Original Article

The effect of smoking on cognition as measured by Cambridge Neuropsychological Test Automated Battery (CATNAB) and brain-derived neurotrophic factor plasma levels

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ABSTRACT

الأهداف: إيجاد العلاقة بين التدخين والقدرات العقلية ومستوى عامل التغذية العصبية المشتق من الدماغ BDNF في البلازما.

المنهجية: أجريت دراسة مقطعية تحليلية بأثر رجعي أجريت خلال الفترة من مارس ونوفمبر 2018م. حيث اشتملت الدراسة على 73 من الذكور31 من المدخنين و 42 من الغير مدخنين تتراوح أعمار عينة الدراسة بين 17 و 33 عاماً. تم تقييم القدرات العقلية لعينة الدراسة باستخدام مصفوفة اختبار الوظائف العصبية المقننة من كامبريدج CANTAB. تم جمع عينات الدم لقياس نسبة عامل التغذية العصبية المشتقة من الدماغ، وتم مقارنة نتائج المجموعتين بالنسبة لمستوى الBDNF و علامات اختبارات القدرات العقلية العصبية . مستوى الدلالة P 0.05>.

النتائج: كان تحصيل مجموعة المدخنين أسوأ بكثير من غير المدخنين في اختبار تعدد المهام، بما في ذلك وقت رد الفعل ومعالجة المعلومات المرئية السريعة. ومع ذلك ، لم يلاحظ أي ارتباط كبير بين المتغيرات المتعلقة بالتدخين ودرجات الاختبار المعرفي. عثرنا على ارتباط ايجابي وحيد بين مستويات BDNF في البلازما وعدد السجائر التي يتم تدخينها يوميًا (r=0.480, *p*=0.024). لم يلاحظ أي ارتباط بين المتغيرات الأخرى المتعلقة بالتدخين ومستويات BDNF في البلازما.

الخلاصة: نستنتج أن مستوى ال BDNF في البلازما مرتبط إيجابياً بعدد السجائر المدخنة يومياً. وأن هناك اعتلال ذا دلالة إحصائية في بعض القدرات العقلية مثل المداومة على التركيز على مهمة ما، والقدرة على التعامل مع المعلومات المتناقضة، وسرعة الاستجابة مع المؤثرات المرئية لدى المدخنين البالغين الذكور.

Objectives: To establish an association between cigarette smoking, cognition, and plasma brain-derived neurotrophic factor (BDNF) levels in healthy young adults.

Methods: This was an ex post facto analytic cross-sectional study conducted between March and November 2018. Participants were 73 healthy males (31 smokers and 42 non-smokers), 17-33 years old. The cognitive function of the participants was assessed through the Cambridge neuropsychological test automated battery (CANTAB). Blood samples were taken to measure the plasma levels of BDNF and the results were compared to identify the association between smoking related variables and cognitive test scores and plasma BDNF levels. A *p*-value of <0.05 was considered statistically significant.

Results: Smokers performed significantly worse than non-smokers in the multitasking test, including reaction time and rapid visual information processing. However, no significant association was observed between smoking related variables and cognitive test scores. The only significant positive correlation was found between plasma BDNF levels and the number of cigarettes smoked per day (r=0.480, *p*=0.024). No correlation was observed between other smoking related variables and plasma BDNF levels.

Conclusion: Plasma BDNF level is positively related to the number of cigarettes smoked per day. Young smokers have significantly impaired sustained attention and less ability to manage conflicting information as compared to age-matched non-smokers.

