/l)	1.52 ± 0.48
LDL-C (mmol/l)	2.54 ± 0.86

*median and interquartile range

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

TABLE 2. Blood pressure and heart rate of the different blood pressure measurement techniques.

Parameter (N = 114) mean ± SD	Attended office BP measurement	Unattended office BP measurement	Home BP measurement (average home BP)	P-value*
SBP (mmHg)	150.52 ± 16.12	139.68 ± 13.80†	126.91 ± 9.80‡⁋	<0.001
DBP (mmHg)	81.77 ± 11.04	78.55 ± 11.71	76.40 ± 8.37‡	0.001
HR** (beats/min)	83.16 ± 17.74	79.74 ± 16.52	72.97 ± 11.79‡⁋	<0.001

*p-value for overall comparison of attended office BP versus unattended office BP versus average home BP

**The presented numbers of the HR of home BP measurement were calculated from 111 subjects' records due to the missing HR data of 3 subjects.

†p-value of <0.001 for comparing attended vs unattended office BP

‡p-value of <0.001 for comparing attended office BP vs average home BP

⁋p-value of <0.005 for comparing unattended office BP vs average home BP

Abbreviations: BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SD, standard deviation

TABLE 3. Bland-Altman plot analysis of blood pressure and heart rate from the different blood pressure measurement techniques.

BP measurement method	Bias	95% CI of bias	Lower 95% LoA	95 % CI of lower LoA	Upper 95% LoA	95% CI of upper LoA
Systolic blood pressure (mmHg)						
Attended versus unattended office BP measurement	10.83	8.51 to 13.16	-13.73	-18.17 to -10.18	35.40	31.84 to 39.84
Attended office versus home BP measurement	23.61	20.42 to 26.79	-10.08	-16.17 to -5.21	57.29	52.42 to 63.38
Unattended office versus home BP measurement	12.77	10.07 to 15.47	-15.77	-20.93 to -11.64	41.31	37.18 to 46.47
Diastolic blood pressure (mmHg)						
Attended versus unattended office BP measurement	3.23	2.05 to 4.40	-9.20	-11.44 to -7.40	15.65	13.86 to 17.90
Attended office versus home BP measurement	5.38	3.52 to 7.23	-14.18	-17.72 to -11.35	24.93	22.10 to 28.47
Unattended office versus home BP measurement	2.15	0.36 to 3.94	-16.75	-20.16 to -14.01	21.04	18.31 to 24.46
Heart rate (beats/minute)						
Attended versus unattended office HR measurement	3.42	2.44 to 4.39	-6.88	-8.74 to -5.39	13.71	12.22 to 15.57
Attended office versus home HR measurement	10.10	7.44 to 12.76	-17.59	-22.67 to -13.53	37.79	33.73 to 42.87
Unattended office versus home HR measurement	6.59	4.18 to 9.00	-18.49	-23.10 to -14.82	31.67	28.00 to 36.28

Abbreviations: BP, blood pressure; CI, confidence interval; LoA, limits of agreement; HR, heart rate

