Cigarette Smoking Reduces BDNF in Heavy Smokers

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Research article

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Abstract

Background: Cigarette smoke is a powerful environmental modifier of DNA methylation. BDNF is a neurotrophin family of growth factors and is important in growth, progression, regeneration, survival, maintenance, and function of neurons. In this study, we aimed to evaluate the epigenetic changes, serum level, and gene expression of BDNF, in cigarette smokers.

Methods: To assess the BDNF DNA methylation, peripheral blood samples were obtained from 237 cigarette smokers and 90 healthy nonsmokers as controls. DNA methylation was evaluated with MS-PCR. Gene expression and serum level were carried out with qRT-PCR and ELISA assay respectively.

Results: The results from MS-PCR showed that DNA methylation of BDNF gene is more significant in heavy smokers than healthy nonsmokers as control (p-value <0.05). Serum level of BDNF and gene expression is significantly lower in heavy smokers than in healthy nonsmokers (p-value <0.05).

Conclusion: In conclusion, heavy smoking can modify BDNF gene epigenetics. The serum levels of BDNF and the gene expression decreases in heavy smokers compared to healthy nonsmokers.

Background:

Smoking is now considered as a preventable cause of death in the United States. There are 480,000 deaths related to smoking each year, according to the Centers for Disease Control. 8.6 millions of people have at least one serious illness due to smoking. Cigarette smoking is a key risk factor for coronary heart disease and the primary factor in lung cancer [1]. Smoking is a powerful environmental modifier of DNA methylation [2]. Brain-derived neurotrophic factor (BDNF) is important for the maintenance, survival, and regeneration of neurons in the adult brain. BDNF, is a member of the neurotrophin family of growth factors that was discovered in 1982. The BDNF gene in human and rat is located on chromosomes (1q14.1) and (2q3) and has 11 exons and 9 exons, respectively [3, 4]. There are two types of BDNF in the human body: pro-BDNF and mature BDNF. The pro-BDNF 32 KD consists of 247 amino acids that break in the cells and form the mature BDNF that secrets from the neurons [5–8]. BDNF is strongly expressed in the central and peripheral nervous systems. It is one of the most abundant neurotrophins in the brain with high concentrations in the cortex and the hippocampus. BDNF has a role in regulating the secretion of neurotransmitters with a key effect on serotonergic, dopaminergic, glutamatergic, and plasticity mechanisms such as long-term potential and mechanisms in learning and memory [9]. Many studies have shown a close link between BDNF and some diseases like schizophrenia, Alzheimer's disease, mood disorders, and Parkinson's disease [10–14]. Some studies have shown that BDNF probably has a necessary role in neuroimmune regulation of mood disorders. The immune cells and immune factors like cytokines play an essential role in the regulation of BDNF; for example, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) affect the behavior through direct functions in the nervous system. These functions activate inflammatory signal pathways and reduce nerve growth factors like BDNF. On the other hand, the regulation of the BDNF-mediated signaling pathways is required for the

process of the immunomodulatory. The mechanism that how BDNF specifically regulates the neuroimmune axis in mood disorders is unclear [15, 16]. Smith PH, et al. found a high prevalence of smoking among patients with depression, general anxiety disorders, and mental disorders. They found that smoking is more common among individuals with mood, anxiety, and substance use disorders, with the highest rates among those with bipolar and substance use disorders [17]. To note the important function of BDNF in the nervous system, we decided to evaluate the epigenetic changes of the BDNF gene in the smokers' individuals. Also, we compared the serum level of BDNF and the expression of the mRNA, between smokers and nonsmokers' subjects.

Methods:

Blood samples were obtained from 237 cigarette smoker individuals (mean age = 34 ± 12) and 90 healthy nonsmokers as controls (mean age = 36 ± 11), with no history of psychiatric illnesses. Before data collection, all respondents were informed about the aims of the study and data confidentiality and gave written informed consent. All participants took part in the survey voluntarily. Exclusion criteria were as follow: a history of primary neurologic diseases such as Parkinson's disease or depressive disorders, schizophrenia, suicide disorder, Alzheimer's and Huntington disease, alcohol usage. Smoker individuals were classified into three groups according to the Fagerstrom protocol: sever, moderate and low users.

