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Correlation between nicotine dependence and inflammatory biomarkers in Thai smokers: eight weeks of synbiotic intervention

Ekasit Lalitsuradej[1,2], Parama Pratummas[1], Phakkharawat Sittiprapaporn[1], Chaiyavat Chaiyasut[2]
[1]Neuropsychological Research Laboratory, Department of Anti-Aging and Regenerative Science, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand
[2]Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand

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ABSTRACT

Cigarette smoke contains thousands of man-made substances, and many may contribute to addiction and inflammation. This study examined the effect of synbiotics on the Fagerstrom test for nicotine dependence (FTND) and inflammatory markers in Thai smokers. 14 smokers with a Nicotine Dependence Fagerstrom Test scores of 4 or higher and no pregnancy or lactation history participated in this study. We gave them surveys about the FTND and continued blood tests for Lipopolysaccharide (LPS), lactulose and mannitol ratios (LMR), Quinolinic acid (QA), and 5-hydroindoleacetic acid (5-HIAA) to record inflammatory marker levels and leaky gut information. Pearson's R-values for LPS and LMR were 0.444 and -0.465. FTND showed a positive correlation with LPS and a negative correlation with leaky gut, but both relationships were weak due to no correlation for LPS but leaky gut. The R^2 of the LPS correlation coefficient was 0.197, p = 0.112, and the R^2 of the leaky gut correlation was 0.217, p 0.001. FTND, LMR, and QA were significantly reduced, while 5-HIAA was elevated. Further investigation is needed to determine the association between smoking and inflammation. In conclusion, synbiotics improved FTND, gut permeability, and inflammation.

Corresponding Author:

Phakkharawat Sittiprapaporn
Neuropsychological Research Laboratory, Department of Anti-Aging and Regenerative Science, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University
36/87-88 P.S. Tower, 25th Floor, Asoke Montri Road, Khlongtoey Nua, Wattana, Bangkok 10110, Thailand
Email: wichian.sit@mfu.ac.th

1. INTRODUCTION

Bioinformatics originated over fifty years ago when deoxyribonucleic acid (DNA) had not yet been sequenced. The primary bioinformatics was peptide sequencing, therefore, developing DNA sequences according to the development of computer technology. A flood of massive data and the rapid proliferation of bioinformatics tools led to the era of big data mining. Today, bioinformatics is tasked with a variety of tasks, including dealing with massive volumes of data, ensuring the duplicability of outcomes, and ensuring proper integration into academic programs [1]. Cigarette smokers are found to provide more bioinformatics data that is utilized for health promotion. Smoking is well-known to include various cancer-induced chemicals, as well as an elevated risk of cardiovascular and chronic obstructive pulmonary disease death (COPD) [2]. Tobacco smoke is made up of hundreds of synthetic compounds that are released when tobacco is chewed or warmed.

Nicotine, carbon monoxide, reactive oxidant substances (ROS), and acrolein are all components of cigarettes that cause this impact. Amongst these substances, nicotine is the most addictive component of cigarette smoking, either directly or indirectly. The rest are more common tobacco smoke toxins with immunomodulatory properties [3]. Nicotine has been suggested to cause cravings and withdrawal symptoms in both humans and animals. This activity of nicotine mostly shows up through explicit nicotinic acetylcholine receptors situated in the mind. It invigorates presynaptic acetylcholine receptors, accordingly, stimulating Ach delivery and digestion. A dopaminergic framework is likewise invigorated by it, expanding the convergence of dopamine in the nuclear accumbens, which enhances behavior change and nicotine dependence. Various researchers have suggested that non-dopaminergic pathways are involved in the rewarding effect of nicotinic abolition [4].

A large-scale genome-wide association study has demonstrated nicotine dependence and never-smoking behavior. This study exhibited the 18 sites of insistent and never-smokers in European Americans, one site in African Americans, and one site in Hispanic Americans, respectively. This massive Genome-wide association studies (GWAS) data of the smoking phenotype in many communities improves understanding of smoking behavior genetic vulnerability [5]. In addition, six genome loci have been identified as the nicotine resilience, such as CHRNB3-CHRNA6 (chr8p11), DBH (chr9q34), CHRNA5-CHRNA3-CHRNB4 (chr15q25), DNMT3B and NOL4L (chr20q11), and CHRNA4 (chr20q11). A better understanding of this genetic activity encourages prediction of the trend of quitting smoking, the harshness of withdrawal treatment response and well-being-related outcomes [6]. The understanding of smoking behavior and the severity of nicotine dependence play a critical role in quitting smoking. The evaluation tool that is widely used, like the nicotine dependence Fagerstrom test, is non-obstructive and friendly for self-report, giving us a frame of nicotine reliance through the physiological and behavioral effects [7]. This tool delivers more of the actual nicotine perceptive rather than a personality trait. Besides, the additional questions have been designed to investigate various aspects of smoking behavior like desire and withdrawal/impulse, the frequency of smoking, the reduction and ceasing of aspiration, automaticity, and mindfulness in smoking, which are the identification criteria for tobacco dependence diagnosis [8]. In addition, GWAS reveals the relationship with smoking weight, lung cancer, and COPD. According to GWAS findings, the 5 subunits of the neuronal nicotinic acetylcholine receptor (CHRNA5) support the prediction of smoking weight and the final time to quit smoking. In severe cigarette users, smoking-related illnesses are associated with cytochrome P450 2A6 (CYP2A6) activity on nicotine metabolites that cause hypertension and lung cancer. To govern the outcome of this expression, minimizing smoking is recommended [9]. The expression of CHRNA5 induces an inflammation process in smokers. The leverage of inflammation modifies genetic information in both DNA and Ribonucleic acid (RNA), protein structure and abilities, changing various mechanisms of action, impacting cells and organs. The phenotypic disparity within inflammation can be clarified by epigenetic alterations [10]. Smoking impacts various natural inflammatory mediators by affecting insensitive burning cells, resulting in immunosuppression. According to the latest research, the NF-kB family is primarily responsible for regulating inflammation generated by smoking by beginning a subordinate and autonomous IκB kinase (IKK) pathway. Several transcription factors, including PAX5 and TGF-β-Smad, were also involved in the release of NF-kB [11]–[13].

