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Original Investigation

Differences in Levels of Biomarkers of Potential Harm Among Users of a Heat-Not-Burn Tobacco Product, Cigarette Smokers, and Never-Smokers in Japan: A Post-Marketing Observational Study

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Abstract

Introduction: Cigarette smoking is associated with the risk of certain diseases, but non-combustible products may lower these risks. The potential long-term health effects of the next-generation non-combustible products (heat-not-burn tobacco products (HNBP) or electronic vapor products) have not been thoroughly studied. The present study aimed to investigate the impact of biomarkers of potential harm (BoPH) of one of HNBP (a novel vapor product: NTV (novel tobacco vapor)), under the conditions of actual use.

Aims and Methods: This study was an observational, cross-sectional, three-group, multi-center study. Exclusive NTV users (NTV, $n = 259$), conventional cigarette smokers (CC, $n = 100$) and never-smokers (NS, $n = 100$) were enrolled. Biomarkers of tobacco smoke exposure (cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)) and BoPH including parameters of physical pulmonary functions relevant to smoking-related diseases were examined, and subjects answered a questionnaire on cough-related symptoms (J-LCQ) and health-related quality of life (SF-36v2®).

Results: Levels of cotinine, total NNAL and BoPH (high-density lipoprotein (HDL)-cholesterol, triglyceride, sICAM-1, WBC count, 11-DHTXB2, 2,3-d-TXB2, 8-epi-PGF2α, forced expiratory volume in 1 second (FEV1), % predicted value of FEV1 (%FEV1) and maximum midexpiratory flow (FEF_{25-75})) were significantly different in the NTV group as compared to levels in CC group ($p < .05$). Significantly higher levels of cotinine, total NNAL, and 2,3-d-TXB2, and lower levels of FEV1 and %FEV1, were observed among NTV users compared to the NS group.

Conclusion: In a post-marketing study under actual use conditions, BoPH associated with smoking-related disease examined in exclusive NTV users were found to be favorably different from those of CC smokers, a finding attributable to a reduction in exposure to harmful substances of tobacco smoke.

Implications: Cigarette smoking is associated with an increased risk of pulmonary diseases like COPD, cardiovascular diseases, and certain cancers. There is a growing body of evidence that HNBP reduces the exposure associated with smoking and that there is a favorable change in BoPH. However, long-term effects regarding the relative health risks to HNBP users compared to CC smokers have not been examined. This study provides post-marketing data under actual use conditions of the effects on biomarkers of potential harm in NTV, one of HNBP, exclusive users

compared to CC smokers and never-smokers. The evidence suggests that exclusive NTV users have favorable levels of BoPH compared to CC smokers, and that is result from a sustained reduction in exposure to harmful substances of tobacco smoke.

Introduction

Cigarette smoking is associated with an increased risk of pulmonary diseases like COPD, cardiovascular diseases (CVD), and cancers in a variety of organs.[1] It is reported that the cause of smoking-related diseases is not directly responsible for exposure to the nicotine itself[2] but long-term exposure to substances emitted in the smoke generated by burning tobacco leaves. Some kinds of non-combustible products are already available such as heat-not-burn tobacco products (HNBP),[3–6] e-vapor products (EVP),[7–10] and smokeless tobacco products (SLTP).[11–14] These products can conceivably deliver nicotine while reducing the harmful materials associated with tobacco combustion. Epidemiological studies have demonstrated that long-term use of SLTP such as snus and snuff is associated with reduced health risks.[13,14] The Food and Drug Administration authorized a manufacturer to market specific snus products with a claim which indicates lower risks of certain diseases by using the products instead of cigarettes.[15] There is a growing body of evidence from clinical studies that HNBP[3–6] and EVP[7,8,10]have the potential to reduce risks from smoking-related diseases by reducing harmful and potentially harmful constituents (HPHCs)[16] and that favorable changes in biomarkers of potential harm (BoPH) occur after conventional cigarette (CC) smokers quit.[4,17–21] However, as HNBP and EVP are relatively new to the world, there is scant information on their influence on actual health risks in users. Therefore, acquiring post-marketing data under actual use conditions on their use over a significant period of time is extremely important to assess the long-term health risks of these relatively new products. Regarding EVP, a report on findings obtained by cross-sectional evaluation has shown risk-reducing potential.[10] A novel tobacco vapor product (NTV: Ploom TECH), one of the HNBP developed by Japan Tobacco Inc., became available in the Japanese market in March 2016. The NTV consisted of a battery, a cartridge with a heater and nicotine-free liquid, and a capsule filled with tobacco blend.[3,22] Analysis of NTV vapor demonstrated that the major constituent in the tobacco capsule is nicotine, along with propylene glycol and glycerol, which are the major liquid components of the cartridge.[22] In the NTV aerosol, neither CO nor most of the 43 Hoffmann analytes (ie aromatic amines, carbonyls, phenolics, PAH, nitrogen oxides, cyanic compound, amine, volatile organic compounds, tobacco specific nitrosamines and metals) except ammonia, formaldehyde, and acetone were found or exceeded the detection limit. When the tobacco capsule was compared with the 3R4F cigarette, ammonia, formaldehyde, and acetone were reduced by 58%, 94%, and 99%, respectively.[22] A five-day confinement longitudinal study in healthy adults in which nicotine equivalents and biomarkers of exposure (BoE) for 14 HPHCs and pyrene were compared among three groups (CC smokers, CC to NTV switchers, and smoking abstainers) showed that the BoE levels for HPHCs in NTV switchers were significantly reduced compared to those of CC smokers, and reached levels comparable to those of smoking abstainers.[3] Although this evidence suggests that NTV may have the potential to reduce the health risks associated with smoking, long-term effects associated with the relative health risks to NTV users compared to CC smokers have not been examined. Therefore, the purpose of the current cross-sectional, observational study is to obtain data under actual use conditions of the effects on biomarkers of potential harm (BoPH) that are reported to be associated with tobacco-related diseases in exclusive NTV users compared to CC smokers and never-smokers (NS). In addition, all subjects answered questionnaires that surveyed cough-related symptoms and health-related quality of life (QOL).

