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Risk Factors of Mood Disorders (Depression and Anxiety) in Smoking Subjects: Reliability with the Age of Smoking Initiation and Inflammatory Processes

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Authors' contributions

Authors may use the following wordings for this section: This work was carried out in collaboration between all authors. Author NF designed the study, performed the statistical analysis, wrote the protocol. Author HF wrote the first draft of the manuscript. Authors AC, KG, BR and RD managed the analyses of the study. Author SF managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of our study was to investigate the causal relationship between smoking and mood disorders (depression, anxiety) and to determine the class at high risk (to developing depression and/or anxiety) depending on age (begin smoking at adulthood (Adt: [23-32] years), childhood (Ch: [5-11] years) or adolescent(Ads: [12-17] years)).
Place and Duration of Study: This study is conducted by Universities of Mentouri Constantine and Badji Mokhtar Annaba, Algeria. Between October 2011 to April 2012.
Methodology: A demographic questionnaire collected data about patient's characteristics

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and medical status, the Hospital Anxiety and Depression Scale and Fagerstrom Test. All subjects underwent a thorough medical evaluation and laboratory exam (WBC count, CRP, ESR, Hct). Our study included 96 smokers male divided into several groups according to the desired statistical analysis.

Results: Multivariate analysis by using regression analysis, showed that 85 % of the variability of depression is explained by duration of cigarette smoking (years), the age of smoking initiation (years) and degree of dependence (Fagertstrom). (R (coefficient de corrélation): 0,921; R^2 (coefficient de détermination): 0,848; DF: 6, F: 82.767, Pr > F: < 0,0001). Therefore, we can conclude with confidence that the three variables do bring a significant amount of information. The correlation is less for anxiety setting (R (coefficient of correlation): 0,759; R^2 (coefficient of determination): 0,576; DF: 6, F: 20,120, Pr > F: < 0,0001). The relation between depression and inflammatory parameters (CRP, ESR and Htc) was examined using the chi-square test for independence (X2-test). This test indicated that depression is clearly related to these inflammatory variables. Furthermore, the ANOVA test indicated that the hematological parameters vary depending the age of smoking initiation.

Conclusion: There is a close relationship between the level of smoking addiction and the emergence of depressive disorders. We suggest an elevated risk of mood disorders in subjects, having a very strong smoking addiction, who began smoking in adulthood and late adolescence versus childhood.

Keywords: Nicotine; depression; anxiety; childhood; adolescent; inflammation; smoking.

1. INTRODUCTION

Smoking has a wide range of biological effects contributing to its negative impact on health [1]. Tobacco smoking, a global epidemic, is one of the greatest challenges of our time. Currently about 1.1 billion people are cigarette smokers worldwide, and their consumption is 5.5 trillion cigarettes annually [2,3]. Approximately 5 million people are killed every year by tobacco in the world, and the number of annual deaths will increase to 10 million by the year 2030 [2,3].

According to Williams and Ziedonis [4], more than 50 to 90% of individuals with mental disorders are highly addicted to tobacco, this rate varying according to the pathologies and comorbidities. Comorbidity of depression and smoking is well recognized, but results from studies that have assessed alternative explanations have varied by the level of smoking and the study method [5]. Major depression, is general regarded as a serious public health problem associated with an increased risk of disability and mortality. According to estimates by the WHO, depression will be the second leading cause of disability worldwide in 2020 [6,7,8,9].

Results of epidemiological studies in general and clinical populations suggest a bidirectional positive relationship between smoking and major depression. For example, in the National Comorbidity Survey nearly 60% of individuals with a life-time history of depression were current or past smokers, while only 39% of the general population were current or past smokers [10,11].

It is known that smoking and anxiety are related, but the causal relationship of the two features are not well defined [1]. Indeed, among the scientific studies, some are going in the

direction of an effect of smoking on anxiety, while others go in the direction of an effect of anxiety on smoking because it was noted that there is a relatively high rate of smokers among patients suffering of anxiety according to the type of anxiety disorder [12], the presence of an anxiety disorder with or without depression is associated with a greater probability of smoking.

It was described that smoking cessation frequently precipitates depressive symptoms that can be reversed with the reintroduction of smoking [13]. Moreover, transdermal nicotine patches exert an antidepressant-like effect in non-smokers [14]. Animal model studies corroborate these effects of nicotine: An acute administration of nicotine elicits an antidepressive-like behavior [15] while long-term nicotine withdrawal promotes increased depressive-like behavior in adult mice [16,17].