Elevation of systemic inflammation also impacts gut microbiota homeostasis, regulating physical and psychological well-being. It is reported that the high prevalence of proinflammatory-induced gut microbiota family *Erysipelotrichaceae* has been observed in smokers. Likely, this strain is also a proinflammatory inducer in dyslipidemic phenotype. Genus *Slackia* is also proven on positive correlation with the numbers of cigarettes consumption and concerns to lipid and xenobiotics metabolism, proinflammation, and individual prediabetes promotor [14]. The imbalance of gut microbial homeostasis, called gut dysbiosis, generates various proinflammatory markers. The elevation of proinflammatory-induced microorganisms activates endotoxin-lipopolysaccharide (LPS) secretion in the peripheral. LPS is a protein present on the outer surface of gram-negative bacteria. Generally, its target is TLR-4, which delivers the action on different receptors [15]–[17]. Human immune cells recognize extracellular LPS through the Toll-like receptor-4 (TLR-4). It is unclear whether the extra sensors are involved in recognizing lipopolysaccharides in the cytoplasm. TLR-4 activation by LPS needs several downstream linkers. This is the mechanism through which receptors are tagged [17]–[19]. The registration of these linkers can lead to the development of an inflammatory pathway, essentially resulting in the induction of a large number of proinflammatory genes [20], [21]. According to this knowledge, the recovery of gut symbiosis and intestinal permeability seems to modulate the effects of smoking. However, the studies on gut microbiome manipulation in smokers are still limited. Consequently, this investigation aims to propose a connection between the effect of synbiotics on gut homeostasis modulation, nicotine reliance as measured by the nicotine dependence Fagerstrom test, and inflammation as stated in Thai smokers in the bioinformatics way.

2. RESEARCH METHODOLOGY

2.1. Study design

The present study used synbiotics to investigate the network connection between gut and brain in the smoker. This study has been designed as an eight-week experimental study and is being carried out under the guidelines of Mae Fah Luang University's Research Ethics Committee. All volunteers were enrolled and screened by the nicotine dependence Fagerstrom test scores. The primary measurement was nicotine dependence data collected by the nicotine dependence Fagerstrom test. The secondary measurements were inflammation, neuronal chemokines, and gut permeability data collected by blood and first-morning urine. All data was collected at the start of the trial and at the end of the eighth week. Participants in this study were adult male or female smokers between 18 and 70 years of age who lived in Mahasarakham Province, Thailand. All participants were male or female who smoked for 1 to 5 years and scored the nicotine dependence Fagerstrom test ≥ 4 as well as non-pregnancy or breastfeeding.

2.2. Synbiotic intervention

The synbiotics preparation comprised 25 x 10^9 CFU of 7 probiotics strains (5 x 10^9 CFU of *Lactobacillus rhamnosus,* 3 x 10^9 CFU of *L. paracasei,* 0.5 x 10^9 CFU of *L. reuteri,* 1 x 10^9 CFU of *L. salivarius,* 8.5 x 10^9 CFU of *Bifidobacterium lactis,* 5 x 10^9 CFU of *B. breve,* and 2 x 10^9 CFU of *B. longum*) and 8.5 grams of prebiotics (4 g. inulin, 2 g. of galacto-oligosaccharide (GOS) and 2 g. of oligofructose (FOS). Lactomason Co., Ltd., Gyeongsangnam-do, South Korea, provided the probiotic strains in the preparation, which were packaged with prebiotics in an aluminum foil sachet. The synbiotics were recommended to be consumed before breakfast for eight weeks.

