Original Article

Comparison of the effect of smoking on cardiac automaticity between pre- and post-menopausal women

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Abstract

Our aim was to compare the effect of smoking on cardiac automaticity between pre- and postmenopausal women by using heart rate variability (HRV) parameters. Thirty three premenopausal (mean age; 32.4 ± 9.2 years) and 29 postmenopausal (mean age; 49.0 ± 6.1 years) smoker women were enrolled. Basic clinical, echocardiographic, Holter parameters and HRV values were compared between the two groups. The high frequency (HF) and low frequency (LF) power spectrum of HRV and HF/LF were significantly higher ($p< 0.05$) and standard deviation of the averages of NN intervals in all 5-min segments of the entire recording (SDANN) from the time-domain criteria was significantly lower ($p< 0.05$) in postmenopausal smokers. Linear logistic regression analysis revealed that smoking duration, oestrogen level and age were significant and independent factors for impaired HRV parameters in postmenopausal smoker women (OR=3.142; 95% CI = 2.78ï 6.89; p = 0.001; OR=2.394; 95% CI = 1.1ï 4.9; p = 0.03; OR=1.4; 95% CI = 1.03ï 2.03; p = 0.04, respectively). Smoking impairs cardiac autonomic function both in pre- and postmenopausal women, while the effect is more prominent in the latter group. Smoking together with aging and decrease in oestrogen levels might be related with a higher degree of cardiac autonomic alterations.

Key words: Post-menopausal, women, smoking, cardiac automaticity, heart rate variability.

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Introduction

The smoking habit is an important and independent risk factor for development of atherosclerosis, coronary heart disease, acute myocardial infarction, and sudden cardiac death for both genders (1, 2). We know from previous studies that cigarette smoking increases the risk of cardiac arrhythmias (3, 4). Cigarette smoking causes acute increases in plasma catecholamine levels and in cardiac norepinephrine spill over, which are potentially arrhythmogenic (5). Smoking has also been correlated with sympathetic hyperactivity, leading to an increased pulse rate, high blood pressure, and ventricular premature contraction (VPC) (6). Menopause, which is characterized by the presence of reduced oestrogens in circulation, leads to various effects in different organs and systems in a woman. Previous studies demonstrated an increased the risk for developing cardiovascular disease and arrhythmias in the postmenopausal period (7, 8). Oestrogen deficiency has been proposed as the mechanism of this relationship although there are also some epidemiologic studies which have shown that oestrogen has cardioprotective properties (8, 9). Interestingly, results of some previous studies indicate that women who smoke cigarettes have an early natural menopause, because of relative oestrogen deficiency (10, 11). Heart rate fluctuations of are indicators of sympathetic and parasympathetic contributions from the autonomic nervous system to the heart rate. Spectral analysis of heart rate variability (HRV) has shown at least two distinct

regions of periodicity in heart rate (12, 13). HRV is assessed noninvasively by some parameters obtained from a 24-h ambulatory (Holter) recording and is used to evaluate cardiac autonomic function (14, 15). Previous studies have shown that HRV measurements are useful for the prediction of sudden cardiac death and ventricular arrhythmias in healthy individuals (16, 17). To our knowledge, no previous study has compared cardiac autonomic function between pre- and postmenopausal women smokers. In this retrospective study, we aimed to investigate the effects of smoking on cardiac autonomic function using HRV parameters in healthy pre- and post-menopausal women.

