GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

STUDY OF THE INTERACTION BETWEEN MARKERS OF OXIDATIVE STRESS AND SMOKING IN MIXED ANXIOUS DEPRESSIVE DISORDER

PhD THESIS ABSTRACT

SCIENTIFIC COORDINATOR
Prof. Univ. Dr. Cristinel ȘTEFĂNESCU

PhD Student
Letiția TROFOR

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LIST OF ABBREVIATIONS

ICD-10	International Classification of Diseases
ROS	Reactive oxygen species
MDA	Malondialdehide
SOD	Superoxide dismutaze
CAT	Catalaze
TRx	Tioredoxine
GPx	Glutation peroxidaze
NO	Nitric oxide
QH	Semichinone
O2	Oxygen
HO	Hidroxide
CO	Carbon monoxide
TBARS	Tiobarbituric acid
F2-IsoPs	F2-Isoprostan
WHO	World Health Organisation
MADD	Mixed anxious and depressive disorder
GABA	Gamma-aminobutiric acid
CSF	Cerebrospinal fluid
BDI	Beck Depression Inventory
HAM-D	Hamilton Depression Scale
HAM-A	Hamilton Anxiety Scale
SSRI	Selective serotonine receptors inhibitors
SSRN	Selective norepinephrine receptors inhibitors
NRT	Nicotine replacement therapy
PY	Packs-years
DL	Detection limit
QL	Quantification limit
VIT C	Vitamin C
UA	Uric acid
CHOL	Cholesterole
TLG	Triglycerides
CREAT	Creatinine
WBC	White blood cells
RBC	White red cells
HB	Hemoglobin
HCT	Hematocrit
PLT	Thrombocytes
VSH	Erythrocyte sedimentation rate

The PhD thesis includes:

- 124 pages – of which 43 pages of General Aspects;
- List of Abbreviations;
- 38 figures divided as follows: chapter VIII – 8 figures, chapter IX – 10 figures, chapter X – 6 figures , chapter XI – 6 figures , chapter XII - 7 figures, chapter XIII – 1 figure;
- 30 tables divided as follows: chapter VIII – 1 table, chapter IX– 14 tables, chapter X – 1 table, chapter XI – 6 tables, chapter XII - 2 tables, chapter XIII – 6 tables;
- 196 bibliographical references;
- 4 Annexes (smoking status evaluation form, Fagerström nicotine dependence test, Hamilton depression rating scale, Hamilton anxiety rating scale).

Note: In the current PhD thesis abstract, the table of contents, the numbering of selected figures and the abbreviation list are kept under the original form, as in the PhD thesis.

Key words: *oxidative stress, smoking, depression, anxiety, nicotine dependence.*

INTRODUCTION. CURRENT STATE OF KNOWLEDGE

The oxidative stress, defined as the unbalance between the oxidants and antioxidants in favor of the oxidants, which leads to a break in signaling and redox control, as well as molecular distructions, has a potential etiopathogenic role in some psychiatric disorders. It seems that the brain is particularly vulnerable to the oxidative stress, aspect explained by the reduced antioxidant levels and high concentrations of polyunsaturated fatty acids, with an increased need for oxygen. (Pădurariu M, 2010).

On the other hand, the oxidative stress has also been associated with tobacco exposure. Thus, smoking determines the formation of oxidative stress through at least two means: direclty, through the free radicals contained in cigarette smoke and indireclty through the inflammatory answer that they generate (Bruno RS, 2006).

The impact of oxidative stress markers in mixed anxious and depressive disorder depresivă (MADD) has been very less studied until now. Tipical for major depressive disorder is the presence of a high level of some oxidative stress markers such as 8-hidroxideoxiguanozin and malondialdehide. Also, there is a low antioxidant level quantified. (Bakunina N, 2015).

Smoking is a delicate aspect in patients with MADD, because of the somatic implications and also through the emotional charge that tobacco consumption and quitting smoking may have on these patients. For these patients, there is a higher smoking prevalence, a more severe nicotine dependence and a lower abstinence rate (Jamal M, 2012).

Showing the relationship between the oxidative stress levels and smoking in patients with mixed anxious and depressive disorder could be the foundation for future studies to evaluate the eficacy of selective antioxidant therapies in patients with MADD, taking into consideration the smoking non-smoking status.