Methods

Study Design

This study was an observational, cross-sectional, three-group, and multi-center study. The study was overseen by PPD-SNBL Inc. (Tokyo, Japan), and it was conducted at Shinanozaka Clinic (Tokyo, Japan) and OCROM Clinic (Osaka, Japan) during two ambulatory visits (screening: Day -28 to Day -1; the survey day: Day 1) between April 2019 and September 2019. The study was conducted in three self-identified groups: exclusive NTV users (NTV group), exclusive conventional cigarette smokers (CC group), and never-smokers (NS group), ie, those who had never used any kind of tobacco or nicotine-containing product. Participant recruitment was conducted by 3H Medi Solution Inc. (Tokyo Japan), and eligible participants were screened. On the day of screening, interested persons visited the clinic, were provided with study details, and then screened by inclusion and exclusion criteria including history of tobacco use, exhaled carbon monoxide (CO) concentration (NTV and NS group), urinary cotinine (CC and NS group), and females underwent urinary human chorionic gonadotrophin testing to ascertain if they were pregnant. Participants found to be eligible by the screening process were informed that they had been enrolled into the study, their next clinic visit for the survey was scheduled, and they were provided with a laboratory kit for urine collection.

Sociodemographic profiles such as age, gender, and Body Mass Index (BMI) are considered potential influential factors on biomarkers such as blood lipids[23] and pulmonary function.[24] To facilitate an appropriate intergroup comparison according to the proportion (%) of each of the background factors (age: 20–30, 31–40, 41–50, 51–65, gender: male, female, BMI: <18.5, ≥18.5 to <25.0, ≥25.0) of the NTV group, all subjects in the CC group and NS group were selected such that the proportion of each background factor matched that of NTV group within a margin of ±2% (calculated using the number of eligible subjects in the NTV group).

On the day of the survey (Day 1), participants visited the clinic and provided a first-void urine sample taken in the morning of Day 1 for biomarker analysis. Compliance after the screening day was checked again using the inclusion and exclusion criteria including tobacco use history, exhaled CO concentration, urinary excretion of cotinine, and testing for pregnancy, and the remaining eligible subjects were enrolled into one of the three groups. Enrolled subjects then underwent the specified tests and examinations (questionnaire, biomarker examination, lung function, etc.).

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki and registered at the UMIN Clinical Trials Registry (UMIN000036304). Prior to the start of the study, the study documents were approved by the

Institutional Review Board of the medical institutions. All participants provided written informed consent to participate in the study.

Participants (Inclusion and Exclusion Criteria)

Participants comprised Japanese men and women (aged 21 to 65 years) living in Japan, who self-identified as an exclusive NTV user, an exclusive CC smoker, or an NS. Participants were required to be in good health (self-identified). Participants who met the following inclusion criteria were enrolled into the study. NTV group: subjects who used NTV exclusively on a daily basis (on more than four days a week) for the immediately preceding three or more months and whose level of exhaled CO was ≤10 ppm according to previous observation.[3] CC group: subjects who used CC exclusively on a daily basis (on more than four days a week) for the immediately preceding one or more years and who tested positive for urinary cotinine at the screening. NS group: subjects who had never used any kind of tobacco or nicotine-containing products and who tested negative for urinary cotinine (One Step Cotinine Test Device DCT-102 (cut-off 200ng/ml), Accuracy-One Inc., California, USA) at screening and whose level of exhaled CO was equal to or less than10 ppm; which might include non-smoker (0.05–30 ng/ml) who was exposed to cigarette smoke constituents from the environment.[25] Also Participants who did not meet the smoking history or age criteria, or who were pregnant or planning to become pregnant, were excluded. The exhaled CO level of subjects was measured using a piCO+™ Smokerlyzer® (Bedfont Scientific Ltd, Maidstone., England) at screening. This measurement was only performed on NTV users and non-smokers to confirm negative results due to the short half-life (1–4 h) of exhaled CO.

Demographics and Tobacco Product Use History

The baseline characteristics of subjects, included gender, age, BMI, history of tobacco use, the ISO tar yield of the subject's usual brand of CC (value printed on each cigarette package), daily cigarette consumption, and frequency of use were all recorded at the screening.

Products

No Study Product was Provided by the Sponsor or Study Investigator

Chemical Analysis of Biomarkers

For the evaluation of tobacco exposure, the following biomarkers were measured. Biomarker of nicotine exposure: plasma cotinine. Biomarkers of 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone (NNK),[26] the HPHCs of smoking exposure: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides, NNAL-O-glucuronide and NNAL-N-glucuronide (total NNAL), measured in first-void urine. For the evaluation of BoPH, the following were measured in plasma samples. Biomarkers for lipid metabolism[27]: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C),[28] and triglyceride (TG). For vascular endothelial function, soluble intercellular adhesion molecule-1 (sICAM-1)[29] was measured. For inflammation, the WBC count[30] was determined in whole blood samples. As biomarkers for platelet activation, 11-dehydrothromboxane B2 (11-DHTXB2)[31] and 2,3-dinor thromboxane B2 (2,3-d-TXB2)[32] were measured in the first-void morning urine samples, and for oxidative stress, 8-epi-prostaglandin F2α (8-epi-PGF2α)[33,34] was measured in the first-void morning urine samples. Plasma cotinine levels, total NNAL, 11-DHTXB2, 2,3-d-TXB2, 8-epi-PGF2α, and creatinine in spot urine were measured by LC-MS/MS using validated methods at Celerion Laboratories (Lincoln, NE, USA) according to applicable Good Laboratory Practice (GLP) standards, and values of total NNAL,11-DHTXB2, 2,3-d-TXB2, and 8-epi-PGF2α were corrected by the urinary creatinine levels. TC, LDL-C, HDL-C, and TG were determined by autoanalyzer, sICAM-1 was determined by enzyme-linked immunosorbent assay (ELISA), and blood WBC count was determined by hemocytometer using validated methods at LSI Medience Inc. (Tokyo, Japan) according to applicable GLP standards.