Over the past two decades numerous studies have indicated that smoking is highly correlated with the development of depression [18,19] and recent studies indicate that both the offspring of women who smoke and adolescent smokers are more susceptible to depression in later life [20,21], whereas those who initiate smoking later in life are not [20]. In this context, the aim of our study was to investigate the causal relationship between smoking and depression/anxiety and to determine the class at high risk of developing depression depending on age: subjects who began the smoking during adolescence and subjects who began adulthood.

2. SUBJECTS AND METHODS

2.1 Subject Assessment

This study was approved by Scientific Council of the Faculty of Sciences, Annaba University, Algeria. Patients give written informed consent to the use of their de-identified clinical and biological data for the purposes of research and program evaluation. The survey was conducted from October 2011 to April 2012 in north eastern Algeria. The records of patients were selected for this study if they were between 18 and 60 years of age. The average age of the patients was 33.5 + - 7.2 with extremes ranging from 21 years to 50 years. The averages duration of cigarette smoking is 19.5 +-2.9; and age of smoking initiation is 14.0 +- 7.3.

A demographic questionnaire collected data about patient's characteristics (age, antecedent) and medical status, the Hospital Anxiety and Depression Scale (HADS) [22] and Fagerstrom Test for cigarette Dependence [23] were used in this study. To facilitate this study, we distributed questionnaires of Fagerstrom and HADS tests to several general practitioner offices, to be completed by anonymous smoking patients. Self-report questionnaires were administered by different clinicians. All subjects underwent a thorough medical evaluation including medical history, physical exam and laboratory exam (complete blood cell count, CRP, ESR, Hct) (Fig. 1). Subjects with clinically meaningful abnormal laboratory values were excluded. All patients selected for the study are healthy and have no apparent illness. 12 patients with some unexpected anomalies were excluded from the study (02 diabetes, 03 hypertension, 07 infections (the blood test)). In the final, 96 patients were divided into several groups according to the desired statistical analysis. To facilitate interpretation of the results we symbolize classes as follows (Table 1, Fig. 1):

Table 1. Frequency distribution of levels of anxiety and depression (HAD scale) depending on the degree of smoking addiction (Fagerstrom test) of 96 subjects

Fagerstrom test		Degree of dependence				
HAD scale		**No dependence**	**Weak dependence**	**Medium dependence**	**High dependence**	**Very high dependence**
Global repartition		-	Ch: 06	Ch: 24	Adt: 06 Ch: 18 Ads: 12	Adt: 12 Ads: 18
Anxiety level	No anxiety (0)	-	Ch: 06	Ch: 12	Ads: 06	-
	Non significant anxiety state (1)	-	-	Ch: 12	Adt: 06 Ch: 18 Ads:06	Adt: 12 Ads: 18
	Significant anxiety state	-	-	-	-	-
Degree of depression	Not depressive (0)	-	Ch:06	Ch: 24	Ch: 06 Ads:06	-
	Early depression (1)	-	-	-	Adt: 06 Ch: 12 Ads: 06	Ads:12
	Strongly depressed state (2)	-	-	-	-	Adt: 12 Ads:06

Adt: *Subjects Begin smoking at adulthood age ([23-32] years) (n=18);*
Ch: *Subjects Begin smoking at childhood ([5-11] years) (n=48);*
Ads: *Subjects Begin smoking at adolescent age ([12-17] years) (n=30).*
NoDep: *No dependence (n=0)*
MedDep: *Weak dependence and Medium dependence (n=30)*
HDep: *High dependence and Very high dependence (n=66)*
Level 0: no anxiety (n=24)
Level 1: Non significant anxiety state and (n=72)
Level 0: Not depressive (n=42)
Level 1: Early depression (n=36)
Level 2: Strongly depressed state (n=18)

2.2 Hospital Anxiety and Depression Scale (HADS)

Comorbidity of anxiety and depression is estimated through the HADS test (Appendix) which contains fourteen items rated from 0 to 3. Seven questions are related to anxiety (HADS A) and seven questions to depressive dimension (HADS D), in order to obtain two scores (maximum score = 21). By adding the points of the 1, 3, 5, 7, 9, 11, 13 responses, we obtain the A total, and for the 2, 4, 6, 8, 10, 12, 14 answers, we obtain the D total. To detect depression or anxiety symptoms, an interpretation is proposed for each A and D scores. A score above 8 on the D scale indicates a depressive state and a score above 12 on the A scale indicates an anxiety disorder [22]. Patients are asked to choose one response from the four given for each interview. They should give an immediate response and be dissuaded from thinking too long about their answers. The questions relating to anxiety are marked “A”, and to depression “D”.

