ผลของการสูบบุหรี่ต่อเซลล์เม็ดเลือด โปรตีนในพลาสมา ไซโตไคน์ และคีโมไคน์ในผู้ใหญ่ที่มีสุขภาพดี

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บทคัดย่อ

ควันบุหรี่ประกอบด้วยสารพิษ สารก่อมะเร็ง และสารเสพติดหลายชนิด ซึ่งการสูบบุหรี่ส่งผลเสียต่อสุขภาพ เสี่ยงต่อการเกิดโรคมะเร็งและโรคอื่น ๆ งานวิจัยนี้ศึกษาผลของการสูบบุหรี่ต่อสารบ่งชี้ทางชีวภาพในเลือดของคนสุขภาพดี โดยคัดเลือกอาสาสมัคร 357 ราย แบ่งเป็นผู้สูบบุหรี่ 135 ราย และไม่สูบบุหรี่ 222 ราย และเก็บตัวอย่างเลือดเพื่อวิเคราะห์สารบ่งชี้ทางชีวภาพ ผลการศึกษาพบว่าผู้สูบบุหรี่มีค่าเกล็ดเลือด เม็ดเลือดขาว และ reticulocyte ผิดปกติ นอกจากนี้ ค่าโปรตีนในพลาสมา (CRP และ PDGF), ไซโตไคน์ (IL-2, IL-6, IFN-γ, และ TNF-α) และคีโมไคน์ (MCP-1 MIP-1β และ RANTES) มีการเปลี่ยนแปลงในผู้สูบบุหรี่ เมื่อเปรียบเทียบระหว่างกลุ่มผู้สูบบุหรี่เพศหญิงและเพศชาย และระหว่างกลุ่มผู้สูบบุหรี่และไม่สูบบุหรี่ที่อายุน้อยกว่า 50 ปี พบว่าไม่มีผลต่อสารบ่งชี้ทางชีวภาพ แต่กลุ่มผู้สูบบุหรี่ที่อายุมากกว่าหรือเท่ากับ 50 ปี มีการเปลี่ยนแปลงของสารบ่งชี้ทางชีวภาพเพศและอายุที่มากขึ้นมีผลเล็กน้อยต่อเซลล์เม็ดเลือดและค่าโปรตีนบางค่า ได้แก่ เกล็ดเลือด เม็ดเลือดขาว, IL-6, PDGF และ TNF-α การสูบบุหรี่ทำให้เกิดการตอบสนองของระบบภูมิคุ้มกันที่ผิดปกติ และยิ่งส่งผลมากขึ้นในผู้สูบบุหรี่ที่อายุมากขึ้น ผลการวิจัยนี้เสนอว่าการวิเคราะห์สารบ่งชี้ทางชีวภาพซึ่งเป็นวิธีที่รวดเร็วอาจจะเหมาะสมในการใช้ตรวจติดตามระดับการทำงานของระบบภูมิคุ้มกันในกลุ่มผู้สูบบุหรี่ และตรวจติดตามการเกิดโรคที่สัมพันธ์กับการสูบบุหรี่ อีกทั้งยังเป็นการสร้างความตระหนักถึงพิษภัยของการสูบบุหรี่และเลิกสูบบุหรี่ในกลุ่มผู้สูบบุหรี่ได้

คำสำคัญ: การสูบบุหรี่ เซลล์เม็ดเลือด โปรตีนในพลาสมา ไซโตไคน์ และคีโมไคน์

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Influence of Cigarette Smoking on Blood Cell Subpopulations, Plasma Proteins, Cytokines, and Chemokines in Adult Healthy Individuals

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Abstract

Cigarette smoke contains various toxic, carcinogenic and addictive substances. Cigarette smoking is an unhealthy lifestyle with increasing risks for cancer and various diseases. We investigated the influence of cigarette smoking on systemic immunological biomarkers in adult healthy individuals. Three hundred fifty-seven adult healthy participants, consisting of 135 cigarette smokers (smokers) and 222 who never have been smoking (non-smokers), were included for this investigation. Rapid and low-cost blood-based biomarkers were analyzed. Abnormal test results of platelets, total white blood cells and its subpopulations and reticulocytes were found in the smoker participants. Alterations of plasma proteins (CRP and PDGF), cytokines (IL-2, IL-6, IFN-γ, and TNF-α) and chemokines (MCP-1, MIP-1β and RANTES) was found. No influence on levels of these plasma biomarkers was observed when comparing neither female and male smokers nor young (< 50 years) smokers and non-smokers Additive Influence of age and smoking on these blood-based biomarkers was detected in the older (≥ 50 years) smokers. Gender and aging have a marginal influence on some systemic blood cell and plasma immune response biomarkers of healthy individuals, including platelets, total white blood cells, IL-6, PDGF, and TNF-α. Cigarette smoking dysregulated systemic immunological response in adult

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healthy individuals, and an apparent increase was observed in old age smokers. Our investigation suggested that the rapid and low-cost blood-based biomarkers might be suitable for monitoring host immunological status in clinical studies of smokers, cigarette smoking-associated diseases and motivate adult healthy smokers or smoking patients to quit smoking.