Respiratory Function Test

For the examination of pulmonary functions,[24,35] forced expiratory volume in 1 second (FEV1), % predicted value of FEV1 (%FEV1), forced vital capacity (FVC), % predicted value of FVC (%FVC), FEV1/FVC ratio (FEV1%), maximum midexpiratory flow (FEF_{25-75})[7] and peak expiratory flow (PEF) were measured at the clinics using a spirometer (type HI-201 or HI-801, Chest Inc. Tokyo, Japan) based on ATS/ERS guidelines.[36]

Questionnaire

The evaluation questionnaire was developed on the basis of a Japanese version of the Leicester cough questionnaire (J-LCQ) for cough-related symptoms, and SF-36 Health Survey Scales were used for assessment of general health status. A validated Japanese version of the Leicester cough questionnaire (LCQ),[37] J-LCQ,[38] was used to estimate cough-related symptoms. The use of J-LCQ was permitted by its author, Dr. Birring[37] and translators, Drs. Niimi and Ogawa. A Japanese version of SF-36[39], SF-36v2® (iHope International Inc., Tokyo Japan) was used to estimate QOL. The composite three-component summary score [Physical component summary (PCS), Mental-component summary (MCS), Role-social-component summary (RCS)] was calculated with Japanese national standard-converted value for each eight-component score according to the supplier's instruction.

Statistical Analysis

Since the purpose of this study is to understand the biological effects of daily use of NTV, no statistical hypothesis has been set. Therefore, no sample size estimation was performed. However, for smokers and non-smokers, there are already many reports from clinical studies investigating BoPH. Referring to these reports, a minimum sample size of 100 (CC: 100, NS: 100) was set to investigate BoPH in the smoker and non-smoker groups.

Differences between the NTV or NS group and the CC group for total NNAL, plasma cotinine, and the score on the Japanese version of SF-36 were evaluated by performing an analysis of covariance (ANCOVA). The ANCOVA model included group and covariates for site and group*site interaction. Differences between the NTV or NS group and the CC group for all BoPH were evaluated by performing an ANCOVA. The ANCOVA model included group and covariates for age, gender, BMI, group*age interaction, group*gender interaction, and group*BMI interaction. The same model was used in the additional analysis to compare the NTV group (with over 3 months of NTV use and a previous smoking

history of over 20 years) with the CC group (with a smoking history exceeding 20 years).

We selected age, gender, and BMI as covariates as they were considered potential influential factors on BoPH such as blood lipid parameters[23] and physical lung function[24] as confounding factors. The differences between the NTV or NS group and the CC group for the LCQ score where the score were not distributed normally were analyzed using the non-parametric Steel test.

Descriptive statistics were presented to describe demographic characteristics, tobacco product use history by group and tobacco product use history before NTV use in the NTV group. For biomarkers not distributed normally, a natural log transformation was applied to ANCOVA, and the geometric LS mean was used in such cases.

The two-sided significance level was set to 0.05. SAS for Windows (SAS Institute, Cary, NC) was used for conducting the statistical analyses.

Results

Subjects

Informed consent was obtained from 568 participants (NTV group: 301, CC group: 134, NS group 133; of which 109 participants did not meet the protocol inclusion/exclusion criteria. The remaining 459 participants (NTV group: 259, CC group: 100, NS group 100) were enrolled into the study, and all enrolled subjects completed the study.

Demographics

A summary of the demographic characteristics is provided in Table 1. Regarding subject background information, the percentage composition by gender, age, and BMI in the NTV, CC, and NS groups were comparable. The average of age and BMI were 45.4 (range: 21–64) years and 23.95 (range: 14.4–40.7) kg/m^2, respectively. There were no differences between groups in terms of sex, age, and BMI. Subject characteristics among the three groups were equally distributed as a result of matching recruitment for CC and NS groups with the NTV group.

History of Tobacco Product Use

A summary of tobacco product use for the NTV group and CC group is provided in Table 1. Subjects in the NTV group exclusively used NTV for an average of 1.2 years (range: 3 months–3.3 years) and consumed an average of 3.56 capsules daily (range: 0.1–15 capsules; a single tobacco capsule can last for approximately 50 puffs, depending on the puff duration of the user). Most subjects (93.1%) in the NTV group used NTV every day. Subjects in the CC group had smoked CC for an average of 24.6 years (range: 1.4–44 years) and consumed on average 16.9 cigarettes daily (range: 5–50 cigarettes). Most subjects (98%) in the CC group smoked CC every day.