2.3 Fagerstrom Test for Nicotine Dependence

The Fagerstrom test (Appendix) consists in six questions to measure the level of nicotine dependence. The total score is be-tween 0 and 10, and the interpretation of test score is:

Score 0 to 2: the subject is not addicted to nicotine, it can often stop smoking without using nicotine replace-ment therapy.
Score of 3 to 4: the subject is weakly dependent on nicotine.
Score of 5 to 6: the subject is moderately dependent on nicotine.
Score of 7 to 8: the subject is highly dependent on nicotine.
Score of 9 to 10: the subject is very heavily dependent on nicotine

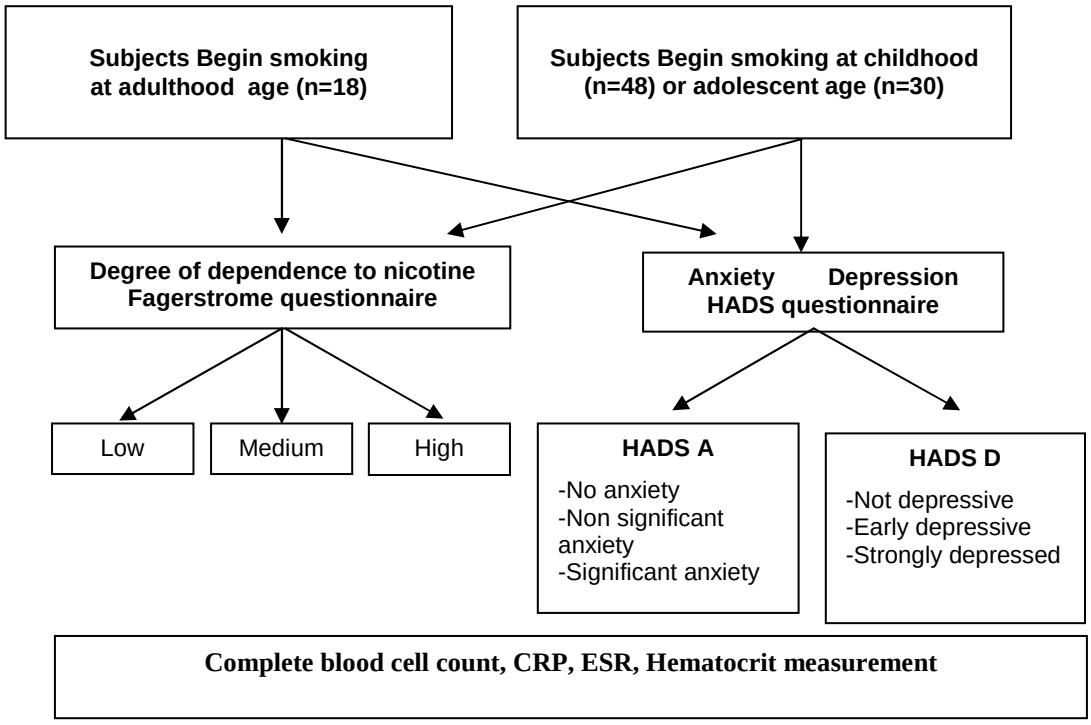

Fig. 1. Experimental Protocol

2.4 Statistical Analysis

The principal methods of statistical analysis were a multivariate analysis by using regression analysis, with depression scale (or anxiety scale) as the dependent variable and as covariates age of smoking initiation, duration of cigarette smoking, and dependence scale from Fagerstrom test. The relation between depression and inflammatory parameters (CRP, ESR and Htc) was examined using the chi-square test for independence (X2-test). For hematological parameters (data), significant changes were determined using ANOVA with age of smoking initiation as main factors. Post-hoc analysis was performed by the Neuman–Keuls test. For simplicity, we reduce the number of different groups, and consider weak and medium dependence as 1 group and high and very high dependence as a second group.