Bisulfite Modification Of Genomic Dna And Methylation Analysis:

Genomic DNA was extracted from whole blood leukocytes according to the protocol described with the (Pars Tous Company, Iran) kit. Briefly extracted DNA samples were treated with sodium bisulfite and then PCR amplified using primers specific for either the methylated and modified unmethylated promoter region of each growth factor genes. The primers are listed in Table 1. In all MSP reactions, DNA from normal leukocytes and universal human methylated DNA standards from Zymo Research (ZYMO Research, Freiburg, Germany) were used as unmethylated (negative) and methylated (positive) controls, respectively.

Methylation Specific Pcr (ms-pcr):

The results from MS-PCR showed that the percentage of the people that their BDNF gene (in exon I, in promoter I (was methylated, was significantly higher in heavy smokers than in healthy nonsmokers, Table 4, (P-value = 0.001). Also, the rate of cigarette dependence directly correlates with the methylation status in the subjects. (Fig. 1).

Figure 1. Correlation of nicotine dependence with BDNF gene methylation status. the percentage of the people that their BDNF gene was methylated, was higher in the heavy smokers > medium smokers > low smokers. ** P-value < 0.001

Quantitative Real-time Polymerase Chain Reaction:

The RT-PCR products were quantified using $2^{-\Delta\Delta ct}$ equation. The threshold cycle of BDNF gene was used to calculate the relative expression of the gene in smokers and control groups. The results showed that the relative expression of BDNF gene in heavy smokers is significantly (p-value < 0.001) lower than the healthy nonsmokers (Fig. 2).

Figure 2. Relative expression of BDNF gene in heavy smokers and healthy control. The results from the groups of moderate and low smokers did not show any significant difference, data not shown. ** P-value < 0.001

Enzyme-linked Immunosorbent Assay (elisa):

The serum level of BDNF was significantly (p-value < 0.05) lower in heavy smokers than healthy nonsmokers; these data confirm the results from real-time PCR (Fig. 3).

Figure 3. Comparison between heavy smokers and healthy nonsmokers in the serum level of BDNF. The results from the groups of moderate and low smokers did not show any significant difference, data not shown. **** P-value < 0.05

Results:

237 cigarette smoker individuals and 90 healthy nonsmokers as controls participated in this study. All participants were men, unfortunately, women were not willing to participate due to social and cultural reasons. According to the Fagerstrom standard protocol, people were divided into three groups as shown in table 3. In summary, the basis of the scale for nicotine dependence is the number of cigarette consumption per day, and a questionnaire that evaluates the level of nicotine dependency in the individual.

Methylation specific PCR (MS-PCR):

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Fig1. Correlation of nicotine dependence with BDNF gene methylation status. the percentage of the people that their BDNF gene was methylated, was higher in the heavy smokers >medium smokers> low smokers.* P-value <0.001

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Enzyme-linked immunosorbent assay (ELISA):

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Fig3. Comparison between heavy smokers and healthy nonsmokers in the serum level of BDNF. The results from the groups of moderate and low smokers did not show any significant difference, data not shown.* P-value <0.05

Discussion:

In this study, we aimed to evaluate nerve growth factor BDNF gene methylation in smokers. Also, we compared the serum level of BDNF and its gene expression between cigarette smokers and healthy nonsmokers. Our results showed that BDNF gene methylation was significantly higher in heavy smokers than healthy nonsmokers. Data from real-time PCR and ELISA assay confirm the results from MS-PCR. BDNF gene expression and serum concentration were significantly lower in heavy smokers than healthy nonsmokers. These data showed that BDNF gene methylation correlated with lower expression of the gene and serum level of the protein. Although serum level of BDNF, mostly originated from the brain, BDNF is stored in the platelets in a significant amount and is released when it is necessary [18]. Our findings are consistent with the previous study by M.Yeom et al. which showed that repeated exposure to the nicotine reduces the BDNF expression and protein level [19]. The lower BDNF level may correlate to the reduction of the beneficial effects of this growth factor. A few studies investigated nerve growth factor BDNF and epigenetics changes in smoker's healthy people. Toledo-Rodriquez et al. found that adult individuals who had been exposed to prenatal smoking showed DNA methylation of the BDNF gene, this strongly leads to long-term downregulation of BDNF expression [20]. In another study, the decrease in BDNF mRNA and protein was contributed to the behavioral changes in male mice exposed to smoking. They suggested that epigenetic modification may underlie long-lasting changes in the plasticity and

development of the brain. These changes may lead to laterneurodevelopmental problems and it may transfer to the next generation [21, 22]. In a study, it has been shown that cigarette smog modifies DNA methylation of BDNF gene, and some other special genes like AXL, PTPRO, C11orf52, FRMD4A, CYP1A1, AhRR, FOXP3, TSLP, IGF2 in fetus, in smoking mothers against nonsmokers [23]. Zhang et al. showed that smoking severity is associated positively with serum BDNF levels, this contradiction may be due to the limited number of samples in their study and difference in the age of the participants, also while they measured fasting BDNF, we checked random BDNF in the serum, nicotine dose of injection may be another factor leading to different results [24]. Kenny et al. demonstrated, in an animal study, that acute nicotine administration significantly decreases the BDNF gene expression in the rat hippocampus [25]. Bhang et al. found that plasma concentration of BDNF is lower in smoker groups but its concentration increases after abstinence during 1–2 months [26]. All together more studies are needed to evaluate the BDNF exchange in the smokers specifically. For the future studies, we suggest to compare BDNF level between the groups of active smokers, former smokers and non-smokers.

Conclusion

Heavy smoking can modify BDNF gene epigenetics and its serum level. To note the role of BDNF in nerve growth, regeneration, and maintenance, it may be an important issue to be considered in heavy smokers to prevent possible damages to the nervous system.

Abbreviations

Brain-derived neurotrophic factor
BDNF
Interleukin-1β
IL-1β
Interleukin-6
IL-6
Tumor Necrosis Factor-α
TNF-α
AXL receptor tyrosine kinase
AXL
PTPRO
Protein Tyrosine Phosphatase Receptor Type O
Chromosome 11 Open Reading Frame 52
C11orf52
FERM Domain Containing 4A
FRMD4A
Cytochrome P450 Family 1 Subfamily A Member 1
CYP1A1

Aryl-Hydrocarbon Receptor Repressor
AHRR
Forkhead box P3
FOXP3
Thymic stromal lymphopoietin
TSLP
Insulin Like Growth Factor 2
IGF2

Declarations

Ethics approval and consent to participate: Prior to data collection, all respondents were informed about the aims of the study and data confidentiality, and gave written informed consent. All participants took part in the survey voluntarily.

Consent for publication: not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors have declared that no conflict of interest exists (financial, non-financial) and they have consented to the publication.

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Ethics committee: Fasa University of medical sciences (belong to the ministry of health), Deputy department of research, ethic code: IR.FUMS.REC.1395.155

Authors' contributions: Conceptualization**:** BM, methodology: BM, SK, FM, AT, data curation and analysis: BM, FM, FK, AT writing—original draft preparation: BM, SK, FM, writing—review and editing: BM, the authors read and approved the final manuscript.

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Tables

Table 1. The sequence of the primers for MS-PCR.