Keywords: smoking, brain-derived neurotropic factor, cognition, neuropsychological tests, CATNAB

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There is currently a global prevalence of tobacco smoking of 21.2% among the adult population (35.8% of males and 6.6% of females).[1] The most recent World Health Organization's (WHO) global report on tobacco smoking found an increase in the prevalence of tobacco smoking is among both genders. Furthermore, the prevalence of tobacco smoking in the Kingdom Saudi Arabia (KSA) is projected to increase to 24% by 2025.[2]

Tobacco smoking is known for its adverse impact on human health, resulting in the deaths of approximately 7 million individuals each year, with over 6 million of these arising from direct tobacco use and approximately 890,000 from passive smoking.[3] A considerable degree of research has highlighted the deleterious effects of tobacco smoking, along with its association with multiple types of cancers, strokes, pulmonary and cardiovascular diseases, and reduced levels of immunity.[4] The high concentration of free radicals in tobacco smoke can increase oxidative stress and damage to deoxyribonucleic acid and other neuronal structures. Furthermore, exposure to tobacco smoke increases oxidative stress by decreasing antioxidants important for maintaining oxidative balance.[5] Smoking can also reduce cerebral perfusion, either directly (by affecting the vasomotor reactivity of cerebral arteries) or indirectly (by accelerating atherosclerosis formation).[6] Smoking also increases homocysteine level in plasma, which is a known risk factor for cognitive impairment and stroke.[7] In addition, tobacco smoking may cause cortical thinning and decrease grey matter density in brain areas significant for cognition, such as, the frontal lobe, thalamus and cerebellum.[8] Furthermore, nicotine (an important chemical agent in tobacco), is known for its influence on cognition through stimulating nicotinic acetylcholine receptors distributed throughout the brain.[9] Smoking-induced cognitive impairment has been reported even in passive smokers.[10]

The brain-derived neurotrophic factor (BDNF) has been proposed to have a significant role in brain development, synaptic plasticity, cognition and memory.[11] A study showed higher BDNF levels in aged mouse as compared to young and BDNF levels were inversely correlated with the spatial memory.[12] The significance of BDNF in the normal development and functioning of nervous system highlights the importance of examining its association with tobacco smoking. Although, an association between chronic smoking and cognitive impairment in elderly and middle-aged subjects is well established,[13] the impact of smoking on cognition of young individuals has remained controversial. This current research therefore focuses on exploring the association between smoking, cognition, and BDNF.

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Methods. This study took the form of an ex post facto analytic cross-sectional study, conducted between March and November 2018 at the Department of Physiology, in College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, KSA.

The calculated sample size was 110 participants (55 smokers and 55 non-smokers). The calculation of the sample size employed OpenEpi, an open source epidemiologic statistics for public health (version 3.01) online tool.[14] The calculation of the sample size was based on the mean scores of spatial working memory strategy (SWMS) and standard deviation (SD) values with a power of 80% and a 95% confidence interval.

We recruited 73 participants (31 smokers and 42 non-smokers) through purposive sampling (non-probability sampling). Participants were recruited from Family and Community Medicine Center, Imam Abdulrahman bin Faisal University (IAU) and an anti-smoking clinic in Al Khobar, KSA.

Inclusion criteria were healthy males aged between 17 and 33 years old. We chose to employ only male participants due to it being simpler to recruit males than females, particularly as the prevalence of male smokers in KSA is far higher than females.[2] The selected participants had a history of smoking at least one cigarette a day over the previous 6 months or more, regardless of the number/duration of previous attempts to give up smoking. The non-smoking participants were those who had never smoked tobacco in their lifetime.

We excluded subjects who had previously smoked but had given up at the time of the study. It also excluded subjects with psychiatric disorders or chronic diseases capable of impacting on cognition (such as, diabetes mellitus, asthma and sickle cell disease), along with alcoholics and those who had previously used (or smoked) substances other than tobacco, such as cannabis, cocaine, and heroin. Users of electronic cigarettes were also excluded.

The ethical approval for this study was obtained from the Institutional Review Board of IAU under the reference number IRB-PGS-2018-01-033. The research was according to the principles of Helsinki Declaration. All participants received a detailed information form

accompanied by a verbal explanation of the procedure. Written consent was obtained from all participants prior to commencement of the study. All participants filled out a self-designed questionnaire comprised of questions related to their smoking and health status. The participants were then assessed for their cognitive functions by means of the Cambridge neuropsychological test automated battery (CANTAB).[15] In addition, blood samples were collected and plasma levels of BDNF were measured.