Materials and Methods

Patients

This study was performed by the retrospective analysis of the medical, laboratory and holter recordings of women patients that had undergone a 24 hour holter recording at the outpatient clinics of Abant Izzet Baysal University Cardiology Department and Duzce State Hospital Cardiology Department between 2010 and 2013. Thirty three premenopausal smoker women (mean age; 32.4 ± 9.2 years) and twenty nine postmenopausal smoker women (mean age; 49.0 ± 6.1 years), in the previous laboratory records around the dates of holter recordings of whom an oestrogen level studied in our hospital was also present, were included in our study. All smokers had a history of incessant smoking for at least 1 year. All enrolled postmenopausal women had their last menstrual period at least one year before their inclusion to the study. In addition, information, including age, race, height, weight, physical examination result, echocardiographic measurement, menopausal status, age at menopause, and use of postmenopausal hormones, was ascertained from the medical recordings. Routine 12-lead electrocardio graphy (ECG) of the patients was also evaluated. Height and weight had been measured for the calculation of the body mass index (BMI = weight (kg)/height^2 (m^2)). Laboratory test results, including fasting glucose, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglycerides (TG) and oestradiol level were investigated from medical recordings. Congestive heart failure (CHF), moderate or severe degrees of any valvular regurgitation or co-existent valvular stenosis, previous MI, angina or angina-like symptoms, hypertension, hyperlipidemia, diabetes mellitus, obstructive sleep apnea and being on any medical therapy at the time of clinical evaluation were accepted as exclusion criteria. Those patients with pacemaker rhythm, atrial fibrillation (AF), left bundle branch block, right bundle branch block, any sign of ischemia on the initial ECG and echocardiographic evidence of LV hypertrophy, systolic dysfunction, wall motion abnormalities or pericardial disease were also excluded from the study.

Echocardiography

Results of the echocardiographic examination of the included patients were also revealed from the medical records. For the echocardiographic exclusion of structural heart disease, all patients had been evaluated by transthoracic M mode and two dimensional (2D), pulsed-wave (PW), continuous wave (CW), colour flow and tissue Doppler imaging (TDI) modalities. All examinations had been performed with the GE- Vivid-3 system (GE Vingmed, Horten, Norway) with a 2-4 MHz transducer at a depth of 16cm. Two-dimensional, Doppler echocardiographic examinations and M-mode measurements were performed according to the recommendations of the American Society of Echocardiography. Systolic dysfunction was defined as an ejection fraction of < 55%.

Measurement of Heart Rate Variability

The 24-hour holter recordings of all patients were analysed to obtain the HRV parameters. Standard deviation of all NN intervals (SDNN), SDNN index (SDNNI), square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), standard deviation of the averages of NN intervals in all 5-min segments of the entire recording (SDANN) from the time-domain criteria and the proportion of differences in successive NN intervals greater than 50 ms (pNN50) were calculated. Spectral analysis of HRV is obtained by summing the powers of each frequency band: very low frequency (VLF) <0.04 Hz that is thought to be influenced by the thermoregulation of vasomotor tone; low frequency (LF) 0.04ï 0.15 Hz that is affected by the baroreceptor reflex and is thought to reflect sympathetic and parasympathetic tone; high frequency (HF) 0.15ï 0.40 Hz that is influenced by respiratory frequency and thought to reflect parasympathetic tone. LF and HF powers were expressed in normalized values in our study. In all patients, the LF/HF was calculated (12).

Statistical analysis

Parametric data were expressed as mean ± SD, and categorical data as percentages. All statistical procedures were performed using SPSS 15.0.

Parametric data were compared using Studentôs t-test and categorical data with the chi-square test Relation between the cigarettes per day, smoking duration and HRV parameters were assessed by Pearsonôs correlation coefficient. Variables, found to have significant differences in univariate analysis (p<0.1) were evaluated for multicolinearity and then enrolled into linear logistic regression analysis (stepwise forward LR). A p-value Ò0.05 was considered to be statistically significant.