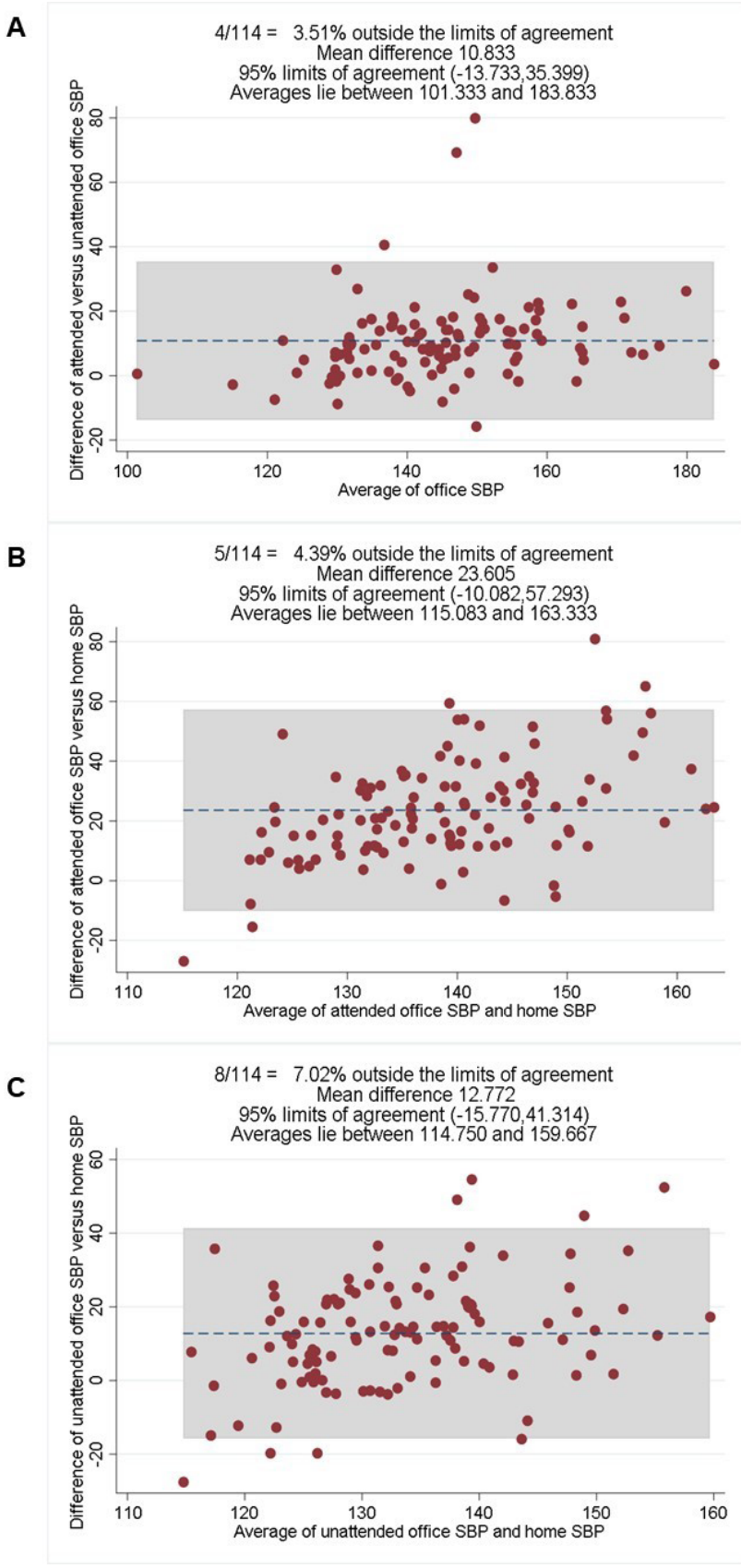


Fig 1. Bland-Altman plot for **(A)** the comparison of attended versus unattended office SBP, **(B)** the comparison of attended office versus home SBP, and **(C)** the comparison of unattended office versus home SBP.