Self-developed questionnaire. The questionnaire was designed to collect information concerning the participants' health status and history of smoking. All participants answered questions about their: i) age; ii) chronic diseases; ii) psychiatric disorders; iv) use of medication; v) smoking; vi) use of alcohol; and vii) use of addictive substances other than tobacco. Smokers were asked additional questions related to their smoking history (such as, age of their initiation into smoking, the number of cigarettes smoked per day and current or past smoking of tobacco products other than cigarettes). Pack-years for smokers (number of cigarettes smoked per day × years as a smoker/20) was calculated to quantify their lifetime exposure to cigarette smoking. A pilot study was conducted with 18 participants (9 smokers and 9 non-smokers) aged between 24 and 34, to test the clarity of the tool and estimate the time required to complete the questionnaire. The piloted participants were not involved, and few changes were made in the final questionnaire in response to the pilot study. The finalized questionnaire used in the study is available in **Appendix A**.

Cognitive assessment. Cambridge neuropsychological test automated battery is a battery of computerized neuropsychological tests by University of Cambridge, England.[15] The CANTAB tests depend on touch screen technology, which provides rapid and non-invasive cognitive assessment. These tests follow a game-like format, in which a feedback is provided following each trial, thus maintaining the motivation of the participants.[15]

Four tests from CANTAB were used to assess the cognitive functions of the current participants, namely: i) reaction time (RTI); ii) spatial working memory (SWM); iii) rapid visual information processing (RVP); and iv) multitasking test (MTT). Testing took place in a quiet room, with the subjects provided an iPad (Apple Inc, USA) and headphones. All the participants received a detailed explanation on how to undertake each cognitive test and the tests were all carried out in the same sequence for each of the subjects.

Reaction time. This test is used to assess mental and motor response speeds and measures reaction time, impulsivity, movement time and response accuracy. This study measured RTI median 5-choice movement time and RTI median 5-choice reaction time.

Spatial working memory. This consists of an executive function and decision-making test used to assess spatial working memory. The test was used to measure spatial working memory between errors (SWMBE468) and SWMS 6-8 boxes.

Rapid visual information processing. This test is used to evaluate attention, in particular sustained attention. It was used to measure rapid visual-information processing (RVPA), rapid visual-information processing median response latency and rapid visual-information processing probability of false alarm (RVPPFA).

Multitasking test. This is an executive function and decision-making test assessing the ability to manage conflicting information and ignore task-irrelevant information. It was used to measure multitasking test reaction latency (median), multitasking test incongruency cost (median), multitasking test multitasking cost (median), and MTT total incorrect.

Brain-derived neurotrophic factor sandwich enzyme-linked immuno-sorbent assay (ELISA). Approximately 3 milliliters of venous blood was collected in a tri-potassium ethylene diamine tetra-acetic acid (EDTA. K3) tube and centrifuged for 15 minutes (min) at 1,500 × g within 45 min of collection. Following centrifugation, the plasma portion was aliquoted into an Eppendorf tube and stored at -80°C. Measurements of plasma BDNF concentrations were carried out according to the manufacturer's instructions in our institutional biochemistry laboratory, using human BDNF sandwich ELISA kits (Aviva systems biology, CA, USA).

Statistical analysis. The data were entered and analyzed using IBM SPSS Statistics for Windows, version 24 (IBM Corp, Armonk, NY, USA). Testing for normality of distribution was performed using the Shapiro-Wilk test. A comparison between the 2 groups (such as, smokers and non-smokers) was undertaken by means of an unpaired student t-test for parametric data and a Mann-Whitney test for non-parametric data. Mean and standard deviations were calculated for parametric variables (RTI, SWM, MTI ,and BDNF), with median and range being calculated for non-parametric variables (RVP). A Chi-square ($\chi 2$) test was used for qualitative data analysis. To determine the association between smoking related variables (such as, age at commencement of smoking; number of cigarettes/day; duration of smoking; and the number

of pack-years) and cognitive test scores and BDNF concentrations, Pearson's correlation was used for parametric data and spearman's correlation was used for non-parametric data. Mean values and standard deviations were also calculated and a *p*-value of <0.05 was taken as statistically significant.