Results

Clinical characteristics of both groups are shown in Table 1. The baseline resting heart rate, systolic-diastolic blood pressure, and BMI were not significantly different between groups. As expected, oestrogen level in postmenopausal women was significantly lower than in premenopausal women (13.4 ± 7.2, 142.2 ± 62.1; p<0.001). When HRV parameters were compared, HF, LF and HF/LF were significantly higher in postmenopausal smokers compared to the premenopausal smokers (HF [ms] 979±112, 219±45, p=0.001; LF [ms] 362±94, 119±51, p=0.011; HF/LF [ms] 3.11 ± 0.73, 1.66 ± 0.32, p=0.034). In addition, SDANN was significantly lower in postmenopausal smokers compared to the premenopausal smokers ([ms] 103.62 ± 32.13, 193.59 ± 65.34, p=0.002). Other parameters of HRV were lower in postmenopausal smokers than in premenopausal smokers, but this difference was statistically insignificant (SDNNI [ms] 108.79 ± 48.14, 121.31 ± 55.13; p=0.094; SDNN [ms] 131.56 ± 51.12, 142.29 ± 76.13, p=0.112; pNN50 [%] 11.63 ± 8.31,14.31 ± 7.89, p=0.143; RMSSD [ms] 92.78 ± 55.41, 98.12 ± 60.07, p=0.211; TRIA [ms]451.82 ± 156.07, 487.51 ± 188.99, p=0.301; postmenopausal and premenopausal, respectively).

The mean value of cigarettes per day in the group of postmenopausal smokers was found to be 16.3 ± 8.1, while the mean value of smoking duration was 7.98 ± 6.31 years. Also, the mean value of cigarettes per day in the group of premenopausal smokers was found to be 10.3 ± 8.1, while the mean value of smoking duration in this group was 4.87 ± 2.83 years. There was a significant difference between the two groups in terms of smoking duration and cigarettes per day (p=0.017, p=0.031; respectively). There was a strong significant positive correlation between smoking duration and the value of SDNNI, SDNN and SDANN in postmenopausal smokers (r: 0.789; r: 0.822; r: 0.897; p< 0.001 respectively). In addition, there was a positive correlation between cigarettes per day and all HRV parameters in postmenopausal smokers, but this correlation was statistically insignificant (p> 0.05). HRV parameters of premenopausal women showed no significant correlation with the smoking duration and cigarettes per day (p> 0.05). After adjustment for other variables (including age, systolic blood pressure, diastolic blood pressure, BMI, oestrogen level, TC, HDL-C, LDL-C, cigarettes per day and smoking duration) through linear logistic regression analysis for the verification of determinants of impaired HRV parameters, smoking duration, oestrogen level and age were found to be significant and independent factors for impaired HRV parameters in postmenopausal smoker women (OR=3.142; 95% CI = 2.78ï 6.89; p = 0.001; OR=2.394; 95% CI = 1.1ï 4.9; p = 0.03; OR=1.4; 95% CI = 1.03ï 2.03; p = 0.04, respectively).

Table 1. The comparison of the basic clinical, echocardiographic, Holter parameters and HRV values between pre- and postmenopausal smoker women.

	Pre-menopausal smoking women (n=33)	Post-menopausal smoking women (n=29)	p value
Age(years)	32.4 ± 9.2	49.0 ± 6.1	**<0.001**
Weight (kg)	64.12± 7.82	66.13±8.12	0.413
BMI(kg/m2)	24.2 ± 3.1	24.5 ± 3.2	0.173
Systolic BP (mm/hg)	123.3 ± 6.4	125.5 ± 7.3	0.232
Diastolic BP (mm/hg)	68.7 ± 7.2	68.3 ± 6.9	0.631
Heart rate (bpm)	79.3 ± 6.7	81.9 ± 7.4	0.104
LVEF(%)	59.9 ± 5.2	56.3 ± 5.6	0.098
LVEDD (cm)	4.12 ± 0.31	4.22 ± 0.31	0.172
Cigarettes/day	10.3 ± 8.1	16.3 ± 8.1	**0.031**
Smoking duration (years)	4.87 ± 2.83	7.98 ± 6.31	0.017
Estrogen (pg/ml)	142.2 ± 62.1	13.4 ± 7.2	**<0.001**
SDNNI [ms]	121.31 ± 55.13	108.79 ± 48.14	0.094
pNN50 [%]	14.31 ± 7.89	11.63 ± 8.31	0.143
RMSSD [ms]	98.12 ± 60.07	92.78 ± 55.41	0.211
TRIA [ms]	487.51 ± 188.99	451.82 ± 156.07	0.301
SDANN [ms]	193.59 ± 65.34	103.62 ± 32.13	**0.002**
SDNN[ms]	142.29 ± 76.13	131.56 ± 51.12	0.112
HF/LF [ms]	1.66 ± 0.32	3.11 ± 0.73	**0.034**
HF [ms]	219±45	979±112	**0.001**
LF [ms]	119±51	362±94	**0.011**