A summary of tobacco product use before NTV use in the NTV group is provided in Table 2. It shows that 92.3% (N = 239) of subjects had used tobacco products daily, 5.8% (N = 15) had stopped

Table 1. Demographics and tobacco product use history

		NTV group	CC group	NS group	Total
		N = 259	N = 100	N = 100	N = 459
Gender [N (%)]	Male	193 (74.5)	75 (75.0)	75 (75.0)	343 (74.7)
	Female	66 (25.5)	25 (25.0)	25 (25.0)	116 (25.3)
Age (years)	Mean	45.4	45.9	44.6	45.4
	SD	9.4	9.7	8.7	9.3
	Median (min., max.)	46.0 (22, 64)	46.0 (21, 64)	46.0 (22, 62)	46.0 (21, 64)
Age group [N (%)]	21~30	18 (6.9)	6 (6.0)	8 (8.0)	32 (7.0)
	31~40	50 (19.3)	20 (20.0)	19 (19.0)	89 (19.4)
	41~50	113 (43.6)	45 (45.0)	47 (47.0)	205 (44.7)
	51~65	78 (30.1)	29 (29.0)	26 (26.0)	133 (29.0)
BMI (kg/cm^2)	Mean	24.03	23.67	24.04	23.95
	SD	3.98	3.35	4.19	3.90
	Median (min., max.)	23.70 (15.4, 40.7)	23.10 (16.1, 32.0)	23.55 (16.3, 39.7)	23.50 (15.4, 40.7)
BMI group [N (%)]	BMI < 18.5	8 (3.1)	3 (3.0)	5 (5.0)	16 (3.5)
	18.5 ≤ BMI < 25.0	165 (63.7)	63 (63.0)	63 (63.0)	291 (63.4)
	BMI ≥ 25.0	86 (33.2)	34 (34.0)	32 (32.0)	152 (33.1)
Period of use (year)	Mean	1.2	24.6	-	-
	SD	0.63	10.1	-	-
	Median (min., max.)	1.08 (0.25, 3.3)	25 (1.4, 44)	-	-
Daily consumption (Capsule/Cigarette)	Mean	3.56	16.9	-	-
	SD	2.14	7.18	-	-
	Median (min., max.)	3 (0.1, 15.0)	20 (5, 50)	-	-
Frequency of use [N (%)]	Every day	241 (93.1)	98 (98.0)	-	-
	6 days/week	5 (1.9)	0 (0.0)	-	-
	5 days/week	8 (3.1)	0 (0.0)	-	-
	4 days/week	5 (1.9)	2 (2.0)	-	-
Tar value (mg)	Mean	-	7.2	-	-
	SD	-	4.5	-	-
	Median (min., max.)	-	7 (1, 19)	-	-

CC = conventional cigarette, NS = never-smokers, NTV = novel tobaaco vapor.

Table 2. Tobacco products use history before novel tobacco vapor (NTV) use in NTV group

Tobacco product use history before NTV use (*N* = 259)	*N* (%)	Type of user	*N* (%)	CC smoking/ quitting period [Year]	CC consumption [cigarette]	CC Tar value [mg]
			N = 233[1]	Mean (SD)	Mean (SD)	Mean (SD)
Tobacco product user	239 (92.3)	Exclusive CC smoker	147 (61.5)	20.5 (10.4)	14.92 (6.61)	4.7 (3.9)
		Exclusive HNBP user	63 (26.4)	-	-	-
		Dual user (CC and HNBP)	23 (9.6)	18.3 (9.5)	11-14.5[2]	3-3.8[2]
Quitter	15 (5.8)	-	-	5.5 (6.2)	-	-
Never-smoker	5 (1.9)	-	-	-	-	-

Tobacco product user: Subject who had used tobacco products on a daily basis.
Quitter: someone who has stopped using tobacco products on a sustained basis.
Never-smoker: Subject who had never used any kind of tobacco products.
CC = Conventional cigarette.
HNBP = heat-not-burn tobacco product.
[1]Data from six subjects were excluded due to the uncertainty that their responses to the questionnaire were reliable.
[2]Varies depending on heated tobacco product types (iQOS, Glo, NTV) with CC combination.

using tobacco products on a sustained basis (average quit period: 5.5 years) and 1.9% (*N* = 5) of subjects had never used any kind of tobacco products previous to the study. Out of those who had used tobacco products, the majority (71%, 170/239) were either exclusive CC smokers (*N* = 147) or dual users (CC and heated tobacco, *N* = 23). The average duration of smoking CC, CC daily consumption, and CC tar value for exclusive CC smokers were 20.5 years, 14.9 cigarettes, and 4.7 mg tar, respectively. The tar value for exclusive CC smokers in the NTV group before NTV use was lower than that of the CC group (4.7 vs 7.2 mg) whereas the values for CC smoking duration and CC daily consumption in both groups were similar (Table 1).

Exposure to Nicotine and NNK

Nicotine and NNK exposure were estimated by measuring plasma cotinine and urinary excretion of total NNAL.

The results of exposure level testing for cotinine and NNAL are shown in Table 3, in which the geometric LS mean values based on the statistical model are shown. Compared to the CC group the levels for plasma cotinine were significantly lower in the NTV group (–73.0%, $p < .0001$) and in the NS group (–99.7%, $p < .0001$). Compared to the CC group the NNAL levels were significantly lower in the NTV group (–94.3%, $p < .0001$) and in the NS group (–97.2%, $p < .0001$). NNAL levels in the NTV group were still significantly higher than those of the NS group (207%, $p < .0001$) and the cotinine levels were intermediate between the CC and NS groups.

Biomarkers of Potential Harm

The BoPH level results are summarized in Table 3, in which the geometric LS mean values based on the statistical model are shown. Regarding blood sample variables, levels of HDL-C, TG, sICAM-1, and the WBC count differed significantly between the NS and CC groups (NS/CC: +12.7%, –25.5%, –16.6%, and –18.9%, respectively). The levels of these variables differed significantly between the NTV and CC groups, by +13.9%, –22.8%, –12.4%, and –17.8% (NTV/CC), respectively, whereas the differences in these variables between the NTV and NS groups were not significant. The levels of TC and LDL-C were not significantly different among the three groups.