Keywords: Cigarette smoking, Blood cells, Plasma proteins, Cytokines, Chemokines

Introduction

Cigarette smoking is an independent lifestyle risk for numerous types of cancer and other diseases.[1, 2] Cigarette smoke contains various toxic, carcinogenic, and addictive substances. *In vitro* experiments showed that smoke extracts alter normal cell phenotypes, gene expression profiles, and induction of massive cell death.[3]

Rapid detection of systemic immune status could be done at low cost by analysing levels of blood cells or plasma biomarkers. Dysregulated levels of circulating white blood cells (Wbc), Wbc subpopulations and neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR) or platelet to lymphocyte ratio (PLR) are suggested to be associated with inflammation and poor clinical outcome in cancer patients.[4, 5]

Plasma proteins, cytokines, and chemokines play an important role in local and systemic host inflammatory responses, leukocyte trafficking and function of lymphocytes, natural killer (NK) cells or monocytes.[6, 7] Independent from tumor status and treatment modulation, increased plasma levels of C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α) were associated with a poor clinical outcome in cancer patients.[8]

In Sweden, approximately 7-12% of the population smoked daily in a recent decade, and cigarette smoking was associated with an increased risk of peripheral artery disease (PAD).[9] The aim of this study was to investigate the influence of cigarette smoking on systemic immunological profile (Wbc and its subpopulations, plasma proteins, cytokines, and chemokines) and hematological parameters of adult healthy individuals at Ryhov Hospital, Sweden. Rapid and low-cost blood-based biomarkers in adult healthy cigarette smokers and adult healthy who never used any type of tobacco products were analyzed for this purpose.

Materials and Methods

1. Study population

A total of 306 adult healthy blood participants and 51 healthy individuals turning up at a healthy center, Ryhov Hospital for health check-up were recruited for this study. None of them had a history of cancer or was using any immunomodulation agents. There were 177 males and 180 females in one Swedish community, and all were Caucasians. Among them, 135 had a history of cigarette smoking (smokers) and 222 had never used any type of tobacco products (non-smokers). There were 85 aged < 50 and 272 aged ≥ 50, according to the middle age period. Peripheral blood, 10 mL, was collected in EDTA containing tubes. The circulating blood cell numbers and phenotype were analysed within three hours. The plasma was stored at -80 °C until analysis for proteins, cytokines, and chemokines.

The study was conducted in accordance with the Declaration of Helsinki. Ethical Board at Linköping University approved this

investigation (Dnr 2015/178-32). Written information and informed consent were obtained from all participants before the blood was drawn.

2. Analysis of circulating blood cell biomarkers

The levels of circulating red blood cells (Rbc), reticuloendothelial cells hemoglobin (Hb), hematocrits (Hct), platelets (Plt), Wbc and Wbc subpopulations were analyzed using a Sysmex XE5000 instrument (Sysmex Corporation, Kobe Japan).

3. Analysis of plasma C-reactive protein (CRP), cytokines, and chemokines

Plasma CRP levels were analyzed by using the latex-enhanced immunoturbidimetric method (Siemens Advia 1800) according to the company protocol (Siemens Healthcare, Erlangen, Germany). Plasma cytokines IL-2, IL-6, IL-10, IL-12, Interferon-gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α)], chemokines [monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein-1 beta (MIP-1β)/Chemokine ligands 4 (CCL4), regulated upon activation, normal T cell expressed and secreted (RANTES)/ CCL5], and platelet-derived growth factor (PDGF) were analysed using the multiplex fluorochrome technique (Luminex xMAP^{TM} Technology, Austin, TX, USA). The customized fluorochrome kit was purchased from Bio-Rad Laboratories (Bio-Rad Laboratories, Hercules CA). The concentrations were determined according to the Bio-Rad Laboratories protocol (www.Bio-Rad.com/bio-Plex/ x-Plex).