Discussion

This study has several important findings. Firstly, HF, LF, HF/LF and SDANN were significantly impaired in postmenopausal smokers compared to the premenopausal smokers; this means that postmenopausal smoker women have more impaired cardiac autonomic function then premenopausal smoker women. Secondly, the smoking duration had a significant positive correlation with the value of SDNN, SDNNI and SDANN in postmenopausal smokers; this means that smoking duration has a direct effect on the cardiac autonomic function in postmenopausal smoker women. Thirdly, smoking duration, oestrogen level and age were statistically significant independent factors for the verification of impaired HRV parameters in postmenopausal smoker women.

In this study, we found that postmenopausal smoker women have more impaired cardiac autonomic function then premenopausal smoker women. Similarly; some previous studies on comparison of HRV of premenopausal women with that of postmenopausal women found that postmenopausal women have more impaired HRV parameters then premenopausal women, with the possibility of ovarian hormonal influences on cardiac autonomic function (18, 19). Moodithaya *et al.* revealed that just age and difference in oestrogen levels were the important confounders, responsible for the differences in all the components of impaired cardiac autonomic function between the pre- and postmenopausal groups (18). They concluded that both ageing and declined oestrogen levels are associated with the autonomic alterations seen among postmenopausal women. Several studies have shown reduction in autonomic modulation with ageing in both genders (18, 20, 21). These studies concluded that, ageing is associated with an increased dependency on sympathetic control of cardiac responses and reduced vagal responsiveness. Previous studies also demonstrated that decline in the level of oestrogen causes a decrease in vagal and increase in sympathetic action (22-24). This study, together with previous reports indicates a reduction in cardiac autonomic modulation in post-menopausal women compared to premenopausal women. In addition to these, we concluded that smoking duration was as much important as other factors for reduction in cardiac autonomic modulation, especially in postmenopausal smoking women.

The second important aim of this study was to show the effect of smoking on cardiac autonomic function in the pre- and postmenopausal period. In our study, HF, LF and HF/LF were higher; other parameters were lower in postmenopausal women than in premenopausal women, similar to the results of the previous studies (18, 19). These results might be associated with smoking. Most previous studies have focused on the effect of cigarette smoking on the cardiac autonomic system and HRV. Eryonucu *et al*. (25) showed that the total HRV parameters were significantly reduced in smokers compared to non-smokers. Kupari *et al*. (26) assigned their subjects to two groups according to the number of cigarettes smoked per day, and found that HRV was lower in people who smoked ten or more cigarettes per day than in both the non-smoker group and in people who smoked fewer than ten cigarettes per day. Barutcu *et al*. (27) found that the time domain parameters of HRV (SDNN and RMSSD) were decreased in heavy smokers compared to non-smokers. This study showed that smoking together with ageing and declined oestrogen levels have more adverse effect to the cardiac autonomic function; also this condition can cause a wide spectrum of cardiac rhythm disorders; including transient sinus arrest and/or bradycardia, sinus tachycardia, atrial fibrillation, sinoatrial block, AV block, and ventricular tachyarrhythmiaôs, and sudden cardiac death. Together with these previous results, our findings suggest that impairment of the cardiovascular autonomic system in postmenopausal smoker women may contribute to the other deleterious effects of smoking. It is already known that smoking has anti-oestrogenic effects which can occur by several mechanisms (28). Some of these include a negative effect on ovarian function and blockade of the aromatase enzyme which leads to decreased conversion of androgens to estrogens (29), and increased metabolism of estrogens in the liver in a pathway that leads to peripherally inactive forms of oestrogen (30, 31). As we mentioned before,