3. RESULTS

3.1 Variation of the Depression (HADS) with Duration of Cigarette Smoking (Years), the Age of Smoking Initiation (Years) and Degree of Dependence (Fagertstrom) (Analysis of Covariance (ANCOVA))

Using the Analysis of Covariance (ANCOVA), we want to find out how the depression (HADS) varies with duration of cigarette smoking (years), the age of smoking initiation (years) and degree of dependence (Fagertstrom), and to verify if a linear model makes sense. The Dependent variable (or variable to model) is here the depression (HADS scale). The qualitative explanatory variables are duration of cigarette smoking (years), the Age of smoking initiation (years) and degree of dependence (Fagertstrom). The Table 2 displays statistics variables of the depression model.

Table 2. Summary statistics variables of the depression model

Variable	Observations	Mean	Std. Deviation	Number of modalities	Modalities	Frequencies
Depression	96	-	-	-	-	-
Age of smoking initiation (years)	-	14,063	7,331	-	-	-
Duration of cigarette smoking (years)	-	19,563	2,930	-	-	-
degree of dependence (Fagertstrom).	-	-	-	3	Med Dep ~ No Dep ~ H Dep	60 ~ 6 ~ 30

The R^2 (coefficient of determination) indicates the % of variability of the dependant variable which is explained by the explanatory variables. R (coefficient of correlation): 0,921; R^2 (coefficient of determination): 0,848

In this particular case, 85 % of the variability of depression is explained by duration of cigarette smoking (years), the age of smoking initiation (years) and degree of dependence (Fagertstrom). The remainder of the variability is due to some effects (other explanatory variables).

It is important to examine the results of the analysis of variance. The results enable us to determine whether or not the explanatory variables bring significant information (null hypothesis H0) to depression model.

The Fisher's F test is used. Given the fact that the probability corresponding to the F value is lower than 0.0001, it means that we would be taking a lower than 0.01% risk in assuming that the null hypothesis (no effect of the two explanatory variables) is wrong (DF: 6, F: 82.767, Pr > F: <0,0001). Therefore, we can conclude with confidence that the three variables do bring a significant amount of information.

We also want to find out if the three variables provide the same amount of information. To do this, we have to examine the Type I SS and Type III SS tables (Table 3, Table 4).

Table 3. Type I Sum of Squares analysis of the depression model

Source	DF	Sum of squares	Mean squares	F	Pr > F
Age of smoking initiation	1	51,019	51,019	262,940	< 0,0001
Duration of cigarette smoking	1	3,302	3,302	17,020	< 0,0001
Fagertstrom	2	30,158	15,079	77,713	< 0,0001
Age of smoking initiation*Fagertstrom	2	7,733	3,866	19,927	< 0,0001
Duration of cigarette smoking*Fagertstrom	2	4,144	2,072	10,680	< 0,0001

The Type I SS table is constructed by adding variables in the model one by one, and by evaluating the impact of each on the model sum of squares (Model SS). In consequence, in Type I SS, the order in which the variables are selected will influence the results. The lower the F probability corresponding to a given variable, the stronger the impact of the variable on the model as it is before the variable is added to it.

Table 4. Type III Sum of Squares analysis of the depression model

Source	DF	Sum of squares	Mean squares	F	Pr > F
Age of smoking initiation	1	20,848	20,848	107,447	< 0,0001
Duration of cigarette smoking	1	5,173	5,173	26,662	< 0,0001
Fagertstrom	2	4,778	2,389	12,312	< 0,0001
Age of smoking initiation*Fagertstrom	2	0,000	0,000	0,000	
Duration of cigarette smoking*Fagertstrom	2	4,144	2,072	10,680	< 0,0001

The Type III SS table is computed by removing one variable of the model at a time to evaluate its impact on the quality of the model. This means that the order in which the variables are selected will not have any effect on the values in the Type III SS. The Type III SS is generally the best method to use to interpret results when an interaction is part of the model. The lower the F probability corresponding to a given variable, the stronger the impact of the variable on the model.

3.2 Variation of the Anxiety (HAD) with Duration of Cigarette Smoking (Years), the Age of Smoking Initiation (Years) and Degree of Dependence (Fagertstrom) (Analysis of Covariance (ANCOVA))

The Dependent variable (or variable to model) is here the anxiety (HADS scale). The qualitative explanatory variables are duration of cigarette smoking (years), the Age of smoking initiation (years) and degree of dependence (Fagertstrom).

R (coefficient de corrélation): 0,759; R^2 (coefficient de détermination): 0,576

The correlation is less for anxiety setting than depression (R (coefficient de corrélation): 0,759; R^2 (coefficient de détermination): 0,576; DF: 6, F: 20,120, Pr > F: < 0,0001).