4. Statistical analysis

Two-sided Student's t-test was used for all comparisons between the adult healthy smokers and non-smokers. Correction for multiple testing was done[10] and the threshold for statistical significance was determined after the correction at $p < 0.05$.

Results

Blood cell biomarkers of adult healthy smokers and non-smokers

Fourteen circulating blood cell biomarkers were analyzed in smokers and non smoker. Variations in 11 out of 14 circulating blood cell biomarkers were found in smokers, compared with the non-smokers (Table 1A). Stratified by gender, smoking influenced nine and eight out of the 14 biomarkers in female and male smokers, respectively, when compared with their corresponding female and male non-smokers (Table 1A). In smokers, a variation of hemoglobin (Hb) and hematocrit (Hct) levels was found in females, compared with males (Table 1B). In non-smokers, a variation in seven blood cell biomarkers was found in females, compared with males (Table 1B).

Stratified by age, a variation was found in eight and ten out of the 14 biomarkers in young (< 50 years) and old (≥ 50 years)

Table 1A. The influence of smoking on circulating blood cell profile of adult healthy cigarette smoking (Smokers) and those who never have used any tobacco product (Non-smokers).

Parameters	Non-smokers (n = 222)	Smokers (n = 135)	*p* value	Female			Male			< 50 years			≥ 50 years		
				Non-smokers (n = 104)	Smokers (n = 76)	*p* value	Non-smokers (n = 118)	Smokers (n = 59)	*p* value	Non-smokers (n = 55)	Smokers (n = 30)	*p* value	Non-smokers (n = 167)	Smokers (n = 105)	*p* value
Rbc	4.74	4.69	0.37	4.57	4.62	0.45	4.89	4.79	0.25	4.66	4.67	0.93	4.77	4.7	0.24
Hb	142.6	143.7	0.55	137	140	0.49	147.6	147.9	0.91	141.1	138.4	0.496	143.1	145.2	0.300
Hct	0.40	0.42	0.02*	0.40	0.41	0.01*	0.42	0.43	0.12	0.39	0.40	0.296	0.41	0.42	0.05
RET	43.40	49.59	<0.0001*	40.76	48.22	0.0003*	45.72	51.36	0.02*	33.65	32.47	0.039*	43.05	49.91	0.0001*
Platelet	226.9	254.4	<0.0001*	240.9	263.2	0.007*	214.7	243.1	0.0004*	217	263	<0.0001*	230.2	251.8	0.0015*
Wbc	5.65	7.08	<0.0001*	5.67	7.21	<0.0001*	5.65	6.93	<0.0001*	6.03	7.18	0.0062*	5.53	7.06	<0.0001*
Lymphocytes	1.78	2.09	<0.0001*	1.86	2.11	0.005*	1.72	2.05	<0.0001*	1.76	2.12	0.0065*	1.79	2.08	<0.0001*
Monocytes	0.51	0.61	<0.0001*	0.49	0.59	0.004*	0.53	0.63	<0.0001*	0.52	0.62	0.0048*	0.51	0.60	<0.0001*
Neutrophils	3.19	4.18	<0.0001*	3.13	4.33	<0.0001*	3.23	3.99	0.0002*	3.60	4.27	0.046	3.05	4.16	<0.0001*
Eosinophils	0.15	0.18	0.034*	0.16	0.17	0.82	0.14	0.19	0.006*	0.13	0.23	0.003*	0.16	0.16	0.66
Basophils	0.01	0.03	<0.0001*	0.02	0.04	0.021*	0.008	0.03	<0.0001*	0.004	0.04	<0.0001*	0.01	0.03	0.005*
MLR	0.30	0.29	0.29	0.28	0.28	0.87	0.32	0.30	0.27	0.31	0.29	0.48	0.3	0.29	0.427
NLR	1.94	2.16	0.048	1.87	2.23	0.031*	2.01	2.06	0.71	2.21	2.1	0.72	1.85	2.16	0.011*
PLR	136	126	0.043	138	127	0.12	134.1	123.3	0.15	133.4	126.3	0.54	136.9	125.7	0.045

Rbc, red blood cell; Hb, hemoglobin; Hct, hematocrit; RET, reticulocyte, Wbc, white blood cell; MLR, monocytes-to-lymphocytes ratio; NLR, neutrophils-to-lymphocytes ratio; PLR, platelets-to-lymphocytes ratio (PLR); * Two-sided Student´s t-test (*p* value < 0.05)

Table 1B. The influence of age and gender on circulating blood profile of adult healthy cigarette smoking (Smokers) and those who never have used any tobacco product (Non-smokers).