decreased oestrogen levels in menopause has adverse effects in cardiac autonomic function in the postmenopausal period and one of the mechanisms for the adverse effects of smoking on cardiac autonomic functions may be its contributions to this hypo-estrogenic environment. The main limitation of our study was the small sample size. A small sample size can result in a low statistical power for equivalency testing, leading to false-negative results. However, establishing a smoking group in pre- and postmenopausal women without co-morbidities (*e.g.*, diabetes mellitus, hypertension, and cardiovascular or renal disorders) is very difficult. Secondly, we did not assess the impact of cyclic hormonal changes that occurs in different phases of the menstrual cycle in premenopausal women on the parameters that we evaluated during the study. Thirdly, we did not assess the impact of circadian variation. Diurnal fluctuations in autonomic tone make it difficult to calculate HRV over 24 h. This may also have influenced our results. Another limitation of the study was its retrospective nature and the need to rely on previous patient records during such studies.

Our data indicate that autonomic modulation of the heart is reduced in pre- and postmenopausal smoker women, but especially in postmenopausal smoker women. Smoking duration, aging and decreased oestrogen levels increases deleterious effects of smoking in postmenopausal women all of which in turn increase the risk of arrhythmias and sudden death in this patient population. To achieve a meaningful reduction in the societal burden of deleterious effects of smoking, cigarette smoking among all age groups and especially among postmenopausal women, must be reduced.

Declaration of interests: The authors report no conflicts of interest.

References

1. Guilbert JJ. The world health report 2002 - reducing risks, promoting healthy life. Educ Health (Abingdon). 2003 Jul;16(2):230.

2. Zhu BQ, Parmley WW. Hemodynamic and vascular effects of active and passive smoking. Am Heart J. 1995 Dec;130(6):1270-5.

3. Goldenberg I, Moss AJ, McNitt S, Zareba W, Daubert JP, Hall WJ, et al. Cigarette smoking and the risk of supraventricular and ventricular tachyarrhythmias in high-risk cardiac patients with implantable cardioverter defibrillators. J Cardiovasc Electrophysiol. 2006 Sep;17(9):931-6.

4. Hallstrom AP, Cobb LA, Ray R. Smoking as a risk factor for recurrence of sudden cardiac arrest. N Engl J Med. 1986 Jan 30;314(5):271-5.

5. Narkiewicz K, van de Borne PJ, Hausberg M, Cooley RL, Winniford MD, Davison DE, et al. Cigarette smoking increases sympathetic outflow in humans. Circulation. 1998 Aug 11;98(6):528-34.

6. Gidding SS, Xie X, Liu K, Manolio T, Flack JM, Gardin JM. Cardiac function in smokers and nonsmokers: the CARDIA study. The Coronary Artery Risk Development in Young Adults Study. J Am Coll Cardiol. 1995 Jul;26(1):211-6.

7. Knowlton AA. Estrogen and cardiovascular disease: aging and estrogen loss at the heart of the matter? Future Cardiol. 2012 Jan;8(1):9-12.

8. Stevenson JC. A woman's journey through the reproductive, transitional and postmenopausal periods of life: impact on cardiovascular and musculo-skeletal risk and the role of estrogen replacement. Maturitas. 2011 Oct;70(2):197-205.

9. Davison S, Davis SR. New markers for cardiovascular disease risk in women: impact of endogenous estrogen status and exogenous postmenopausal hormone therapy. J Clin Endocrinol Metab. 2003 Jun;88(6):2470-8.

10. Baron JA. Smoking and estrogen-related disease. Am J Epidemiol. 1984 Jan;119(1):9-22.

11. Baron JA, La Vecchia C, Levi F. The antiestrogenic effect of cigarette smoking in women. Am J Obstet Gynecol. 1990 Feb;162(2):502-14.

12. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996 Mar 1;93(5):1043-65.

13. Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. Med Biol Eng Comput. 2006 Dec;44(12):1031-51.

14. Balcioglu S, Arslan U, Turkoglu S, Ozdemir M, Cengel A. Heart rate variability and heart rate turbulence in patients with type 2 diabetes mellitus with versus without cardiac autonomic neuropathy. Am J Cardiol. 2007 Sep 1;100(5):890-3.