Table 5. Analysis of differences between groups with a confidence interval of 95.00% *(Tukey test Depression model): Tukey value: 3,370*

Modalités	Difference	Difference reduced	Critical value	Pr. > Diff	Significant
H Dep ~ No Dep	2,200	11,168	2,384	< 0,0001	Yes
H Dep ~ Med Dep	1,800	18,275	2,384	< 0,0001	Yes
Med Dep ~ No Dep	0,400	2,121	2,384	0,091	Non
Age of smoking initiation *Fagertstrom-H Dep ~ Age of smoking initiation *Fagertstrom-Med Dep	0,727	33,575	2,384	< 0,0001	Yes
Age of smoking initiation*Fagertstrom-H Dep ~ Age of smoking initiation*Fagertstrom-No Dep	0,110	5,068	2,384	< 0,0001	Yes
Age of smoking initiation*Fagertstrom-No Dep ~ Age of smoking initiation*Fagertstrom-Med Dep	0,617	14,253	2,384	< 0,0001	Yes
Duration of cigarette smoking *Fagertstrom-H Dep ~ Duration of cigarette smoking*Fagertstrom-Med Dep	0,126	6,462	2,383	< 0,0001	Yes
Duration of cigarette smoking*Fagertstrom-H Dep ~ Duration of cigarette smoking*Fagertstrom-No Dep	0,077	6,100	2,383	< 0,0001	Yes
Duration of cigarette smoking*Fagertstrom-No Dep ~ Duration of cigarette smoking*Fagertstrom-Med Dep	0,049	1,972	2,383	0,125	Non

Table 6. Type I Sum of Squares analysis of the anxiety model

Source	DF	Sum of squares	Mean squares	F	Pr > F
Age of smoking initiation	1	2,098	2,098	24,446	< 0,0001
Duration of cigarette smoking	1	3,646	3,646	42,477	< 0,0001
Fagertstrom	2	4,238	2,119	24,689	< 0,0001
Age of smoking initiation*Fagertstrom	2	0,002	0,001	0,014	0,986
Duration of cigarette smoking*Fagertstrom	2	0,377	0,188	2,196	0,117

Table 7. Type III Sum of Squares analysis of the anxiety model

Source	DF	Sum of squares	Mean squares	F	Pr > F
Age of smoking initiation	1	0,000	0,000	0,000	1,000
Duration of cigarette smoking	1	0,000	0,000	0,000	
Fagertstrom	2	0,421	0,210	2,450	0,092
Age of smoking initiation*Fagertstrom	2	0,000	0,000	0,000	1,000
Duration of cigarette smoking*Fagertstrom	2	0,377	0,188	2,196	0,117

Table 8. Analysis of differences between groups with a confidence interval of 95.00% *(Tukey test Anxiety model): Tukey value: 3,370*

Modalités	Difference	Difference reduced	Critical value	Pr. > Diff	Significant
H Dep ~ No Dep	1,000	7,633	2,384	< 0,0001	Yes
H Dep ~ Med Dep	0,300	4,580	2,384	< 0,0001	Yes
Med Dep ~ No Dep	0,700	5,580	2,384	< 0,0001	Yes
Age of smoking initiation *Fagertstrom-H Dep ~ Age of smoking initiation *Fagertstrom-Med Dep	0,187	12,969	2,384	< 0,0001	Yes
Age of smoking initiation*Fagertstrom-H Dep ~ Age of smoking initiation*Fagertstrom-No Dep	0,121	4,207	2,384	0,000	Yes
Age of smoking initiation*Fagertstrom-No Dep ~ Age of smoking initiation*Fagertstrom-Med Dep	0,066	4,554	2,384	< 0,0001	Yes
Duration of cigarette smoking *Fagertstrom-H Dep ~ Duration of cigarette smoking*Fagertstrom-Med Dep	0,071	5,427	2,383	< 0,0001	Yes
Duration of cigarette smoking*Fagertstrom-H Dep ~ Duration of cigarette smoking*Fagertstrom-No Dep	0,026	2,576	2,383	0,031	Yes
Duration of cigarette smoking*Fagertstrom-No Dep ~ Duration of cigarette smoking*Fagertstrom-Med Dep	0,044	6,683	2,383	< 0,0001	Yes

Table 9. Hematological parameters in three categories of subjects: Begin smoking at adulthood (n=18)([23-32]), Begin smoking at childhood (n=48)([5-11]) and Begin smoking at adolescent age (n=30)([12-17])