2.3. Data acquisition and determination

2.3.1. Nicotine dependence Fagerstrom test

We initially appointed participants and explained the objectives and procedures. After the participant answered the informed consent, they provided the individual nicotine dependence Fagerstrom test. Six questions make up the nicotine dependence Fagerstrom test. The following are the results of the scoring: (a) how quickly do you light up your first cigarette after waking? [Within the first five minutes (3 points), between six and thirty minutes (2 points), between thirty-one and sixty minutes (one point), after sixty minutes (zero point)]; (b) are you having difficulty abstaining from smoking in areas where smoking is prohibited? [Yes (one point), no (zero point)]; (c) which cigarette would you be most resentful of giving up? [The first morning (one point), the second (zero point)]; (d) how many cigarettes do you smoke each day? [ten (zero points), ten to twenty (one point), twenty-one to thirty (two points), thirty-one to three or more (three points)]; (e) are you more likely to smoke in the morning? [Yes (one point), no (zero point)]; (f) even when you're unwell and confined to your bed, do you still smoke? [Yes (one point), no (zero point)], respectively. The details of questions and scores are presented in Table 1. These six questions should be scored in the range from 0 to 10. The higher the score, the more ingrained smoking dependence [22].

Table 1. Nicotine dependence Fagerstrom test

Questions	Answers	Points
How quickly do you light up your first cigarette after waking?	In the first five minutes	3
	Approximately 6 and 30 minutes	2
	Approximately 31 and 60 minutes	1
	Following 60 minutes	0
Are you having difficulty abstaining from smoking in areas where smoking is prohibited?	Yes	1
	No	0
Which cigarette would you hate to give up?	the morning's first	1
	Another	0
How many smokes do you consume on a daily basis?	fewer than 10	0
	10 to 20	1
	21 to 30	2
	greater than 31	3
Are you have a higher proclivity to smoke in the morning?	Yes	1
	No	0
Even when you're unwell and confined to your bed, do you still smoke?	Yes	1
	No	0

2.3.2. Leaky gut lipopolysaccharide assessment and determination

Lactulose and mannitol ratios (LMR) were used for the assessment. All participants received 5 g of mannitol and 10 g of lactulose dissolved with 150 ml of water after fasting for 8 hours. Before analysis, urine

was collected with no fasting (except receiving water) for 6 hours and stored at 4 ºC. The samples were aliquoted in microtubes and stored at -80 ºC before calculation. The Enzy™ Chrome Intestinal Permeability Assay kit (Universal Biological Ltd., Cambridge, UK) was used for saccharide determination. The protocol was followed as per the manufacturer's instructions. For Lipopolysaccharide, the serum sample was collected to determine LPS. The human LPS ELISA Kit (MyBioSource®, San Diego, CA, USA) was used for the analysis, and the manufacturer's instructions were followed.

2.3.4. Tryptophan metabolites determination

The determination of tryptophan metabolites was made from first morning urine samples. For Quinolinic acid (QA) testing, a Human Quinolinic Acid ELISA kit (Fivephoton BiochemicalsTM, San Diego, CA, USA) was chosen. 5-hydroindoleacetic acid (5-HIAA) was measured using the 5-HIAA-ELISA-Kit-Urine-Fast-Track (Immusmol, Bor-deaux, France). The manufacturer's instructions were used to determine the procedure.

2.4. Data analysis

To review and analyze the results and determine the strength of their link, the mean, standard deviation, and Pearson correlation were employed in the descriptive analysis. Before and after the intervention, a paired t-test or Wilcoxon signed-rank test was used to determine the results. Statistically significant was considered as a *p*-value less than 0.05.

3. RESULTS AND DISCUSSIONS

3.1. Demographic information of the participants

Demographic data has been displayed in Table 2. The participants in this study comprised 13 (92.9%) males and 1 (7.1%) female. This group was mostly aged 20–59 years. The average age was 40.57 ± 13.32 years. Half of the participants were single, and the rest were married.

Table 2. Demographic information

Characteristics	Number (Percentage)	Mean ± SD
Gender		
Male	13 (92.9)	-
Female	1 (7.1)	-
Age		
Under 20	1 (7.1)	
20-39	5 (35.7)	40.57 ± 13.32
40-59	6 (42.9)	
60 and above	2 (14.3)	

3.2. Effect on nicotine dependence Fagerstrom test

In this study, it was found that twelve people had eight scores (85.7%) in the nicotine dependence Fagerstrom test, whereas two people scored nine (14.3%). The mean score was 8.14 ± 0.35, as shown in Table 3. Fagerstrom's synbiotic intervention reduced nicotine dependence. The test scores went from 8.08 ± 0.49 at baseline to 7.23 ± 0.93 at 8th weeks (p = 0.001) of intervention as shown in Figure 1.

Table 3. Nicotine dependence Fagerstrom test results

Scores	Number (Percentage)	Mean ± SD
4	-	8.14 ± 0.35
5	-	
6	-	
7	-	
8	12 (85.7)	
9	2 (14.3)	
10	-	

3.3. Leaky gut assessment (LMR) and LPS level

At the end of the study, synbiotics modulated LMR from 0.17 ± 0.14 to 0.08 ± 0.04 (p = 0.017) as shown in Figure 2. The effects of lipopolysaccharide levels in plasma were estimated to be around 42.9% in the range of 10 to 19 pg/mL, and 35.7% and 14.3% in the ranges of 20 to 29 pg/mL and 30 to 39 pg/mL, respectively. Only one person had a lipopolysaccharide level of 40 or higher, accounting for 7.1% of all subjects. The mean LPS concentration was 25.48 ± 12.36 pg/mL as displayed in Table 4.