Similarly, levels of 11-DHTXB2, 2,3-d-TXB2, and 8-epi-PGF2α in urine were significantly lower in the NS group (–32.6%, –44.4%, –28.9%, respectively) and the NTV group (–24.5%, –34.4%, –21.6%, respectively) compared to the CC group. The levels of 11-DHTXB2 and 8-epi-PGF2α in the NTV group were similar to those in the NS group with no significant difference, while significantly higher levels of 2,3-d-TXB2 (+18.1%, $p = .0312$) was observed in the NTV group compared to the NS group.

Regarding pulmonary functional parameters, the levels for FVC and %FVC were significantly higher in the NS group (+6.5, $p = .0062$; +6.4%, $p = .0028$, respectively) compared to CC group, but were no significantly different from the levels seen in the NTV group. The levels of FEV1, %FEV1 and FEF_{25-75} were significantly higher in the NS group (+8.7%, $p = .0002$; +8.5%, $p < .0001$; +14.0%, $p = .0053$, respectively) and the NTV group (+4.1%, $p = .0355$; +5.0%, $p = .0039$; +8.9%, $p = .0345$, respectively) compared to the CC group. The levels of FEF_{25-75} in the NTV group were similar to those in the NS group with no significant difference, while significantly lower levels of FEV1 and %FEV1 was observed in the NTV group compared to the NS group (–4.2%, $p = .0261$; –3.3%, $p = .0439$, respectively). The levels of FEV1% and PEF were not significantly different among the three groups. The relative values of the geometric LS mean in the CC group compared to the NTV and NS groups are shown in Supplementary Figure 1.

Additional Analysis

In this study, the smoking history of the CC group (over 1 year) and the use period of the NTV group (over 3 months) are different. It is not clear whether the observed favorable difference of some BoPH between the NTV group and the CC group can be attributed to NTV use or to the difference in exposure length of the study subjects to the two types of product (NTV vs. CC). Therefore, we performed an additional analysis to compare the NTV group with over 3 months of NTV use and a previous smoking history of over 20 years with the CC group with a smoking history exceeding 20 years. The results are summarized in Supplementary Table S1. Compared to the CC group the levels for plasma cotinine and total NNAL were significantly lower in the NTV group (–72.8%, $p < .0001$; –94.4%, $p < .0001$; respectively). Levels of HDL-C, TG, sICAM-1, WBC count, 11-DHTXB2, 2,3-d-TXB2, 8-epi-PGF2α, FEV1, %FEV1, FEV1%, FEF_{25-75}, and PEF differed significantly between the CC and NTV groups (NTV/CC; +13.3%, $p = .0008$; –22.1%, $p = .0153$; –16.3%, p

Table 3. Biomarkers of exposure and potential harm

Biomarker	Matrix/physical test	Biomarker/parameter	Group	*N*	Geometric LS mean [95% CI]	Geometric LS mean ratio (%) [95% CI] (NTV/CC or NS/CC)	*p* value[1]	Geometric LS mean ratio (%) [95% CI] (NTV/NS)	*p* value[1]
BoE[2]	Blood	Plasma cotinine	CC	100	193 [147, 254]				
		(ng/mL)	NTV	259	52.3 [43.1, 63.4]	27.0 [19.3, 37.8]	<.0001	10261.8 [7340.4, 14345.8]	<.0001
			NS	100	0.51 [0.39, 0.67]	0.3 [0.2, 0.4]	<.0001		
	Urine	Total NNAL	CC	100	93.0 [75.5, 115]				
		(ng/g . Cr)	NTV	259	5.34 [4.61, 6.20]	5.7 [4.4, 7.4]	<.0001	207.1 [160.3, 267.6]	<.0001
			NS	100	2.58 [2.09, 3.18]	2.8 [2.1, 3.7]	<.0001		
BoPH[3]	Blood	Total cholesterol	CC	100	207.5 [199.9, 215.4]				
		(mg/dL)	NTV	259	209.6 [204.3, 215.1]	101.0 [96.6, 105.7]	.655	103.3[98.8, 108.1]	.156
			NS	100	202.9 [195.5, 210.6]	97.8 [92.8, 103.1]	.402		
		LDL-C	CC	100	124 [116.9, 131.6]				
		(mg/dL)	NTV	259	124.6 [119.6, 129.8]	100.4 [93.5, 107.9]	.903	103.8 [96.6, 111.6]	.311
			NS	100	120 [113.1, 127.3]	96.8 [89.0, 105.2]	.442		
		HDL-C	CC	100	52.9 [50.3, 55.7]				
		(mg/dL)	NTV	259	60.3 [58.2, 62.5]	113.9 [107.0, 121.3]	<.0001	101.1 [95.0, 107.7]	.725
			NS	100	59.6 [56.6, 62.8]	112.7 [104.7, 121.2]	.0015		
		Triglyceride	CC	100	116.7 [103.8, 131.2]				
		(mg/dL)	NTV	259	90.1 [83.1, 97.7]	77.2 [67.0, 89.1]	.0004	103.6 [89.9, 119.5]	.623
			NS	100	87 [77.3, 97.8]	74.5 [63.1, 88.0]	.0005		
		sICAM-1	CC	100	463.6 [438.2, 490.5]				
		(ng/mL)	NTV	259	405.9 [390.4, 422]	87.6 [81.8, 93.8]	.0002	105.0 [98.0, 112.5]	.162
			NS	100	386.5 [365.3, 409]	83.4 [77.0, 90.3]	<0.0001		
		WBC count	CC	100	6635 [6301, 6987]				
		(/μL)	NTV	259	5454 [5263, 5652]	82.2 [77.2, 87.5]	<.0001	101.4 [95.2, 108.0]	.664
			NS	100	5378 [5107, 5664]	81.1 [75.3, 87.2]	<.0001		
	Urine	11-DHTXB2	CC	100	867.98 [778.76, 967.41]				
		(ng/g . Cr)	NTV	259	655.60 [608.28, 706.58]	75.5 [66.2, 86.2]	<.0001	112.0 [98.2, 127.8]	.0916
			NS	100	585.20 [524.89, 652.43]	67.4 [57.8, 78.6]	<.0001		
		2,3-d-TXB2	CC	100	438.39 [387.24, 496.29]				
		(ng/g . Cr)	NTV	259	287.78 [264.15, 313.52]	65.6 [56.5, 76.3]	<.0001	118.1 [101.5, 137.3]	.0312
			NS	100	243.74 [215.23, 276.03]	55.6 [46.6, 66.3]	<.0001		
		8-epi-PGF2α	CC	100	232.20 [213.20, 252.89]				
		(ng/g . Cr)	NTV	259	181.98 [171.56, 193.02]	78.4 [70.6, 86.9]	<.0001	110.3 [99.4, 122.4]	.0646
			NS	100	165.00 [151.46, 179.74]	71.1 [63.0, 80.2]	<.0001		
BoPH[3]	Lung function	FVC	CC	99	3.523 [3.412, 3.638]				
		(L)	NTV	259	3.62 [3.541, 3.701]	102.8 [98.8, 106.8]	.169	96.5 [92.8, 100.3]	.0688
			NS	100	3.754 [3.635, 3.876]	106.5 [101.8, 111.5]	.0062		
		%FVC	CC	99	108.42 [105.37, 111.56]				
		(%)	NTV	259	111.97 [109.79, 114.19]	103.3 [99.8, 106.9]	.0688	97.1 [93.8, 100.5]	.0933
			NS	100	115.34 [112.09, 118.68]	106.4 [102.2, 110.8]	.0028		
		FEV1	CC	99	2.858 [2.771, 2.949]				
		(L)	NTV	259	2.977 [2.914, 3.041]	104.1 [100.3, 108.2]	.0355	95.8 [92.2, 99.5]	.0261
			NS	100	3.108 [3.012, 3.206]	108.7 [104.0, 113.6]	.0002		
		%FEV1	CC	99	98.89 [96.26, 101.59]				