Hematological	WBCmm3	Lymphocytes %	Monocytes %	Neutrophiles %	Hte (%)	HB
Ch	9087,5±2420,359	31,025±4,835	9,9125±1,8193	59,0625±4,939	40,935±15,889	15,375±0,963
Ads	10040±2332,321	30,38±7,636	7,84±1,714	61,78±9,254	35,708±17,996	14,28±0,748
Adt	11433,333±540,152	34,4±0,222	7,9±0,550	57,7±0,436	43,566±1,480	13,9±0,877

We can see that the p-value for each pamaremer > 0.05. This confirms the weak impact of all parameters on the anxiety model.

3.3 Variation of Hematological Parameters in Three Categories of Subjects: Begin Smoking at Adulthood (n=18)([23-32]), Begin Smoking at Childhood (n=48)([5-11]) and Begin Smoking at Adolescent Age (n=30)([12-17]) (ANOVA Analysis)

We recorded a significant decrease of WBC (p< 0, 0001), lymphocytes (p< 0, 0001) and Htc (p< 0, 0001) in Ch versus Adt and significant increase of monocytes (p< 0,0001) and Hb (p< 0,0001)(Tables 9 &10).

Table 10. Comparison between hematological parameters (Newman-Keuls (SNK) test) in three categories of subjects: Begin smoking at adulthood (n=18)([23-32]), Begin smoking at childhood (n=48)([5-11]) and Begin smoking at adolescent age (n=30) ([12-17])

Hematological	Modalités	Difference	Difference reduced	Critical value	Pr. > Diff	Significant
WBCmm3	Adt ~ Ch	2345,833	3,911	2,382	0,001	Yes
	Adt ~ Ads	1393,333	2,153	1,986	0,034	Yes
	Ads ~ Ch	952,500	1,886	1,986	0,062	No
Lymphocytes (%)	Adt ~ Ads	4,020	2,461	2,382	0,041	Yes
	Adt ~ Ch	3,375	2,229	1,986	0,028	Yes
	Ch ~ Ads	0,645	0,506	1,986	0,614	No
Monocytes (%)	Ch ~ Ads	2,073	5,475	2,382	< 0,0001	Yes
	Ch ~ Adt	2,013	4,477	1,986	< 0,0001	Yes
	Adt ~ Ads	0,060	0,124	1,986	0,902	No
Neutrophiles (%)	Ads ~ Adt	4,080	2,189	2,382	0,078	No
	Ads ~ Ch	2,717	1,868			No
	Ch ~ Adt	1,363	0,789			No
Hct	Ch ~ Adt	3,308	4,202	2,382	0,000	Yes
	Ch ~ Ads	2,395	3,612	1,986	0,000	Yes
	Ads ~ Adt	0,913	1,075	1,986	0,285	No
HB	Ch ~ Adt	1,475	6,025	2,382	< 0,0001	Yes
	Ch ~ Ads	1,095	5,311	1,986	< 0,0001	Yes
	Ads ~ Adt	0,380	1,439	1,986	0,154	No

3.4 Relationship between Depression Inflammatory Parameters (χ^2-test)

Tables 11 shows the frequency and percentage of different levels of depression (level 0: Not depressive (n=42), level 1: Early depression (n=36), level 2: Strongly depressed state (n=18)). We note that the depression (level 2) is the most representative with the following frequencies: ESR ≥20mm First hour: 12; ESR ≥60mm Second hour: 12; CRP - positive: 12.

Table 11. Relationship between depression inflammatory parameters: Table combinations of terms

Line	Column	Frequency	Percentage
ESR ≥20mm First hour - 0	Depression - 0	36	12,50
	Depression - 1	30	10,42
	Depression - 2	6	2,08
ESR ≥20mm First hour - 1	Depression - 0	6	2,08
	Depression - 1	6	2,08
	Depression - 2	12	4,17
ESR ≥60mm Second hour - 0	Depression - 0	36	12,50
	Depression - 1	30	10,42
	Depression - 2	6	2,08
ESR ≥60mm Second hour - 1	Depression - 0	6	2,08
	Depression - 1	6	2,08
	Depression - 2	12	4,17
CRP - Négative	Depression - 0	36	12,50
	Depression - 1	36	12,50
	Depression - 2	6	2,08
CRP - positive	Depression - 0	6	2,08
	Depression - 1	0	0,00
	Depression - 2	12	4,17
	Total	288	100

χ^2-test : χ^2 (observed value) ≥ 77,128; χ^2 (critical value): 18,307: DF: 10; p-value unilateral: < 0,0001; Alpha: 0,05. this demonstrates that depression is highly dependants of these inflammatory parameters.