15. Lombardi F, Stein PK. Origin of heart rate variability and turbulence: an appraisal of autonomic modulation of cardiovascular function. Front Physiol. 2011;2:95.

16. Buchner T, Petelczyc M, Zebrowski JJ, Prejbisz A, Kabat M, Januszewicz A, et al. On the nature of heart rate variability in a breathing normal subject: a stochastic process analysis. Chaos. 2009 Jun;19(2):028504.

17. Wong JS, Lu WA, Wu KT, Liu M, Chen GY, Kuo CD. A comparative study of pulse rate variability and heart rate variability in healthy subjects. J Clin Monit Comput. 2012 Apr;26(2):107-14.

18. Moodithaya SS, Avadhany ST. Comparison of cardiac autonomic activity between pre and post menopausal women using heart rate variability. Indian J Physiol Pharmacol. 2009 Jul-Sep;53(3):227-34.

19. Ribeiro TF, Azevedo GD, Crescencio JC, Maraes VR, Papa V, Catai AM, et al. Heart rate variability under resting conditions in postmenopausal and young women. Braz J Med Biol Res. 2001 Jul;34(7):871-7.

20. Aydın G, Sarıkaya S, Turgut OO, Şahin Ş, Çakmak NY, Yılmaz MB, et al. Assesment of Autonomic Function in Metabolic Syndrome using Combination Heart Rate Variability and Heart Rate Turbulence. Acta Med Anatol. 2013;1(1):21-25.

21. Ziegler D, Laux G, Dannehl K, Spuler M, Muhlen H, Mayer P, et al. Assessment of cardiovascular autonomic function: age-related normal ranges and reproducibility of spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses. Diabet Med. 1992 Mar;9(2):166-75.

22. Mercuro G, Podda A, Pitzalis L, Zoncu S, Mascia M, Melis GB, et al. Evidence of a role of endogenous estrogen in the modulation of autonomic nervous system. Am J Cardiol. 2000 Mar 15;85(6):787-9, A9.

23. Ottesen B, Sorensen MB. Women at cardiac risk: is HRT the route to maintaining cardiovascular health? Int J Gynaecol Obstet. 1997 Oct;59 Suppl 1:S19-27.

24. Stampfer MJ, Colditz GA, Willett WC, Manson JE, Rosner B, Speizer FE, et al. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. N Engl J Med. 1991 Sep 12;325(11):756-62.

25. Eryonucu B, Bilge M, Guler N, Uzun K, Gencer M. Effects of cigarette smoking on the circadian rhythm of heart rate variability. Acta Cardiol. 2000 Oct;55(5):301-5.

26. Kupari M, Virolainen J, Koskinen P, Tikkanen MJ. Short-term heart rate variability and factors modifying the risk of coronary artery disease in a population sample. Am J Cardiol. 1993 Oct 15;72(12):897-903.

27. Barutcu I, Esen AM, Kaya D, Turkmen M, Karakaya O, Melek M, et al. Cigarette smoking and heart rate variability: dynamic influence of parasympathetic and sympathetic maneuvers. Ann Noninvasive Electrocardiol. 2005 Jul;10(3):324-9.

28. Tanko LB, Christiansen C. An update on the antiestrogenic effect of smoking: a literature

review with implications for researchers and practitioners. Menopause. 2004 Jan-Feb;11(1):104-9.

29. Blackburn CW, Peterson CA, Hales HA, Carrell DT, Jones KP, Urry RL, et al. Nicotine, but not cotinine, has a direct toxic effect on ovarian function in the immature gonadotropin-stimulated rat. Reprod Toxicol. 1994 Jul-Aug;8(4):325-31.

30. Meek MD, Finch GL. Diluted mainstream cigarette smoke condensates activate estrogen receptor and aryl hydrocarbon receptor-mediated gene transcription. Environ Res. 1999 Jan;80(1):9-17.

31. Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N Engl J Med. 1986 Nov 20;315(21):1305-9.