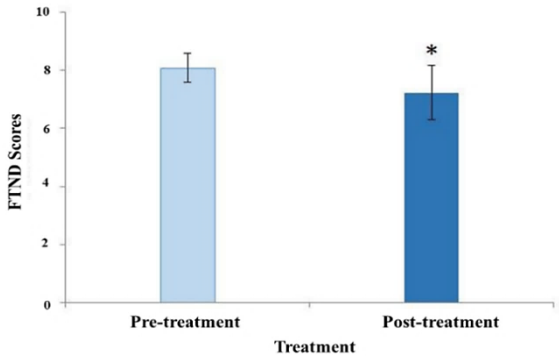

Figure 1. The modification of the nicotine dependence Fagerstrom test scores at the end of the study; p = 0.001.
* Statistically significant distinction (p < 0.05).

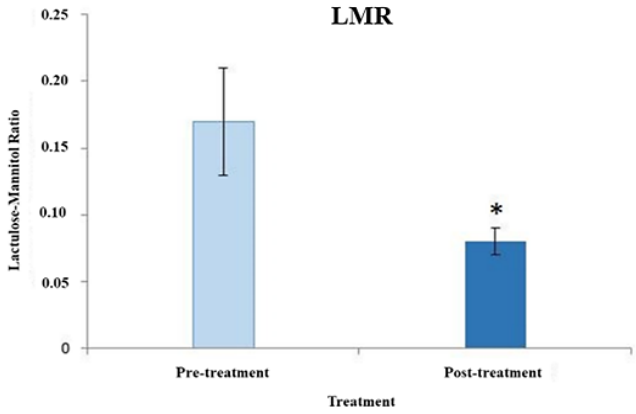

Figure 2. The modulation of LMR at the end of the study; p = 0.017.
* Significant difference (p < 0.05).

Table 4. The performance of LPS level

LPS level (pg/mL)	Number (Percentage)	Mean ± SD
Under 10	-	
10-19	6 (42.9)	
20-29	5 (35.7)	25.48 ± 12.36
30-39	2 (14.3)	
40 and above	1 (7.1)	

3.4. Effects on tryptophan metabolites

The effect had been investigated in two routes. The neurotoxic pathway presented by QA was modulated from 8.84 ± 3.34 ng/mL at baseline to 6.79 ± 1.25 ng/mL after eight weeks (p = 0.025) of intervention. In comparison, neuroprotective pathway presented by 5-HIAA was modified 1,970.27 ± 1463.93 ng/mL before intervention to 4,190.59 ± 3,144.60 ng/mL (p = 0.006) at the post invention as Figure 3.

3.5. Correlation between the nicotine dependence Fagerstrom test and LMR level

The correlation between the nicotine dependence Fagerstrom test and LMR was determined by Pearson correlation coefficient (R), which determined the linear link between two variables from 1 to -1. The correlation coefficient of 1 denoted perfect positive correlation and -1 denoted an utterly negative correlation. The following formula denotes Pearson's correlation coefficient:

$$R = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2}\sqrt{\sum(y-\bar{y})^2}}$$

where;

x = nicotine dependence Fagerstrom test

y = LMR level

$\bar{x}$ = mean of the nicotine dependence Fagerstrom test

$\bar{y}$ = mean of LMR level

therefore;

The outcome displays in Table 5.

$$R = \frac{-0.153}{\sqrt{(1.714)}\sqrt{(0.625)}}$$

$$R = -0.465$$

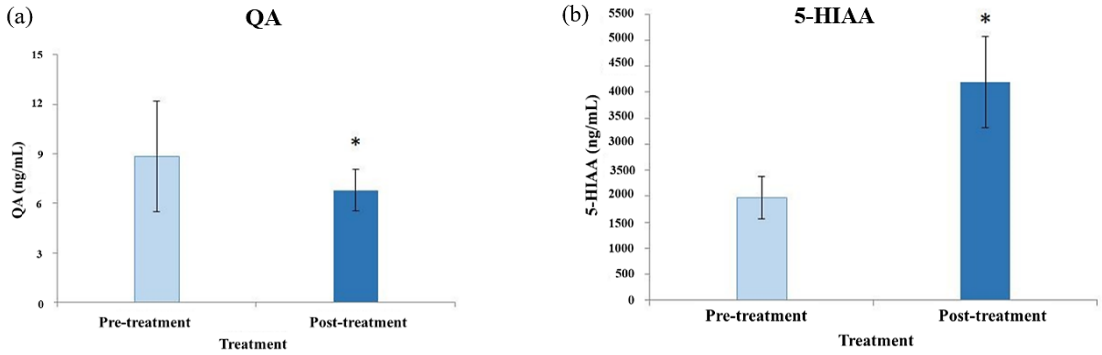

Figure 3. Effect of synbiotics on mean tryptophan after eight weeks of intervention. *Significant difference between samples ($p < 0.05$). (a) Quinolinic acid (QA); $p = 0.025$. (b) 5-hydroindoleacetic acid (5-HIAA); $p = 0.006$