Abbreviation: SBP, systolic blood pressure

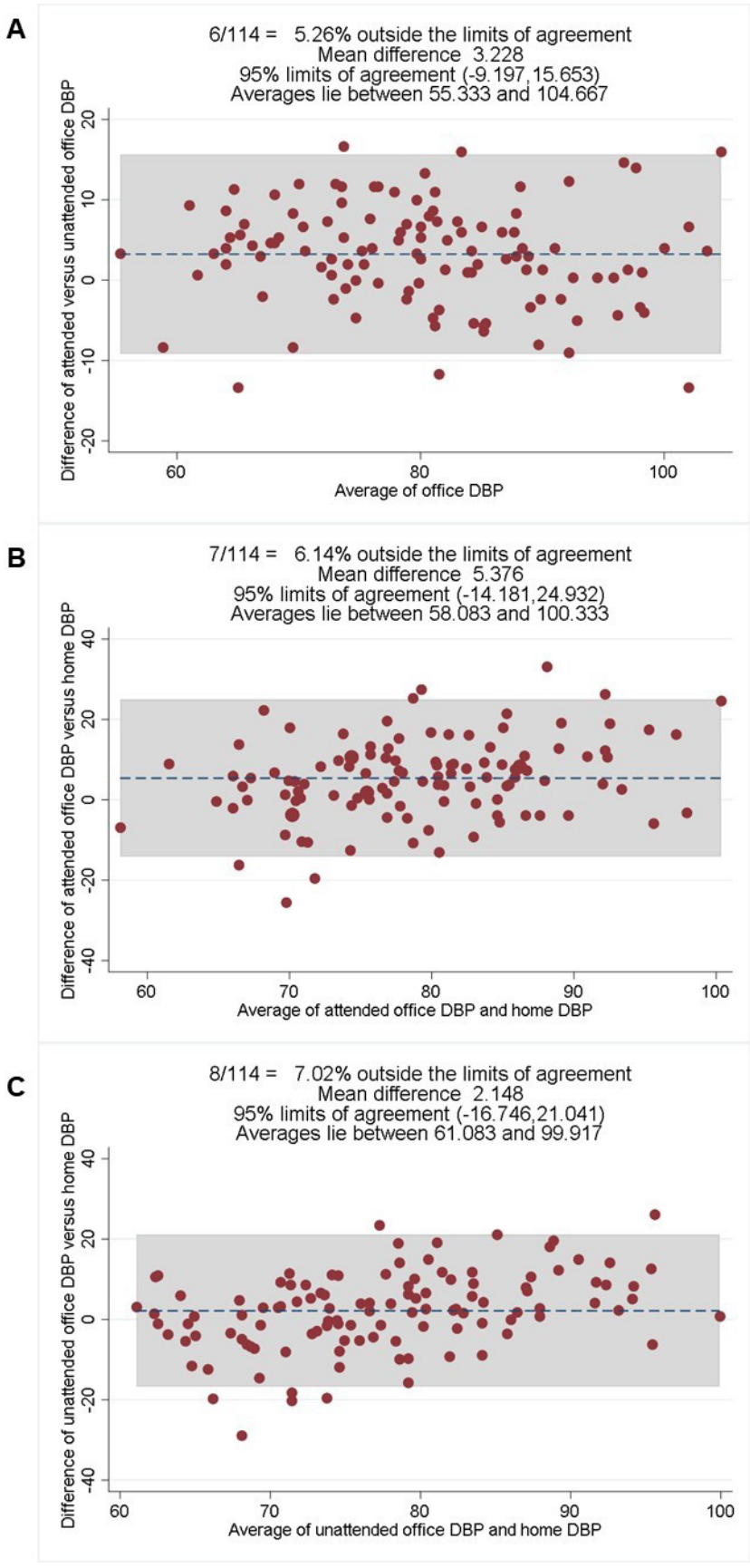


Fig 2. Bland-Altman plot for **(A)** the comparison of attended versus unattended office DBP, **(B)** the comparison of attended office versus home DBP, and **(C)** the comparison of unattended office versus home DBP.