Parameters	Non-smokers			Smokers			Non-smokers			Smoker		
	Age < 50 (n = 55)	Age ≥ 50 (n = 167)	*p* value	Age < 50 (n = 30)	Age ≥ 50 (n = 105)	*p* value	Female (n = 104)	Male (n = 118)	*P* value	Female (n = 76)	Male (n = 59)	*P* value
Rbc	4.66	4.77	0.17	4.67	4.7	0.69	4.57	4.89	<0.0001*	4.62	4.79	0.13
Hb	141.1	143.1	0.51	138.4	145.2	0.005*	137	147.6	<0.0001*	140	147.9	0.0002*
Hct	0.39	0.41	0.07	0.40	0.42	0.009*	0.40	0.42	<0.0001*	0.41	0.43	0.004*
RET	33.65	43.05	0.54	32.47	49.91	0.62	40.76	45.72	0.013*	48.22	51.36	0.18
Platelet	217	230.2	0.045	263	251.8	0.35	240.9	214.7	<0.0001*	263.2	243.1	0.06
Wbc	6.03	5.53	0.031*	7.18	7.06	0.76	5.67	5.65	0.89	7.21	6.93	0.43
Lymphocytes	1.76	1.79	0.81	2.12	2.08	0.71	1.86	1.72	0.26	2.11	2.05	0.51
Monocytes	0.52	0.51	0.97	0.62	0.60	0,67	0.49	0.53	0.26	0.59	0.63	0.23
Neutrophils	3.60	3.05	0.004*	4.27	4.16	0.75	3.13	3.23	0.48	4.33	3.99	0,26
Eosinophils	0.13	0.16	0.11	0.23	0.16	0.09	0.16	0.14	0.18	0.17	0.19	0.23
Basophils	0.004	0.01	<0.0001*	0.04	0.03	0.40	0.02	0.008	0.01*	0.04	0.03	0.56
MLR	0.31	0.3	0.64	0.29	0.29	0.64	0.28	0.32	0.03*	0.28	0.30	0.15
NLR	2.21	1.85	0.02*	2.12	2.16	0.96	1.87	2.01	0.21	1.223	2.06	0.40
PLR	133.4	136.9	0.60	126.3	125.7	0.95	138	134.1	0.47	127	123.3	0.61

Rbc, red blood cell; Hb, hemoglobin; Hct, hematocrit; RET, reticulocyte, Wbc, white blood cell; MLR, monocytes-to-lymphocytes ratio; NLR, neutrophils-to-lymphocytes ratio; PLR, platelets-to-lymphocytes ratio (PLR); * Two-sided Student's t-test (p value < 0.05)

smokers, respectively, when compared with their corresponding young and old non-smokers (Table 1A). In smokers, a variation in Hb and Hct level was found when comparing young and old smokers (Table 1B). A variation in five blood cell biomarkers was seen when comparing young and old non-smokers (Table 1B).

Plasma C-reactive protein (CRP), platelet-derived growth factor (PDGF), cytokines and chemokines

Among the 11 plasma biomarkers nine were higher in smokers compared to non-smokers (Table 2A). Stratified by gender, seven and six variations in levels of the 11 plasma biomarkers were found in female and male smokers, respectively, when compared with their corresponding female and male non-smokers (Table 2A). No variation in these plasma biomarkers was found between female and male smokers or between female and male non-smokers (Table 2B).

Stratified by age, no variation in these 11 plasma biomarkers was found in young (< 50 years old) smokers, compared with young non-smokers. Highest variation, nine out of these 11 plasma biomarkers were found in old smokers, compared with old non-smokers (Table 2A). Only two plasma biomarkers, PDGF and TNF-α, differed between young and old age in smokers and non-smokers (Table 2B).

Discussion

Increasing life expectancy of the healthy population was reported world-wide.[11, 12] In our investigation, gender (male vs female) and aging (< 50 vs ≥ 50 years) have a marginal influence on the studied plasma protein, cytokine, and chemokine profile of adult healthy non-smokers. Thus, chronological age is not a good indicator of immunological status of individuals with healthy lifestyle.