Table 5. Pearson correlation coefficient (R) between the nicotine dependence Fagerstrom test and LMR level

x	y	$x-\bar{x}$	$y-\bar{y}$	$(x-\bar{x})(y-\bar{y})$	$(x-\bar{x})^2$	$(y-\bar{y})^2$
8	16.008	-0.143	-0.100	0.014	0.020	0.010
9	60.499	0.857	0.014	0.012	0.735	0.000
8	18.782	-0.143	0.354	-0.051	0.020	0.125
8	26.337	-0.143	0.099	-0.014	0.020	0.010
8	26.885	-0.143	0.325	-0.046	0.020	0.105
8	27.849	-0.143	0.138	-0.020	0.020	0.019
8	10.747	-0.143	0.481	-0.069	0.020	0.231
8	35.343	-0.143	0.079	-0.011	0.020	0.006
8	36.842	-0.143	0.123	-0.018	0.020	0.015
9	17.325	0.857	0.133	0.114	0.735	0.018
8	11.391	-0.143	0.255	-0.036	0.020	0.065
8	27.16	-0.143	0.105	-0.015	0.020	0.011
8	18.251	-0.143	0.094	-0.013	0.020	0.009
8	23.272	-0.143	-0.100	0.014	0.020	0.010
$\bar{x}$ = 8.143		$\bar{y}$ = 0.17				
		$\sum$		-0.153	1.714	0.625

The Pearson relationship had a value of -0.465 for R. Even though there was a positive association, there is a modest link between the nicotine dependence Fagerstrom test and LMR levels. Additionally, the coefficient of determination (R^2) was estimated to be 0.217 ($p \leq 0.001$). As a result, there was no link between the nicotine dependence Fagerstrom test and LMR levels as shown in Figure 4.

3.6. Correlation between the nicotine dependence Fagerstrom test and LPS level

Likewise, Pearson correlation coefficient (R) for measuring the relationship between the nicotine dependence Fagerstrom test and LPS, where all variables are replaced as,

where;

x = nicotine dependence Fagerstrom test

y = LPS level

$\bar{x}$ = mean of the nicotine dependence Fagerstrom test

$\bar{y}$ = mean of LPS level
therefore;
The outcome displays in Table 6.

$$R = \frac{26.868}{\sqrt{(1.714)}\sqrt{(2137.628)}}$$

$$R = 0.444$$

The Pearson relationship R-value was 0.444. The association between the nicotine dependence Fagerstrom test and the lipopolysaccharide level was minimal, even though positive. Furthermore, R^2, the coefficient of determination, was estimated to be 0.197 (p = 0.1119). There was no longer any link between the nicotine dependence Fagerstrom test and lipopolysaccharide levels. This relationship is shown in Figure 5.

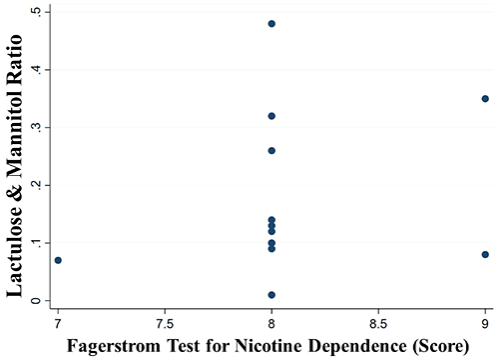

Figure 4. Correlation between the nicotine dependence Fagerstrom test and LMR level

Table 6. Pearson correlation coefficient (R) between the nicotine dependence Fagerstrom test and LPS level

x	y	$x-\bar{x}$	$y-\bar{y}$	$(x-\bar{x})(y-\bar{y})$	$(x-\bar{x})^2$	$(y-\bar{y})^2$
8	16.008	-0.143	-9.470	1.353	0.020	89.680
9	60.499	0.857	35.021	30.018	0.735	1226.48
8	18.782	-0.143	-6.696	0.957	0.020	44.835
8	26.337	-0.143	0.859	-0.123	0.020	0.738
8	26.885	-0.143	1.407	-0.201	0.020	1.980
8	27.849	-0.143	2.371	-0.339	0.020	5.622
8	10.747	-0.143	-14.731	2.104	0.020	217.000
8	35.343	-0.143	9.865	-1.409	0.020	97.320
8	36.842	-0.143	11.364	-1.623	0.020	129.142
9	17.325	0.857	-8.153	-6.988	0.735	66.470
8	11.391	-0.143	-14.087	2.012	0.020	198.442
8	27.16	-0.143	1.682	-0.240	0.020	2.829
8	18.251	-0.143	-7.227	1.032	0.020	52.228
8	23.272	-0.143	-2.206	0.315	0.020	4.866
$\bar{x}$ = 8.143			$\bar{y}$ = 25.478			
		$\sum$		26.868	1.714	2137.628

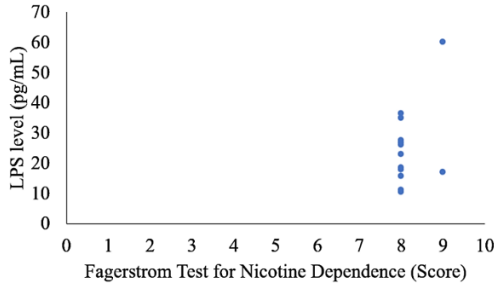

Figure 5. Correlation between the nicotine dependence Fagerstrom test and LPS level