Abbreviation: DBP, diastolic blood pressure

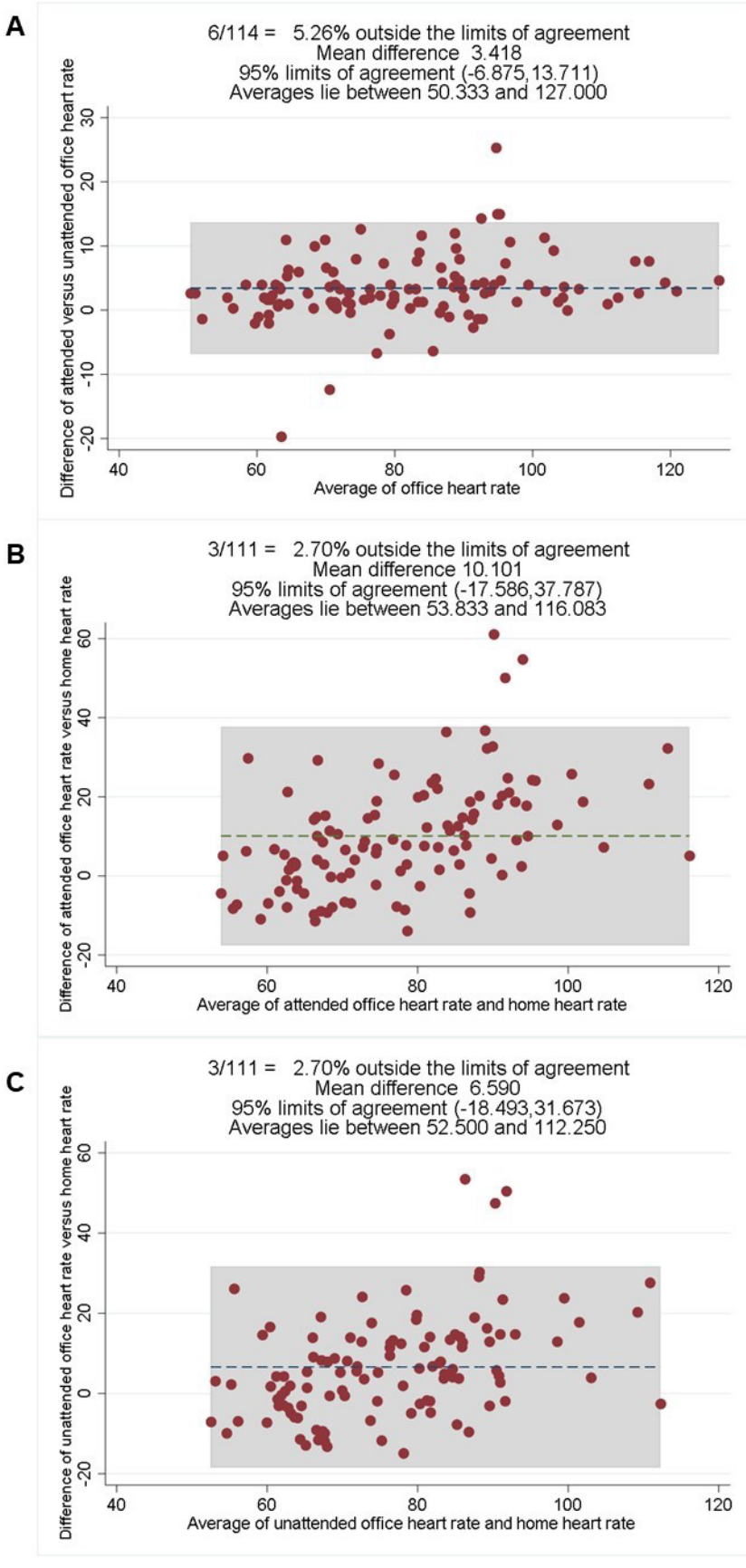


Fig 3. Bland-Altman plot for **(A)** the comparison of attended versus unattended office heart rate, **(B)** the comparison of attended office versus home heart rate, and **(C)** the comparison of unattended office versus home heart rate.

TABLE 4. The correlations of blood pressure and heart rate between the different blood pressure measurement techniques.

Parameters	Pearson's r	P-value
Systolic blood pressure		
Attended versus unattended office BP measurement	0.66	<0.001
Attended office versus home BP measurement	0.19	0.041
Unattended office versus home BP measurement	0.28	0.003
Diastolic blood pressure		
Attended versus unattended office BP measurement	0.85	<0.001
Attended office versus home BP measurement	0.50	<0.001
Unattended office versus home BP measurement	0.58	<0.001
Heart rate		
Attended versus unattended office HR measurement	0.96	<0.001
Attended office versus home HR measurement	0.62	<0.001
Unattended office versus home HR measurement	0.64	<0.001