Table 3. Continued

Biomarker	Matrix/physical test	Biomarker/parameter	Group	*N*	Geometric LS mean [95% CI]	Geometric LS mean ratio (%) [95% CI]		Geometric LS mean ratio (%) [95% CI]	
						(NTV/CC or NS/CC)	*p* value[1]	(NTV/NS)	*p* value[1]
		(%)	NTV	259	103.79 [101.88, 105.73]	105.0 [101.6, 108.4]	.0039	96.7 [93.6, 99.9]	.0439
			NS	100	107.34 [104.49, 110.28]	108.5[104.5, 112.8]	<.0001		
		FEV1%	CC	99	81.136 [79.793, 82.502]				
		(%)	NTV	259	82.225 [81.285, 83.176]	101.3 [99.3, 103.4]	.1967	99.3 [97.3, 101.3]	.506
			NS	100	82.792 [81.421, 84.186]	102.0 [99.7, 104.5]	.0933		
		FEF_{25-75}	CC	99	2.879 [2.698, 3.072]				
		(L/s)	NTV	259	3.134 [2.997, 3.277]	108.9 [100.6, 117.8]	.0345	95.5 [88.3, 103.4]	.254
			NS	100	3.281 [3.075, 3.501]	114.0 [104.0, 124.9]	.0053		
		PEF	CC	99	7.51 [7.185, 7.85]				
		(L/s)	NTV	259	7.884 [7.647, 8.128]	105.0 [99.5, 110.8]	.0768	98.6 [93.4, 104.1]	.607
			NS	100	7.996 [7.649, 8.358]	106.5 [100.0, 113.4]	.0501		

BoE = biomarkers of exposure; BoPH = biomarkers of potential harm; CC = conventional cigarette; FEF = maximum midexpiratory flow; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; HDL-C = High-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NS = never-smokers; NTV = novel tobacco vapor; PEF = peak expiratory flow; sICAM-1 = soluble intercellular adhesion molecule-1; 11-DHTXB2 = 11-dehydrothromboxane B2; 2,3-d-TXB2 = 2,3-dinor thromboxane B2.

[1]*p*-value for comparison between groups from ANCOVA.

[2]The ANCOVA model included group, group*site interaction and site as covariates.

[3]The ANCOVA model included group, group*age interaction, group*gender interaction, group*BMI interaction, group*site interaction and covariates for age, gender, BMI, and site.

< .0001, –21.5%, p < .0001; –25.1%, p = .0012; –27.7%, p = .0008; –22.9%, p = .0001; +6.6%, p = .0086; +6 .5%, p = .0051; +3.6%, p = .0057; +17.6%, p = .0023; +9.3%, p = .0055; respectively).

Questionnaire for Assessment of Cough Status

The total score results for the Japanese version of the cough questionnaire (J-LCQ) are shown in Table 4. The LCQ scores were calculated based on results of the breakdown of each three-domain score (Physical, Psychological, and Social); the higher scores are associated with better health status. LCQ scores were significcantly higher in the NTV group compared to those for the CC group (p < .0001). No significant difference was observed in the scores between the NTV and NS groups.

Questionnaire for Assessment of QOL

Results for the three-component summary score on the SF-36 questionnaire are summarized in Supplementary Table S2, in which the LS mean values based on the statistical model are shown. The three-component summary score was calculated based on results of the breakdown of eight subscale scores (Role physical, General health perceptions, Vitality, Role emotional, Mental health, Physical functioning, Bodily pain, and Social functioning). Among the three-component summary scores, only the Mental component (MCS) was significantly higher in the NTV group compared to the CC group (p = .0008). No statistical difference was observed in any of the three-component summary scores between the NTV group and the NS group.