4. DISCUSSION

Several studies found that smoking is highly correlated with the development of depression [19] and recent studies indicate that both the offspring of women who smoke and adolescent smokers are more susceptible to depression in later life [20, 21]**,** whereas those who initiate smoking later in life are not [20].

In our study we have two interesting points emerged. Firstly we found that there is a close relationship between the level of nicotine addiction, anxiety levels, and the emergence of depressive disorders (Tables 5,6,7,8). Secondly results of our studies clearly suggest an elevated risk of mood disorders in subjects who began smoking in adulthood age and late adolescence versus childhood and having a very strong nicotine addiction (Tables 3,4,6,7). It is important to note that subjects, who started smoking at age child, did not develop depression.

Three complementary hypotheses can be advanced to explain these associations:

Cholinergic theory of depression evoked by Peter et al. [9]. This is based on the results of the last decades, namely that physostigmine (an acetylcholinesterase inhibitor (AChE-I)) could exacerbate a depressed mood (although some later investigations with other acetylcholinesterase inhibitors found the opposite trend) and that elevated choline (the rate-limiting precursor to acetylcholine) levels were found in the brains of patients with depression [24,25,26]**.** The neurobiological link between depression and the cholinergic system is also supported by the potent antidepressant activity of muscarinic antagonist scopolamine.

Moreover, investigations have found exaggerated neuroendocrine and pupillary responses among patients with mood disorders after administration of cholinomimetic agents. In addition, ACh facilitates the release of several stress-sensitive transmitter molecules (i.e. corticosterone, ACTH, and CRF) [26,27]**.** Serotonin plays a key role in the coordination of responses to stress [28] and there is a good evidence that in several brain areas nicotine increases release of 5-HT, which, in its turn affects as a neurotransmitter the manifestation of nicotine effects [29].

One theory suggests the ‘‘depressogenic’’ effect of some tobacco ingredients is responsible for the frequent co-occurrence of depression and smoking, and there are several results that support this. Malone et al. [30] reported that cigarette smoking is associated with impaired serotonin function in depressed patients [30]**.** Chronic stress and depression are associated with the dysregulation of the hypothalamo–pituitary–adrenal (HPA) axis [31]. While smoking has a strong influence on the activity of the HPA axis, this effect could be another link between smoking and depression [32,33].

It is increasingly evident that brain development, in the form of cell acquisition, apoptosis, synaptogenesis and programming of synaptic activity, all continue into adolescence [34,35]**.** It is possible that nicotine, acting on cholinergic receptors (ie at the synaptic level) is involved in the maturation of the nervous system or in the implementation of the stress response system (CNS, HPA axis, ...) and nicotine deprivation in the adult can cause damage in CNS. Studies with psychoactive drugs other than nicotine show that the adolescent brain responds differently from that of the adult [36,37].

The field involving the immune system and its interactions with CNS and depression is not sufficiently explored. Several studies have suggested that inflammatory responses play an important role in the pathophysiology of depression. In fact, depressed patients show higher levels of biomarkers of inflammation [38]. Some of the effects of smoking on immune function reflect the direct actions of nicotine [39,40]. Far less information is available concerning developmental exposure to nicotine. In the offspring of women who smoke, there are significant alterations in the cellular makeup of cord blood samples and in IgE levels [41,42,43] as well as a high rate of gene mutations in T cells [44], although it is obviously difficult to ascertain whether these effects reflect the actions of nicotine as compared to other smoke components. With the same prenatal nicotine exposure model that elicits changes in CNS function in the offspring, mitogenic responses of T cells and B cells showed impairment lasting into adulthood [45]. The key question, then, is whether nicotinic cholinergic stimulation in adolescence similarly causes long-term impairment of immune function.