Genetic assessment in human is the most reliable way to distinguish between many genetic variations that linked to the nicotine addiction trend and the dangers of tobacco use. Additionally, various biomarkers, for instance, pharmacology and behavior, that may be examined using biological samples generate the precision for medicinal and individualized treatment [23]. In cigarette smokers, nicotine is classified as a substance-induced/related addition. The gut microbiome-HPA-axis is proposed as a novel therapeutic target, delivering utility bioinformatics on additive manipulation and cessation [24]. Nicotine is the pivotal toxic compound related to craving, addiction behavior, and CYP450 gene expression. Approximately 90% of nicotine is metabolized through CYP2A4, which is correlated with smoking behavior, nicotine dependence, cessation, and lung cancer. CYP2A4 variants are associated with greater or lower mRNA expression and enzyme activity. The prevalence of nicotine craving directly relates to CYP2A4 expression assessed with the nicotine dependence Fagerstrom test. However, the relation between the scores of the nicotine dependence Fagerstrom test and CYP2A4 activity is still controversial. Multiple studies have led us to believe that the intensity of smoking may be a more crucial influencer than the number of cigarettes per day (CPD). In comparison, the nicotine dependence Fagerstrom test evaluates the number of cigarettes rather than smoking intensity. Interestingly, smokers who have a CYP2A4 gene deletion show the advantage of fewer CPD. This is recent knowledge, showing that CYP2A4 genetic variation determines expression level [25]. Concerning CYP2A4 expression, gut microbiota homeostasis cannot be neglected. Principally, the activity of gut microbes' influences gene expression through postbiotics. The understanding is elucidated in the mouse model. The study has been conducted with germ-free mice, colonized GF, and conventional mice. The colonized GF exhibited better CYP2A4 expression than GF mice after prebiotic treatment for 14 weeks. Likewise, better CYP2A4 gene expression in the colonized GF mice was observed [26]. The further investigation in this study was focused on butyrate intervention.

More knowledge of smoking behavior requires phenotypic analysis and genetic considerations to complete the picture of addiction and cessation. CHRNA5/CHRNA3 mutations can interfere with the behavior and hazards of smoking by regulating the receptor response to nicotine. However, the rs16969968 mutation in CHRNA5 is linked to the severity of tobacco-related illnesses. It solves a variety of genetic risks problems. The CHRNA5 gene on chromosome 15 plays a crucial genetic link to cigarette smoking. Various polymorphisms in this region are a correlation on risk of heavy smoking elevation. Moreover, protein containing 5 nicotine acetylcholine receptor subunits is linked to the risk of excessive smoking and completed regulation of protein quality expression [27]. The genotype variation displayed the severity of smoking behavior and side effect. It is suggested that the variants of CHRNA5 rs16969968 exhibited a greater risk of smoking in women. While CHRNA3 rs578776 genotype exhibits lower risk for smoking in general population and female. The results are highly influenced by sexual orientation and the likelihood polymorphic variation homozygosity [28]. The investigation by Lestrat *et al.* has suggested that there are four SNPs of CHRNA5/A3/B4 acetylcholine receptor gene linked to nicotine addiction. However, the investigation of French students suggested a new finding. The nicotine resilience has a correlation with genome rs637137, rs3813567, and rs16969968 dependent "AGG" haplotypes). This evidence is indicated that acetylcholine receptor gene cluster CHRNA5/A3/B4 shows the unique marker in the young population and useful in the treatment of nicotine addicts [29].

Synaptic receptor subunits related to numerous cycles, such as cholinergic autonomic nerve activity and inflammation, are encoded by CHRNA5 quality. Excitation of the vagus nerve (VN) in the lung model causes cell proliferation in lung cancer via nicotine acetylcholine receptors (nAChRs). The proinflammatory-induced microorganisms play this role by activating TLR-4 expression and signaling VN to mediate inflammation through bacterium products [30], [31]. Nicotine and acetylcholine activate nAChR are found in neurons and non-neuronal cells. It's worth noting that 5 nAChR and 7 nAChR are the two primary subunits in heart tissue and lung epithelial cells with the most mRNA and protein linkages. The majority of 7 receptors are homopentamers, critical regulators of the anti-inflammatory pathway induced by VN intervention. The downregulation of 5 nAChR pathway induces the 7 nAChR activity, and the 5 receptor forms heteropentamers with 7 nAChR and other subunits. Interestingly, the variants of CHRNA5, coding for the quality of nAChR 5, are correlated to the use of cigarette and other degenerative disease risks, including diabetes, COPD, atherosclerotic peripheral vascular disease (PVD), and lung cancer, respectively [32]. According to the recent GWAS, there are 45 susceptible sites are indicated the correlation with lung cancer. Numerous investigation of single nucleotide polymorphisms (SNPs) displayed a reliable outcome on human leukocyte antigen (HLA) areas, TERT, CHRNA5, and CHRNA3 [33]. The hyperexpression of these SNPs elevates cancer-related inflammation. The proven evidence has been conducted by Dutkowska *et al.* [34] showing the effect of high mRNA expression after the activation inducing miR-122, miR-9 and interleukins IL-17, IL-6, IL-1 in the non-small cell lung cancer (NSCLC). This evidence guided us that inflammatory elevation by smoking inducing the interleukin expression which promotes carcinogenesis [34]. The activation of CHRNA5 expression stimulates the inflammation process. Therefore, this activity induces the alteration of tryptophan metabolism. Basically, tryptophan can be metabolized into three pathways: kynurenine,