Results. Of the 73 participants, 42.5% (n=31) were smokers and 57.5% (n=42) were non-smokers. The mean age of smokers was 24.7±4.1 years, compared to 20.9±2.7 years for non-smokers (Table 1).

Cognitive function. Significant difference was observed between smokers and non-smokers in 3 cognitive test scores related to RVP test and MTT. Non-smokers performed significantly better than smokers in RVPA (p=0.001), and RVPPFA (p=0.027) and MTTLMD (p=0.007) (Table 2).

No significant associations were observed between smoking related variables and cognitive test scores (Table 3).

Plasma BDNF levels. No statistically significant difference was found between the 2 groups with regards to plasma BDNF levels (Table 2). A statistically significant and moderately positive correlation was observed between plasma levels of BDNF and the number of cigarettes/day (r=0.480, p=0.024). However, no significant association was observed between BDNF and other smoking related variables (example, starting age, duration of smoking, and number of packs per year) (Table 3).

Discussion. This study compared the cognitive functioning and plasma BDNF levels of healthy young smokers and non-smokers, to establish the association between cigarette smoking and cognition and plasma BDNF levels. We found that young healthy smokers demonstrated impaired sustained attention and ability to manage conflicting information while ignoring task-irrelevant information (an executive function). However, no significant difference was identified between the plasma BDNF level of smokers and non-smokers. In addition, no significant association was observed between smoking-related variables and the cognitive performance of smokers. Finally, we found only a moderately positive association between plasma BDNF levels and number of cigarettes smoked per day.

Smoking and cognition. The finding of impairment of sustained attention in young smokers is consistent

Table 1 - Characteristics of study participants (N=73).

Demographics	Smokers (n=31)		Non-smokers (n=42)	
	Mean±SD	Range	Mean±SD	Range
Age (years)	24.7 ± 4.1	18-33	20.9 ± 2.7	17-26
Age at starting smoking (years)	17.4 ± 3.3	8-24		
Number of cigarettes/day	14.8 ± 9.2	1-40		
Duration of smoking (years)	7.3 ± 5	1-21		
Pack-years	6.44 ± 8.35	0.15-40		

Table 2 - Comparison of cognitive tests' scores and plasma BDNF levels between smokers and non-smokers (N=73).

Variables	Smokers (n=31)	Non-Smokers (n=42)	*P*-value
Reaction time			
RTIFMDMT	201.63 ± 41.64	198.91 ± 38.54	0.784
RTIFMDRT	368.09 ± 37.06	356.30 ± 34.61	0.186
Spatial working memory			
SWMBE468	7.41 ±7.48	7.29 ± 8.34	0.954
SWMS	7.04 ± 2.32	5.90 ± 2.95	0.098
Rapid visual information processing			
RVPA	0.869 (0.087)	0.909 (0.050)	0.001*
RVPMDL	443 (102.3)	426 (53.8)	0.308
RVPPFA	0.0063 (0.1102)	0.0058 (0.0068)	0.027*
Multitasking test			
MTTICMD	65.80 ± 48.64	46.47 ± 41.72	0.093
MTTLMD	620.7 ± 109.4	555.97 ± 77.45	0.007*
MTTMTCMD	212.38 ±179.49	145.0 ± 90.51	0.091
MTTTIC	7.32 ± 8.47	3.73 ± 3.54	0.053
BDNF (pg/ml)	338.44 ± 266.4	281.91 ± 141.1	0.245

Data presented as mean±SD or median (IQR) as appropriate. RTIFMDMT: reaction time median five-choice movement time, RTIFMDRT: reaction time median five-choice reaction ime, SWM: spatial working memory, SWMBE468: spatial working memory between errors; SWMS: spatial working memory strategy, RVP: rapid visual information processing, RVPA: rapid visual information processing A', RVPMDL: rapid visual information processing median response latency, RVPPFA: rapid visual information processing probability of false Alarm, MTTICMD: multitasking test incongruency cost (median), MTTLMD: multitasking test reaction latency (median), MTTMTCMD: multitasking test multitasking cost (median); MTTTIC: multitasking test total incorrect, BDNF: brain derived neurotrophic factor, *Statistically significant difference (p<0.05).