Discussion

Cross-sectional post-marketing data under actual use conditions of BoE to nicotine and NNK and BoPH for adult exclusive NTV users, CC smokers, and NS under actual use conditions were explored. The results showed that NTV users were significantly less exposed to nicotine and NNK, and to have significantly lower levels of BoPH (TG, sICAM-1, WBC count, 11-DHTXB2, 2,3-d-TXB2, 8-epi-PGF2α,) than CC smokers. Conversely, levels of HDL-C, FEV1, %FEV1, and FEF_{25-75} were higher than those seen in CC smokers. Moreover, the levels of BoPH (HDL-C, TG, sICAM-1, WBC count, 11-DHTXB2, 8-epi-PGF2α, and FEF_{25-75}) in the NTV group were comparable to those in the NS group. BoPH levels, which are closely correlated with smoking-related diseases such as CVD[27–34] and COPD,[24,35] and levels of NNK, a carcinogen,[26] were significantly and favorably different in exclusive NTV users compared with CC smokers. Among these markers, significant differences between NTV and NS were found in the levels of NNAL, 2,3-d-TXB2, FEV1, and %FEV1. In additional analysis, NTV users with a past smoking history of more than 20 years were significantly less exposed to nicotine and NNK, and to have significantly favorable levels of BoPH (HDL-C, TG, sICAM-1, WBC count, 11-DHTXB2, 2,3-d-TXB2, 8-epi-PGF2α, FEV1, %FEV1, FEV1%, FEF_{25-75}, and PEF) compare to CC smokers who have smoked for over 20 years, consistent with the results shown in Table 3, with the exception of FEV1% and PEF. These findings suggest that the differences observed in this study were due to the use of NTV.

In a search of the history of tobacco product use in subjects belonging to the NTV group, 92.3% (N = 239) had used tobacco products daily among the 61.5% (N = 147) that had been exclusive CC smokers. The average of tar levels in the NTV switchers from the exclusive CC smokers was lower than that of the CC group

Table 4. Japanese version of the Leicester cough questionnaire (J-LCQ) total score

		NTV vs. CC *			NTV vs. NS *		
	Group	*N*	Rank average	*p* value	*N*	Rank average	*p* value
LCQ (total score)	NTV	259	201.48	<.0001	259	175.31	.2952
	CC	100	124.38	-	-	-	-
	NS	-	-	-	100	192.16	-

* Steel test

CC = conventional cigarette; NS = never-smokers; NTV = novel tobacco vapor.

(CC → NTV: *N* = 147, 4.7±3.9 mg vs. CC: *N* = 100, 7.2 ± 4.5 mg) even though the values for smoking duration and daily consumption of tobacco products had been similar between the two groups. Therefore, the data in the NTV group are considered to reflect, for the most part, the positive consequences of switching from CC to NTV.

Cotinine levels observed in the NTV group were about one-fourth those of the CC group who smoked their own products. One of the reasons for the lower cotinine levels of the NTV group could be simply due to the delivery of less nicotine than CC.[22] We previously reported a five-day confinement longitudinal study in which nicotine equivalents were compared among three groups (CC continuation, CC to NTV switcher, or smoking abstainers).[3] Another study reported that not only urinary excretion of nicotine equivalents but also plasma cotinine concentration were reported to be predictive of the nicotine dose (r = 0.75).[40] Levels of relative nicotine equivalents observed in NTV switchers on day 5 were about half those of CC smokers, and NTV switchers consumed an average of 6.1 capsules per day on day 5[3] Relative cotinine levels in the NTV group in the present study were one-fourth of those in the CC group, and the NTV group consumed an average of 3.6 capsules per day, which was 59% of the capsule consumption in the previous study. Therefore, observed differences in nicotine exposure in the NTV and CC groups in the present study may be consistent with the previous study when the number of capsules consumed is taken into consideration. In the previous study just mentioned,[3] a reduction in NNAL levels of 59% was observed in NTV switchers. NNAL levels were markedly lower (−94.3%) in the NTV group than in the CC group. The difference can be explained by the long NNAL half-life of 18 days,[41] and a significant amount of NNAL still remained in NTV switchers after five days. Similar to our data, a major difference in NNAL levels (−86.2%) was found in exclusive EVP users under actual use conditions.[10] Therefore, the present data may represent the actual use conditions of an exclusive user of HNBP. The 2010 Surgeon General's report states that oxidative stress and chronic inflammation are a common thread among the three major smoking-related diseases.[1] Based on the assumption of the reported underlying mechanisms of CVD and COPD, differences and reversible changes by smoking status, association with disease endpoints and according to review articles,[42,43] BoPH, comprising variables related to lipid metabolism, endothelial function, inflammation, oxidative stress, platelet activation, and pulmonary function, were finally selected. These BoPH have been used for HNBP and EVP evaluation in previous studies.[4–7,10] Blood lipids are biomarkers used to evaluate the risks for CVD.[27] In general, higher levels of HDL-C reduce the risk of CVD.[28] Lower levels of HDL-C[44,45] and higher levels of TG[46] in smokers than in non-smokers in healthy subjects have been found, but this was not the case for TC and LDL-C.[43] Significantly lower levels of TG, and higher levels of HDL-C, were observed among NTV users compared to the CC group.