serotonergic, and indole pathways. Among these routes, kynurenine and indoles correspond to various neuropsychiatric disorders. Focusing on the kynurenine pathway, kynurenic acid (KA) is the crucial metabolite in this pathway and is recognized to be an α7nAChR antagonist. The expression of the kynurenine 3-monooxygenase enzyme by encoding the kynurenine 3-monooxygenase (KMO) gene has an inverse correlation with kynurenic acid, which is a pivotal substance on smoking dependence treatment. The modulating KMO gene expression is suggested to be the tactic of tobacco initiation or dependence [35].

Smoking increases transcription of the NF-kB signaling pathway with gut barrier disruption. The intestinal epithelial dysfunction promotes LPS translocation to perform endotoxemia that is linked to metabolic diseases, autism, Alzheimer's disease, asthma, and several autoimmune diseases [36]. LPS translocation is also linked to COPD and lung cancer [37]. Moreover, smoking also alters the balance of commensal and pathogenic microorganisms. It is known that the gut microbiome regulates epigenomes that influence host metabolism. *Lactobacillus* and *Bifidobacterium* modulate the epigenome by engaging a one-carbon donor for DNA methylation. Postbiotics like SCFAs butyrate enhance DNA methylation by enhancing phosphorylation of ERK MAP (kinase 1). Histone methylation, which correlates with obesity and dyslipidemia, is also regulated by gut microbial metabolites. Likewise, histone deacetylase (HDAC) is regulated by butyrate and propionate. The high activity of histone deacetylases (HDACs) is associated with neurological and inflammatory diseases [38]. Intestinal permeability stability correlates with chronic inflammatory disease. The connection between intestinal epithelium and submucosa traffic is also called a tight junction (TJ). TJ comprises a complex of more than 150 proteins, such as occludin, zonulin, and claudin. Among these proteins, zonulin pathways play a critical role in permeability regulation. TJ: Immunoglobulins and LPS can trigger TJ. Gut hyperpermeability contributes to a variety of chronic inflammatory diseases and impairs gut metabolism [39]. The standard assessment of the gut barrier is measured by the ability of sugar absorption and excretion. In the validation study, four saccharides (mannitol, rhamnose, lactulose, and sucralose) were measured in excreted urine at 0–2, 2–8, 8–24, and 0–24 hours. The results exhibited high excretion. After the oral had been investigated by urine excretion in healthy subjects, Mannitol displayed the highest excretion, whereas lactulose showed the lowest in all groups. Lactulose and mannitol are used for leaky gut assessment. Low lactulose is found in the urine in healthy gut permeability, whereas mannitol displays high excretion. Lactulose: mannitol ratios (LMR) are recommended as a potent gut permeability index [40]. At the end of this study, synbiotic intervention attenuated the LMR ratio. This finding demonstrated the benefit of synbiotics on gut permeability regulation. In addition, LMR negatively correlates with Fagerstrom test for nicotine dependence (FTND) as a weak correlation. The result was implied: leaky gut correlates with nicotine dependence. The better leaky gut manipulation, the better regulation of nicotine dependence.

Gut homeostasis is important for regulating neurotransmitter secretion. The enterochromaffin cell (EC) produced a lot of serotonins, which is derived from tryptophan. The release of serotonin helps stabilize gut permeability, gut motility, gut signaling, platelet activation, immune cells, and central serotonin activity. Gut dysbiosis disrupts intestinal serotonin production and distorts tryptophan metabolism. The conversion contributes other metabolites that disturb cognitive function and develop neuropsychiatric disorders. Serotonin converts to 5-Hydroxyacetic acid (5-HIAA) in the brain and is excreted by urine [41]. In this study, synbiotics performed better in terms of LMR, which refers to gut permeability improvement. Moreover, the elevated 5-HIAA and the nicotine dependence Fagerstrom test improvement after the intervention indicate that brain function has shifted to the serotonergic pathway to regulate nicotine activity. On the contrary, tryptophan can be converted to quinolinic acid by the indoleamine-2,3-dioxygenase (IDO) enzyme [42]. This route produces neurotoxin, which is known as the inflammation process. At the end of the study, QA was downregulated, corresponding with better inflammatory regulation.