Table 3 - Correlations between smoking related variables and cognitive test scores and plasma BDNF level (N=31).

Variables	Age at starting smoking		Cigarettes/day		Duration of smoking (years) †		Pack-years‡	
	r	*P*-values	r	*P*-values	r	*P*-values	r	*P*-values
Reaction time								
RTIFMDMT	-0.018	0.929	-0.072	0.726	-0.158	0.431	-0.173	0.399
RTIFMDRT	0.109	0.589	-0.103	0.615	-0.181	0.367	-0.144	0.482
Spatial working memory								
SWMBE468	-0.182	0.363	-0.046	0.824	0.169	0.400	0.036	0.862
SWMS	0.322	0.102	-0.045	0.828	-0.168	0.401	-0.125	0.543
Rapid visual information processing								
RVPA	0.289	0.161	-0.070	0.745	-0.083	0.692	-0.112	0.603
RVPMDL	-0.043	0.839	0.306	0.145	-0.037	0.859	0.240	0.259
RVPPFA	-0.092	0.662	0.025	0.909	0.031	0.882	0.078	0.717
Multitasking test								
MTTICMD	-0.163	0.436	0.218	0.307	0.147	0.482	0.236	0.268
MTTLMD	0.237	0.254	0.252	0.235	0.015	0.944	0.054	0.802
MTTMTCMD	0.283	0.171	-0.003	0.987	-0.027	0.897	0.007	0.975
MTTTIC	0.147	0.482	0.270	0.201	0.035	0.869	0.064	0.766
BDNF (pg/ml)	-0.129	0.558	0.480	0.024*	0.294	0.173	0.375	0.085

Data presented as mean±SD or median (IQR) as appropriate. RTI: reaction time, RTIFMDMT: reaction time medianfive-choice movement time, RTIFMDRT: reaction time median 5-choice reaction time, SWM: spatial working memory, SWMBE468: spatial working memory between errors, SWMS: spatial working memory strategy, RVP: rapid visual information processing, RVPA: rapid visual information processing A', RVPMDL: rapid visual information processing median response latency, RVPPFA: rapid visual information processing probability of false alarm, MTTICMD: multitasking test incongruency cost (median), MTTLMD: multitasking test reaction latency (median), MTTMTCMD: multitasking test multitasking cost (median), MTTTIC: multitasking test total incorrect, BDNF: brain derived neurotrophic factor, *Statistically significant difference ($p<0.05$). †Duration of smoking was calculated by subtracting age at starting smoking from participant current age. ‡Pack-years were calculated as [the number of cigarettes/day × years as a smoker/20].

with the results of previous study conducted by Vajravelu et al.[16] In their study, they reported impaired attention in moderate and heavy smokers only. Mild smokers demonstrated improved sustained attention and alertness over non-smokers.[16]

In executive functioning, we found that, in comparison to non-smokers, smokers demonstrated an impaired ability to manage conflicting information and ignore task-irrelevant information. Our results agree with the previous studies that have reported impairment in executive functions in middle aged and elderly smokers.[17,18]

The insignificant difference in spatial working memory and reaction time tests between young smokers and non-smokers is inconsistent with a previous study conducted on young individuals.[16] This discrepancy in results could be due to our small sample size or presence of a number of confounding factors we were unable to control, example, baseline cognitive ability, age, education, and the use of caffeinated beverages prior to the cognitive assessment.

Lack of association between smoking and cognitive performance, as found in our study, is inconsistent with studies conducted before.[19-21] Studies by Depp et al[19] and Hickling et al[20] were cross-sectional; whereas Vermeulen et al[21] conducted a large prospective study to explore the association between smoking behavior and cognition at baseline, at 3-6-year follow-ups. All these studies reported an inverse relationship between smoking and cognition.