Gene	Sequence(5'→3')	Product size
BDNF Methylated Forward	GCGGGGAGTAGTATCGCGAC	127 bp
BDNF Methylated Reverse	ATTCCCAACGCTTACCTACCTCG	127 bp
BDNF, Unmethylated Forward	GTTGGTGGGGAGTAGTATTGTGAT	132 bp
BDNF, Unmethylated Reverse	CATTCCCAACACTTACCTACCTCTCA	132 bp

Table 2. Primers of BDNF and beta actin for qRT-PCR

Gene	Forward (5'to3')	Reverse (5'to3')
BDNF	TACTTTGGTTGCATGAAGGCTGCC	ACT TGACTACTGAGG ATCACCCTG
Bata-actin	GCCTTTGCCGATCCGC	GCCGTAGCCGTTGTCG

Table3. nicotine dependence according to the Fagerstrom questionnaire.

	Nicotine dependence, (up to 4 Low, up to 5 Medium and more than 6 High)		
	Low (n)%	Moderate(n)%	High(n)%
Smoker(n)%	(25)10%	(85) 36.5%	(127) 53.5%
n: the number of smokers			

Table 4. Comparison between heavy smokers and healthy nonsmokers in the status of BDNF (exon I, promoter I) gene methylation. The results from the groups of moderate and low smokers did not show any significant difference, data not shown. %: percentage of the number of people.

Groups	M	UM	P-value
Heavy smokers	90%	10%	0.001
Nonsmoker	30%	70%	

Figures

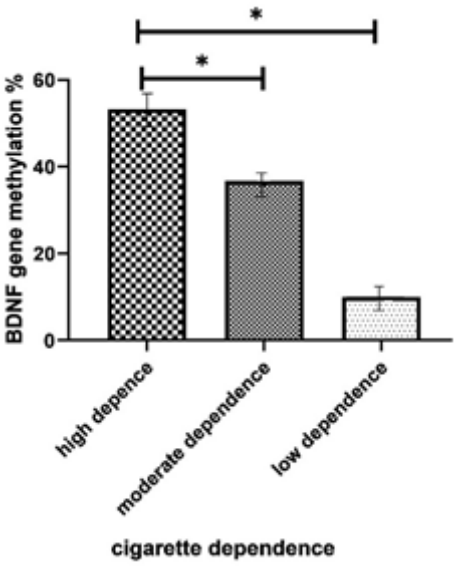

Figure 1

Correlation of nicotine dependence with BDNF gene methylation status. the percentage of the people that their BDNF gene was methylated, was higher in the heavy smokers >medium smokers> low smokers.* P-value <0.001

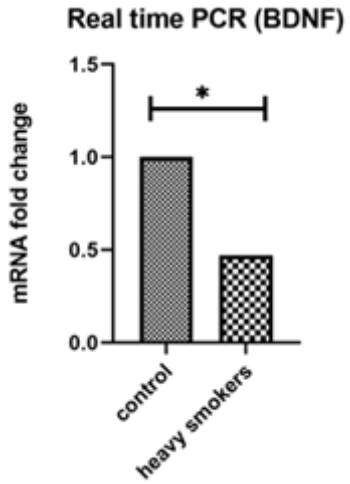

Figure 2

Relative expression of BDNF gene in heavy smokers and healthy control. The results from the groups of moderate and low smokers did not show any significant difference, data not shown.* P-value <0.001

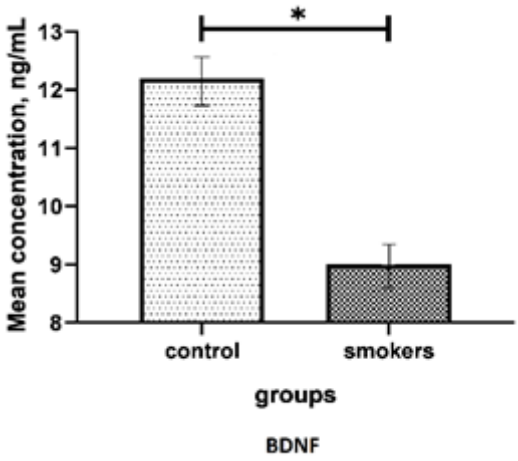

Figure 3

Comparison between heavy smokers and healthy nonsmokers in the serum level of BDNF. The results from the groups of moderate and low smokers did not show any significant difference, data not shown.* P-value <0.05