Smoking cigarettes creates an airborne unhealthy environment with long term toxic and carcinogenic exposure, risk of serious negative health effects and diseases.[13] We have previously shown that cigarette smoke condensate induces massive cell death *in vitro*.[3, 14] Autoantigens and toxic agents released from smoking-induced cell death *in vivo* could provoke a local and systemic host immune response, as detected in this study. We found that total white blood cells and its subpopulations (lymphocytes, monocytes, neutrophils, eosinophils, and basophils) were significantly increased in smokers compared to non-smokers. Thus, dysregulation of systemic immune response occurs in the body of adult healthy smokers. Our assumption is supported by previous investigations.[15, 16] Increased Hct, with no change in circulating Rbc, higher levels of reticuloendothelial cells and platelets in adult healthy smokers suggest smoking influence on bone marrow erythropoiesis capacity and quality.[17, 18]

Table 2A The influence of smoking on circulating plasma profile of adult healthy cigarette smoking (Smokers) and those who never have used any tobacco product (Non-smokers).

Parameters	Non-smokers (n = 222)	Smokers (n = 135)	*p* value	Female			Male			< 50 years			≥ 50 years		
				Non-smokers (n = 104)	Smokers (n = 76)	*p* value	Non-smokers (n = 118)	Smokers (n = 59)	*p* value	Non-smokers (n = 55)	Smokers (n = 30)	*p* value	Non-smokers (n = 167)	Smokers (n = 105)	*p* value
CRP (mg/L)	1.64	2.97	<0.0001*	1.87	2.75	0.019*	1.44	3.26	0.0003*	1.67	2.87	0.081	1.63	3	<0.0001*
IL2 (pg/mL)	17.58	23.56	0.032*	19.26	25.83	0.08	15.46	19.60	0.32	19.37	17.01	0.92	17	25	0.618
IL6 (pg/mL)	20.80	38.55	0.0003*	21.84	39.55	0.003*	19.48	36.02	0.045	24.03	22.04	0.51	20	43	0.73
IL10 (pg/mL)	17.51	14.55	0.631	22.63	16.23	0.53	11.02	11.61	0.83	14.5	15	0.77	18	14	<0.0001*
IL12 (pg/mL)	38.18	41.29	0.0831	45.93	43.05	0.90	28.35	38.22	0.27	40.54	31.33	0.75	38	44	<0.0001*
IFN-γ (pg/mL)	119.9	267.3	<0.0001*	111	280	<0.0001*	130.7	245.5	0.029*	163	180	0.095	109	289	<0.0001*
MCP-1 (pg/mL)	119.8	182.6	<0.0001*	125.7	193.8	0.0004*	112.3	163.2	0.052	149	158	0.96	112	188	0.0074*
PDGF (pg/mL)	738.7	1145.7	<0.0001*	666.1	1049.7	0.0005*	830.7	1312.8	0.0065*	492	717	0.697	798	1252	0.0054*
MIP-1β (pg/mL)	40.16	50.56	0.013*	40.53	51.05	0.036*	39.69	49.71	0.1781	43	44	0.34	40	52	0.0005*
RANTE (pg/mL)	5323.6	6094.4	0.011*	5644.9	6052.8	0.28	4916.6	6166.8	0.011*	4921	5237	0.697	5420.6	6308.7	0.0054*
TNF-α (pg/mL)	89.03	118.9	0.0005*	85.69	112.3	0.011*	93.24	130	0.011*	67	83	0.34	94	128	0.0005*

*Two-sided Student's t-test (p value < 0.05)

Table 2B The influence of age and gender on circulating plasma profile of adult healthy cigarette smoking (Smokers) and those who never have used any tobacco product (Non-smokers).

Parameters	Non-smokers			Smokers			Non-smokers			Smokers		
	Age < 50 (n = 55)	Age ≥ 50 (n = 167)	*p* value	Age < 50 (n = 55)	Age ≥ 50 (n = 167)	*p* value	Female (n = 104)	Male (n = 118)	*p* value	Female (n = 104)	Male (n = 118)	*p* value
CRP (mg/L)	1.67	1.63	0.88	2.87	3	0.83	1.87	1.44	0.08	2.75	3.26	0.45
IL2 (pg/mL)	19.37	17	0.55	17.01	25.19	0.06	19.26	15.46	0.2	25.83	19.60	0.25
IL6 (pg/mL)	24.03	20	0.44	22.04	42.67	0.012*	21.84	19.48	0.58	39.55	36.02	0.73
IL10 (pg/mL)	14.5	18	0.54	14.96	14.44	0.87	22.63	11.02	0.18	16.23	11.61	0.09
IL12 (pg/mL)	40.54	38	0.84	31.33	43.77	0.16	45.93	28.35	0.39	43.05	38.22	0.64
IFN-γ (pg/mL)	163	109	0.17	179.6	289.2	0.051	111	130.7	0.46	280	245.5	0.63
MCP-1 (pg/mL)	149	112	0.05	158.1	188.8	0.27	125.7	112.3	0.38	193.8	163.2	0.34
PDGF (pg/mL)	492	798	0.0005*	717.5	1252.7	0.004*	666.1	830.7	0.08	1049.7	1312.8	0.19
MIP-1β (pg/mL)	43	39	0.5	43.5	52.3	0.39	40.53	39.69	0.85	51.05	49.71	0.87
RANTES (pg/mL)	4921	5420	0.35	5237.3	6308.7	0.07	5644.9	4916.6	0.86	6052.8	6166.8	0.82
TNF-α (pg/mL)	67	94	0.008*	82.69	128	0.005*	85.69	93.24	0.87	112.3	130	0.31