Abbreviations: BP, blood pressure; HR, heart rate

The classification of BP phenotypes by using the different techniques of AOBPM

The proportions of all patients' BP phenotypes are separately presented according to the different BP thresholds (Table 5). According to the thresholds of normal office and home BPs, which were less than 130/80 mmHg, the proportions of patients with normotension, white-coat hypertension, masked hypertension, and sustained hypertension by attended AOBPM were 5.26%, 50%, 0.88%, and 43.86%, respectively. When unattended AOBPM was performed, the proportions of normotensive, white-coat, masked, and sustained hypertensive patients were changed to 14.91%, 40.35%, 4.39%, and 40.35%, respectively. These results were found to be different when using the other BP thresholds, which consisted of a normal office BP of less than 140/90 mmHg and a normal home BP of less than 135/85 mmHg. Of all patients, the BP phenotypes which were classified by attended AOBPM consisted of 20.18% normotensive patients, 51.75% white-coat hypertensive ones, 1.75% masked

TABLE 5. Classification of blood pressure phenotypes based on the different techniques of office blood pressure measurements and the different blood pressure thresholds of the 2017 ACC/AHA and 2018 ESC/ESH hypertension guidelines in all 114 patients.

BP phenotype Number (%)	2017 ACC/AHA hypertension guideline		P-value*	2018 ESC/ESH hypertension guideline		P-value*
	Attended method	Unattended method		Attended method	Unattended method	
Normotension	6 (5.26)	17 (14.91)	<0.001	23 (20.18)	47 (41.23)	<0.001
White-coat HT	57 (50.00)	46 (40.35)	<0.001	59 (51.75)	35 (30.70)	<0.001
Masked HT	1 (0.88)	5 (4.39)	0.044	2 (1.75)	4 (3.51)	1.000
Sustained HT	50 (43.86)	46 (40.35)	<0.001	30 (26.32)	28 (24.56)	<0.001

*p-value for comparing attended versus unattended method

Abbreviation: ACC/AHA, American College of Cardiology/American Heart Association; ESC/ESH, European Society of Cardiology/European Society of Hypertension; BP, blood pressure; HT, hypertension

hypertensive ones, and 26.32% sustained hypertensive ones. The proportion of the normotensive group was significantly increased in proportions (41.23%; p-value of <0.001) while the proportions of the others (i.e., white-coat and sustained hypertensive groups (30.7%; p-value of <0.001 and 24.56%; p-value of <0.001, respectively)) were significantly decreased in proportion by using the technique of attended AOBPM regardless of BP thresholds. The ratio of identified masked hypertension classified by the two methods of AOBPM was only significantly different as regards the BP threshold of the 2017 American hypertension guideline (p-value of 0.044). Furthermore, we separately reveal the results of the classification of BP phenotypes in patients who were and were not taking antihypertensive medications in Supplementary Table 1 & 2.

DISCUSSION

Since the results of SPRINT were published, there has been strong interest in the methods of BP measurement, especially unattended AOBPM, and such methods have been widely debated. Standardized BP measurement is emphasized in both office and home settings. The observer's effect may confound BP which is measured at the clinic more than that measured at home. The results of this study show a significant difference in office SBP between both techniques of OBPM. SBP measurements from unattended AOBPM were significantly lower than those from attended AOBPM. It indicated that the observer's effect was an important confounding factor of inaccurate OBPM. Our results were concordant with the findings from the previous study of Keeley EC., et al.[18] which showed a significantly higher attended office SBP. Moreover, the study of Fanelli E., et al.[19] highlighted the higher attended office SBP, particularly in patients who initially measured their office BP by attended technique. The sequence of AOBPM techniques might not have affected the results in our study because the process for identifying the initial AOBPM technique in each participant was randomly performed. Although the difference in office DBP and heart rate between both AOBPM techniques did not reach statistical significance, attended office DBP and heart rate tended to be higher than unattended ones. A possible underlying mechanism is more activation of the sympathetic nervous system during attended OBPM compared with unattended OBPM.[20]