In a meta-analysis of smoking cessation studies, a trend was identified wherein the maximum increase in HDL-C occurred three weeks after smoking cessation, possibly because of changes in cessation-induced dietary variations and/or body weight gain.[17] In the present study, the average period of NTV use was 1.2 years, a much longer period than three weeks. Some studies have reported a significant correlation between the increase in sICAM-1 levels and future coronary events.[29] A systematic review of the association between WBC counts and coronary artery disease investigated in a number of epidemiological and clinical studies showed that the WBC count is an independent predictor of future cardiovascular events in both healthy individuals without CVD at baseline and subjects with CVD.[30] There is a trend for sICAM-1[45,47] and WBC levels[44,45] to be higher in smokers than in non-smokers in healthy subjects. Elevated sICAM-1 and WBC levels in smokers are reported to decrease following smoking cessation.[18,19,43] The present study found that sICAM-1 and WBC levels were significantly lower in NTV users than in CC smokers, and the levels were equivalent to those in NS subjects. Increased thromboxane A2(TXA2) production is generally believed to be intrinsically involved in the development of thrombogenesis and atherosclerosis.[48] Since TXA2 is unstable in blood, two stable metabolites of TXA2 (11-DHTXB2 and 2,3-d-TXB2) in urine were measured clinically.[32] A positive correlation between levels of 11-DH-TXB2 and several CVD events has been reported previously.[31] Eight-epi-PGF2α is a reliable marker when evaluating exposure levels for oxidative stress.[49] Zhang et al. performed a systematic review of papers (from 1966 to February 2012) that investigated the relationship between 8-epi-PGF2α levels (in urine and blood) and CVD. Of the 22 studies, 20 studies found a significant association between 8-epi-PGF2α levels and CVD.[33] There is a trend for levels of 11-DHTXB2, 2,3-d-TXB2, and 8-epi-PGF2α to be higher in smokers than in non-smokers in healthy subjects.[20,44,45,50] Elevated levels of 11-DHTXB2, 2,3-d-TXB2, and 8-epi-PGF2α in smokers were reported to decrease following smoking cessation.[4,20] In the present study, significantly lower levels of 11-DHTXB2, 2,3-d-TXB2, and 8-epi-PGF2α in NTV users compared to CC smokers were found and there was no difference between the NTV users and the NS subjects in biomarker levels except for 2,3-d-TXB2 (2,3-d-TXB2: NTV > NS, p = .0312). There is a report that 2,3-d-TXB2 could detect the effect of smoking cessation more sensitively than 11-DHTXB2, which may mean that the full effects of NTV remain to be ascertained and differences between NTV and NS may still be found.[20]

Among pulmonary function variables, %FEV1 is mainly used for diagnosis of COPD. An epidemiological cohort study revealed that FEV1 declines faster in smokers than in non-smokers.[24] A systematic review of smoking cessation studies has shown that for FEV1 to reach

the same level as that of non-smokers takes more than two years following smoking cessation.[21] Significantly favorable changes in FEF_{25-75} levels were reported in smokers who switched to EVP from CC for one year, although FVC and FEV1 did not change.[7] Significantly favorable differences were observed in three variables (FEV1, %FEV1, and FEF_{25-75}) in the NTV group compared to the CC group. Compared to the NS group, significantly lower levels of FEV1 and %FEV1 were observed in the NTV group, while no significant difference in the levels of FEF_{25-75} were found. This implies that although NTV users have better in lung function compared to CC smokers, differences between the NTV and NS groups are still observed. These results are surprising because the improvement in pulmonary function brought about by smoking cessation is believed to take a considerable amount of time (more than two years) except for FEF_{25-75}. The period over which NTV was used was from three months to approximately three years, with an average of 1.2 years, which is shorter than that in the previous cessation study. The results of the cough questionnaire survey (J-LCQ) supported this observation.

As for the SF-36 results, the LS mean value of the three-summary component scores in the NTV, CC, and NS groups were all above Japan's national standard value (>50). This means that the average score for self-reported health was better than the national standard values.

Among such healthy subjects, mental QOL was significantly higher in the NTV group than in the CC group, with no difference found between the NTV group and the NS group. Differences in the SF-36 score among never-smokers, ex-smokers, and light, moderate, and heavy smokers were reported to increase in this order in the previous study.[51] It is interesting result that even though the average of self-reported health-related quality of life of each group in this study exceeds the national standards, QOL values might be changed with reduced exposure to toxic substances in cigarette smoke. However, it is considered that further investigations will be needed to ascertain whether the statistically significant difference has clinical significance.

Limitations

There are limitations to this study to consider when interpreting the findings. Since this is a cross-sectional study, changes in biomarker levels over time could not be investigated, as they can in a longitudinal study. Since the baselines of BoE and BoPH are not available, the favorable results for BoPH need to be carefully interpreted as they may have been caused by the sustained reduction in exposure to harmful substances of tobacco smoke with HNBP use. We attempted to address this concern by measuring BoE and BoPH in the CC group and the NS group as a benchmark. Regarding measured BoPH, this study did not directly measure disease endpoints but rather measured BoPH that are associated with pathomechanistic pathways underlying the development of diseases associated with smoking. Therefore, this study demonstrated significantly favorable differences in many BoPH in the NTV group relative to the CC group; however, the clinical significance of the differences is not clear. In order to clarify the reduction in health risks associated with smoking by NTV use, further studies including clinical studies with disease-related endpoints will be necessary.

Conclusion

Although further research is definitely required for next-generation non-combustible products, the present study has provided evidence suggesting that some BoPH including respiratory function and QOL exhibit differences in level between the CC group and the NTV group, and switching completely to NTV may lower the harmful effects associated with tobacco leaves combustion. The study adds to a growing body of evidence suggesting that exclusive HNBP users, compared to CC smokers, have favorable levels of BoPH, which are indicative of pathomechanistic pathways underlying the development of diseases associated with smoking, and that this results from a sustained reduction in exposure to harmful substances of tobacco smoke by HNBP use.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Declaration of Interests

All authors are employees of Japan Tobacco Inc. The authors declare no potential conflicts of interest.

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