* Two-sided Student´s t-test (*p* value < 0.05)

Cytokines are small proteins which are produced from a variety of cells (lymphocytes, macrophages, NK cells, mast cells, and stromal cells) to regulate host responses to infection, immune responses, inflammation, and trauma, such as Interleukin (IL)-6, tumor necrosis factor alpha (TNFa), Interferon (IFN)-g.[19-20] The release of pro-inflammatory cytokines activates immune cells and production of further cytokines, which serve as biomarkers for many diseases.[21] Smoking influence systemic inflammation as indicated by higher level of circulating lymphocytes, monocytes, neutrophils, CRP, IFN-γ and TNF-α of adult healthy smokers.[22] Our current study shows that CRP and pro-inflammatory mediators including of IL-6, IFN-γ and TNF-α in smokers were significantly higher than non-smokers. This finding was supported by previous reports showing that the increased expression of inflammatory analytes (CRP, TNF-α, IL-1β, and IL-17) and chemokines including of MCP-1 and RANTES were observed in smokers.[23-24] Increased levels of plasma IL-6, IFN-γ, PDGF, MCP-1, MIP-1β and RANTES suggests high angiogenesis, cell migrations and inflammation in healthy smokers. This might associate to poor clinical outcome of the cancer patients with smoking habits.[25-27]

This study showed that platelet counts were higher in smokers, compared with non-smokers ($p < 0.0001$). The increase in the platelet count in smokers, reported by Pujani *et al.*, is consistent with our finding.[28] Previous research demonstrated that smoker group had NLR while PLR was significantly higher for the non-smoker group.[29-30] In this study, we found that MLR, NLR, and PLR were not significantly different between smokers and non-smokers. Dysregulated levels of circulating blood lymphocytes or monocytes and its subpopulations indicates impaired homeostasis in adult healthy smokers.

A recent study found that smoking prevalence was the lowest among East Asians (22.7%) and the highest among non-Hispanic whites (38.5%) and there are significant associations between smoking-related traits and genetic ancestry.[31] In this study, we investigated the influence of cigarette smoking on blood cell subpopulations, cytokines, and chemokines in Swedish smokers. However, there are no studies have examined the effect of cigarette smoking on circulating blood cells, cytokines, and chemokines based on ethnicity and genetic ancestry. Further studies examining effects of smoking in different racial/ethnic will be very interesting.

Conclusion

Without the unhealthy airborne environment created by cigarette smoke, gender and aging have a marginal influence on blood-based immune response profile of healthy individuals. Adult healthy smokers dysregulated the levels of various inflammatory blood cells and plasma biomarkers. The influence of

cigarette smoking might increase with time since marginal influences were detected between young healthy smokers compared with their corresponding young healthy non-smokers. Long-term exposure to cigarette smoke might have pronounced effects on blood cells and plasma biomarkers as indicated in old healthy smokers compared either with young healthy smokers or with their corresponding old healthy non-smokers. The intensity and duration time of these systemic pathological immune responses of airborne toxic and carcinogenic substances such as cigarette smoking, contribute to various diseases need further investigation.

Conflict of interest

The authors declair no potential conflict of interests.

Ethic approval

The study was conducted in accordance with the Declaration of Helsinki. Ethical Board at Linköping University approved this investigation (Dnr 2015/178-32).

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Author contributions

NL, SL, MN and FL conceived and designed the study and NL draft the original manuscript. TL and LS administrated, performed experiments and analyzed data. All authors reviewed the data and edited the manuscript.

Availability of data and materials

To protect the confidentiality of the study participants, raw data are available from author (NL) on reasonable request.

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