According to synbiotics, the inflammatory modulation in smokers involves the expression of the gut microbiome on VN. The inflammatory mediation between the gut and brain is governed by VN activity. In this role, the gut microbiome modulates VN to be anti- or proinflammatory. In addition, VN also senses gut microbial metabolites, irritants, gut hormones, and neurotransmitters. Like pathogenic microorganisms, beneficial microflora activates TLR-4, signaling through the cholinergic anti-inflammatory pathway. Then, Ach released at the distal of VN suppresses TNF-α through α7 nAChR [30], [42]. Consequently, various proinflammatory cytokines from CHRNA5 gene expression through VN can be modulated in the same way. VN regulates inflammation by sensing the gut microbial metabolites, irritants and neurotransmitters. The key modulators are diet, nutrition and likewise probiotics. Like pathogenic microorganisms, beneficial microflora activates TLR-4, signaling afferent fiber to downregulate the inflammatory process through α7 nAChR [30], [37]. Smoking tobacco is reported as the gut microbiome alteration promotor. In the human study, the model exhibited the elevation of most gram-negative microbes: Firmicutes and Proteobacteria, whereas decreasing anaerobe microbe Bacteroidetes [43]–[46]. This alteration is associated with LPS high prevalence, increasing TLR-4 mRNA expression, signaling VN through α5 nAChR resulting in proinflammatory mediator secretion

like IL-1β, TNF-α, and IFN-γ. Although LPS has a strong correlation with degenerative diseases, a low level of LPS delivers the neuroprotective effect on microglia [47]. In this study, over half of the participants exhibited LPS lower than 29 pg/ml. The mean was revealed 25.48 with an SD of 12.36 pg/ml. Synbiotics intervention showed a better LPS regulation in smokers. Conversely, the correlation between LPS and the nicotine dependence Fagerstrom test was still weak. Although LPS has a minimal link with the nicotine dependence Fagerstrom test in this study, LPS remains a focus of our investigation due to the treatment unique ability to maintain an optimal level of LPS. If there were any links, it would be utilized as a bioinformatics-based treatment of nicotine dependence. The recent evidence has indicated us that the regulation CHRNA5 provides the benefits on inflammation and nicotine dependence [29]. However, there is a genetic variation concern on CHRNA5 expression between sex and age to investigate. Although there are no absolute treatment approaches for nicotine addiction, a few approaches have been studied. Tolerance to nicotine can be reduced by using receptor-targeting drugs, allowing pharmacotherapeutic and holistic treatment methods to be applied [48].

4. CONCLUSION

The summary results showed that the nicotine dependence Fagerstrom test and LPS had a positive correlation in the parts that were thought not to correlate at all. The findings could impact scientific works in the realm of association as there is no correlation between the nicotine dependence Fagerstrom test with LPS. However, it might associate with other inflammatory markers other than LPS; otherwise, the genetic deviation of race and sex may be of concern. It is advised that additional research be conducted on the novel bioinformatics-based inflammatory indicators. While this primer discovery encompasses a broad spectrum of data, it may not reflect all the relationships between the gut microbiome and smoking-induced inflammation. There was furthermore a limitation in sample size. Further exploration needs to be done on more sample size, gut microbiome diversity and broadened inflammatory markers.

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CONTRIBUTORS

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BIOGRAPHIES OF AUTHORS

Ekasit Lalitsuradej received Bachelor of Pharmacy from Chiang Mai University, Master of Science in Anti-Aging and Regenerative Science, from Mae Fah Luang University. He is currently a Ph.D. Candidate in Pharmacy, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand. His research interests are synbiotics, probiotics, brain health, and anti-aging and regenerative medicine. He can be contacted at email: linekasit@gmail.com.

Parama Prathummas received Bachelor of Allied Health Sciences (Medical Technology) (First Class Hons.), Doctor of Medicine (First Class Hons.) from Chulalongkorn University, Thailand, and Ph.D. in Anti-Aging and Regenerative Medicine, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Thailand. His research interests are aesthetic medicine, anti-aging and regenerative medicine. He can be contacted at email: we_dont_know_for_sure@hotmail.com.

Phakkharawat Sittiprapaporn received Bachelor of Arts (Second Class Hons.) in English from Srinakharinwirot University, Thailand, Master of Arts in Linguistics, Institute of Language and Cultural for Research and Development from Mahidol University, Thailand, and Ph.D. in Neurosciences, Neuro-Behavioural Biology Center, Institute of Science and Technology for Research and Development Mahidol University, Thailand. He is currently a Head of Brain Science and Engineering Innovation Research Group, Mae Fah Luang University, and Neuropsychological Research Laboratory, as well as a lecturer at Department of Anti-Aging and Regenerative Science School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand. His research interests are cognitive psychology, cognitive neurosciences, cerebral mechanisms in perception and cognition, brain mechanism of music and language perception and cognition, and neurobiology of learning and memory. He can be contacted at email: wichian.sit@mfu.ac.th.

Chaiyavat Chaiyasut received Bachelor of Pharmacy from Chiangmai University, Master of Science in Pharmaceutical Sciences from Chulalongkorn University, Thailand and Ph. D. in Applied Chemistry from Nagoya Institute of Technology, Japan. He is lecturer at Department of Pharmaceutical Sciences, as well as a Head of Innovation Center for Holistic Health, Nutraceuticals and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand. His research fields and special interests are probiotics, prebiotics and synbiotics, microbiota on health and beauty, natural products from microorganisms and medicinal plants, fermentation technology. He also researched about bioactive ingredients for cosmeceuticals, holistic health, and the applications. He can be contacted at email: chaiyavat@gmail.com.