5. CONCLUSION

In our study, the inflammatory profile estimated by measuring the white blood cells, lymphocytes, monocytes, neutrophiles and Hb (Tables 9 and 10) and Serum C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR) (Table 11), we found that inflammation primarily affects the Adt subjects and secondarily the Ads subjects. The inflammatory profile appears very interesting in the management of subjects when stop smoking [35]. Future studies will need to address the functional consequences of immune impairment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Hospital Anxiety and Depression Scale (HADS)

A/ I feel tense or "wound up":
Most of the time 3
A lot of the time 2
From time to time, occasionally 1
Not at all 0

D/ I still enjoy the things I used to enjoy:

Definitely as much 0
Not quite so much 1
Only a little 2
Hardly at all 3

A/ I get a sort of frigHctned feeling as if something awful is about to happen:

Very definitely and quite badly 3
Yes, but not too badly 2
A little, but it doesn't worry me 1
Not at all 0

D/ I can laugh and see the funny side of things:

As much as I always could 0
Not quite so much now 1
Definitely not so much now 2
Not at all 3

A/ Worrying thoughts go through my mind:

A great deal of the time 3
A lot of the time 2
From time to time, but not too often 1
Only occasionally 0

D/ I feel cheerful:

Not at all 3
Not often 2
Sometimes 1
Most of the time 0

A/ I can sit at ease and feel relaxed:
Definitely 0
Usually 1
Not Often 2
Not at all 3

D/ I feel as if I am slowed down:

Nearly all the time 3
Very often 2
Sometimes 1
Not at all 0

A/ I get a sort of frigHctned feeling like 'butterflies' in the stomach:

Not at all 0
Occasionally 1
Quite Often 2
Very Often 3

D/ I have lost interest in my appearance:

Definitely 3
I don't take as much care as I should 2
I may not take quite as much care 1
I take just as much care as ever 0

A/ I feel restless as I have to be on the move:
Very much indeed 3
Quite a lot 2
Not very much 1
Not at all 0
D/ I look forward with enjoyment to things:

As much as I ever did 0
Rather less than I used to 1
Definitely less than I used to 2
Hardly at all 3

A/ I get sudden feelings of panic:

Very often indeed 3
Quite often 2
Not very often 1
Not at all 0

D/ I can enjoy a good book or radio or TV program:

Often 0
Sometimes 1
Not often 2
Very seldom 3
Scoring (add the As = Anxiety. Add the Ds = Depres-sion). The norms below will give an idea of the level of Anxiety and Depression.
0 - 7 = Normal
8 - 10 = Borderline abnormal
11 - 21 = Abnormal

Fagerstrom Test for Nicotine Dependence

The Fagerstrom test consists in six questions to measure the level of nicotine dependence. The total score is be-tween 0 and 10, and the interpretation of test score is:
Score 0 to 2: the subject is not addicted to nicotine, it can often stop smoking without using nicotine replace-ment therapy.

Score of 3 to 4: the subject is weakly dependent on nicotine.
Score of 5 to 6: the subject is moderately dependent on nicotine.
Score of 7 to 8: the subject is highly dependent on nicotine.
Score of 9 to 10: the subject is very heavily dependent on nicotine

Questions of the Test

Is smoking "just a habit" or are you addicted? Take this test and find out your level of dependence on nicotine.
1. How soon after you wake up do you smoke your first cigarette?
After 60 minutes (0)
31 - 60 minutes (1)
6 - 30 minutes (2)
Within 5 minutes (3)
2. Do you find it difficult to refrain from smoking in places where it is forbidden?
No (0)
Yes (1)
3. Which cigarette would you hate most to give up?
The first in the morning (1)
Any other (0)
4. How many cigarettes per day do you smoke?
10 or less (0)
11 - 20 (1)
21 - 30 (2)
31 or more (3)
5. Do you smoke more frequently during the first hours after awakening than during the rest of the day?
No (0)
Yes (1)
6. Do you smoke even if you are so ill that you are in bed most of the day?
No (0)
Yes (1)

Scores of the Fagerstrom Test for Nicotine Depen-dence

Your score was: _____
Your level of dependence on nicotine is:
0 - 2 Very low dependence
3 - 4 Low dependence
5 Medium dependence
6 - 7 High dependence
8 - 10 Very high dependence

Scores under 5: “Your level of nicotine dependence is still low. You should act now before your level of de-pendence increases.”

Score of 5: “Your level of nicotine dependence is moderate. If you don’t quit soon, your level of depend-ence on nicotine will increase until you may be seriously addicted. Act now to end your dependence on nicotine.”

Score over 7: “Your level of dependence is high. You aren’t in control of your smoking it is in control of you. When you make the decision to quit, you may want to talk with your doctor about nicotine replacement therapy or other medications to help you break your addiction.”

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