Smoking and plasma BDNF levels. We did not find any significant difference in plasma BDNF levels of smokers compared to non-smokers; a finding contrary to Neves et al,[22] who reported significantly higher BDNF in heavy smokers compared to non-smokers. The underlying cause of discrepancy could be due to

the possibility that our study participants were light-smokers and not the heavy-smokers. Nevers et al[22] failed to observe any significant difference in BDNF levels of mild smokers compared to non-smokers. We found a moderately positive association between plasma BDNF levels and the number of cigarettes/days. This is consistent with Colle et al[23] who reported a positive correlation of plasma BDNF with number of cigarettes/day and number of pack/years. Zhang et al[24] also reported smoking severity to be positively associated with BDNF levels. This positive association between plasma BDNF levels and the number of cigarettes/days could also be indicative of a compensatory neuroprotective mechanism, or an underlying development and maintenance of nicotine dependence.

Future studies are required to examine the relationship between smoking and BDNF levels in the brain and to explore the mechanisms underlying this relationship and to enhance understanding of the interrelationship between smoking, cognition, addiction and the BDNF system.

Study limitations. We used a relatively small sample size. The lack of statistically significant correlations may be due to small sample size leading to low power; the correlation between RVPA and age at start (r=0.289): the power is as low as 35%.

The *p*-values are also affected by the sample size (smaller the sample size, larger is the *p*-values). Our study was limited to male subjects only. The participants were recruited by non-probability sampling technique, and they may not have been representative of the entire population. This was an observational study and so did not allow for examination of genetic associations or neurobiological mechanisms underlying the influence of smoking on cognition. Self-reported medical, psychiatric, smoking, medication, and addiction histories were recorded that were not based on physical or laboratory examinations. We did not assess any other confounders that may influence cognition or BDNF levels, example nutrition, consumption of caffeinated beverages, and passive smoking, and so on.

In conclusion, young smokers have significantly impaired sustained attention and less ability to manage conflicting information as compared to age-matched non-smokers. Plasma BDNF level is positively related to the number of cigarettes smoked per day. No association was found between plasma BDNF levels and the starting age of smoking, duration of smoking and the number of pack-years.

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Appendix A - Self-made questionnaire (for current smokers and non-smokers).

1. Age _____
2. Are you suffering from any chronic illness?
   - No
   - Yes, please mention which disease _____
3. Are you currently using any medicines or treatments on a regular basis (such as vitamins, soothing pills or hypnotic pills, treatments for chronic illness, treatments to help stop smoking, etc.)
   - No
   - Yes, please write down the name and purpose of the treatment

Name of treatment	Purpose

4. Are you currently suffering from any disorders or psychiatric illnesses diagnosed by a psychiatrist?
   - No
   - Yes, please mention it
5. In the past, have you ever experienced any disorders or psychiatric illnesses diagnosed by a psychiatrist?
   - No
   - Yes, please mention it

Name of disorder or disease	When did it start?	The duration or period of disorder

6. Do you smoke?
   - No *(The questionnaire ends here for non-smokers)*
   - Yes
7. At what age did you start smoking? _____
8. Currently, how many cigarettes do you smoke per day? _____
9. Before you smoked cigarettes, did you use other means of tobacco use (eg, hookahs, cigars, chewing tobacco in the mouth, etc.)?
   - No
   - Yes, please mention it _____
10. In addition to cigarettes, are you currently using other means of tobacco use (eg, hookahs, cigars, chewing tobacco in the mouth, etc.)?
    - No
    - Yes, please provide the details of your use as shown in the following table

Means of tobacco use (Other than cigarettes)	At what age did you start using it?	Number of times of use in a specified period of time (eg, once a day, 5 times a month, 20 times per year)	Duration of use per session

11. Are you currently using or have used non-tobacco addiction items (eg, alcohol, narcotic substances, etc.)?
    - No
    - Yes (please mention the name) .....

*****End of questionnaire. Thank you for your participation*****