The office BP and heart rate from both attended and unattended AOBPMs were statistically higher than home BP and heart rate. This finding supported the presence of the white-coat effect that resulted from patient response to hospital situations, including BP measurement. Office heart rate is still obviously higher than home heart rate despite the fact that some patients took beta-blockers. It implies that the sympathetic nervous system plays a major role in the pathophysiology of the white-coat effect.[21,22] The discrepancies of the biases of office SBP versus home SBP from the Bland-Altman analysis were large in attended AOBPM (23.61 mmHg) and, to a lesser extent, in unattended AOBPM (12.77 mmHg). Our finding indicated unattended AOBPM was not completely devoid of the white-coat effect because office BP, especially SBP, was higher than home BP. This was different from some previous studies.[19,23] The systematic review and meta-analysis of Pappaccogli M., et al.[23] revealed similar BP levels between unattended AOBPM and HBPM. Nevertheless, there should be some caution taken in interpreting this result because of the high heterogeneity of the included studies for meta-analysis. The study of Fanelli E., et al.[19] showed that the BP levels from both AOBPM techniques were lower than home BP. There is a limitation of missing data on the complete home BP of 32 subjects (approximately 22% of the total 118 subjects); therefore, the results may be interfered with by this missing data. In addition, the different baseline characteristics of participants, different methodologies of study, and the environment in each study site may result in different findings. The high correlation of attended and unattended office BP in our study is similar to that found in other studies.[17,18,24,25] Our study showed that BP measured by AOBPM and HBPM was significantly correlated. This is consistent with some previous studies. However, the degree of correlation between office SBP and home SBP was low. The exact reason for the weak relationship between office SBP and home SBP is not well known. A previous study[25] showed similar results of association to our study. Most of the enrolled patients in the previous study (96.4%)[25] and our study (93.86%) were taking at least one antihypertensive medication. We suppose that taking antihypertensive medications[25] and several different drug regimens may result in increased variability of office SBP.[26,27]

Due to the differences in office BP between attended and unattended techniques, we investigated whether the classification of BP phenotypes was affected by them. Our results showed a significant change in the proportion of all BP phenotypes. Unattended AOBPM resulted in decreased numbers of white-coat hypertensive patients because it lowered patients' white-coat effect. The slightly weak correlation between the unattended office and home BP indicated that unattended AOBPM could not replace HBPM. Therefore, there were increased normotensive and masked hypertensive patients. Nevertheless, we

cannot evaluate the diagnostic performance, especially accuracy, for the identification of BP phenotypes because of the lack of ambulatory BP monitoring (ABPM), which is the gold standard for the diagnosis of hypertension.

The strengths of this study consist of using the same standardized protocol of BP measurement for all participants and the randomized order of AOBPM. The measured office BP is not confounded by these factors. The design of our pragmatic study can reflect the real-life outcome in routine clinical practice. There are some limitations in our study. First, most of the participants were taking antihypertensive medications, so their different regimens of such medications including types and numbers may have affected office BP and home BP. Moreover, they may be also affected by the different measured and unmeasured factors of the patients' baseline characteristics, such as age, gender, comorbidities, physical activity and dietary pattern, etc. This limitation may result in high discrepancies in BP parameters and heart rate between the three measured techniques. Second, home BP was collected from the data recorded on paper because the home BP devices used in this study do not send BP data via an online system. Some patients may have failed to record some high BP measurements in their records. Therefore, home BP may not represent actual BP at home. Third, both attended and unattended AOBPM were measured and averaged in a single visit, whereas HBPM was recorded and averaged over 7 days. Given the daily variation of the measurement, the differences between each technique were significant. Finally, ABPM is not performed in our study so there was no gold standard method used to identify abnormal elevated BP, as previously mentioned.

CONCLUSION

Unattended AOBPM results in a lesser white-coat effect than attended AOBPM, however, unattended office BP remains higher than home BP in our study. The office BP measurement method affects the classification of BP phenotypes. The technique of unattended AOBPM can be applied to routine clinical practice.

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Author contributions

Chotruangnapa C created the research question, performed the literature review and then designed the research methodology. All authors conducted and collected the data. The data were statistically analyzed and the manuscript was written by Chotruangnapa C. All authors critically reviewed and approved the final manuscript.

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