PERSONAL CONTRIBUTIONS

CHAPTER VI. MOTIVATION AND RESEARCH OBJECTIVES

Research motivation

In the current context of research on the importance of smoking and oxidative stress in psychiatric pathology associated with depressive and anxiety disorders, the present study aims to advance the problem by analyzing the relationship between smoking and the level of oxidative stress in psychiatric disorders.

Our scientific activity is justified by the frequency of the disorder studied in psychiatric practice, but also by the impact of the mixed anxious-depressive disorder on the patient's functionality. Highlighting potentially modifiable factors, such as oxidative stress or smoking, which negatively affect the evolution of the condition, could lead to an efficient management of these patients. Also, this study may be useful for further research on targeted psychiatric therapies, applied and depending on the smoking status of the patient.

Most of the current research regarding smoking, mental illness and oxidative stress is focused on each aspect, not emphasizing the importance of the link between these factors.

Given the exponential increase in the number of psychiatric patients diagnosed with depression and anxiety, smoking disorder and the recent discovery of the links between these factors, the study may be considered appropriate under these conditions.

Research objectives

The present study aims to investigate the importance of oxidative stress in patients with mixed anxious and depressive disorder. The assessment of oxidative stress level in patients diagnosed with mixed anxious and depressive disorder was performed by dosing of the main antioxidant enzymes and lipid peroxidation markers such as malondialdehyde (MDA).

The second objective is justified by the importance and frequency of smoking in patients suffering from anxiety and depression, as well as the possible complications in the prognosis and clinical evolution of these conditions. Thus, the second part of the study includes an extensive analysis on the impact of smoking in patients with mixed anxiety and depressive disorder.

The third objective concerns the correlation between oxidative stress and smoking in patients with mixed anxious and depressive disorder, following aspects such as: intensity of smoking (quantified by number of packs-years, score of Fagerström nicotine dependence test, assessment of recent consumption - smoking in the last 12 months - and nicotine withdrawal syndrome), the study of biomarkers of exposure to smoking, the HAM-A / HAM-D score for the evaluation of symptoms of anxiety and depression.

CHAPTER VII. STUDY DESIGN

Work hypothesis

Smoking patients with mixed anxious and depressive disorder have a much higher level of oxidative stress than non-smokers and respond much harder to psychiatric treatment as well as to nicotine dependence treatment.

Patients with mixed anxious and depressive disorder have a higher level of oxidative stress, as demonstrated by dosing the main antioxidant enzymes and lipid peroxidation markers, such as MDA, as well as two non-enzymatic biomarkers, vitamin C and uric acid.

The impact of oxidative stress on depressive and anxiety disorders may have different implications depending on the presence of tobacco consumption and its intensity.

Also, oxidative stress could influence the clinical evolution and severity of these psychiatric disorders directly proportional to the severity of the depression rate score, respectively anxiety, evaluated by the specific scales.

Nevertheless, exposure to passive smoking could result in changes in serum MDA concentrations.

Source population and patients studied

The studied population included patients adressed to the Institute of Psychiatry „Socola" Iasi, for depressive and anxiety symptoms, between October 2017 and March 2018

Aspects studied

- Demographic data such as: age, birth place, sex, education etc.
- Intensity of tabacco consumption (quantified by the number of packs-year, Fagerström nicotine dependence score, evaluation of recent tobacco consumption)

- Study of biomarkers of tobacco exposure (carbon monoxide in the exhaled air, uric acid)
- Anxiety and depression levels, quantified by the specific rating scales: HAM-A, HAM-D
- Study of oxidative stress biomarkers.

The oxidative stress markers that are easy to identify in biological fluids are the aldehides. One of the most studied aldehides is the malondialdehide (MDA) (Prelipceanu D 2011). In the present study we determined the MDA concentrations, as well as two non-enzymatic markers, the vitamin C and seric uric acid.

Methodology

The results of personal contributions are presented in six chapters, which analyze the clinical aspects of oxidative stress in smokers and non-smokers patients diagnosed with MADD.

Thus, in Chapter VIII of the personal part, a demographic study of the selected patients was performed, with a statistical analysis, to be able to quantify the severity of the depressive symptoms in the MADD according to demographic factors such as: age, social status, marital status and area of residence.

In Chapter IX, the biochemical determination of lipid peroxidation markers was performed, as well as oxidative and antioxidant parameters of major importance in the assessment of oxidative stress in MADD; the chapter covers the methodology for developing and validating the method for determining the serum MDA using UV-VIS spectrophotometry, as well as developing and validating the method for quantifying plasma Vitamin C and determining the biochemical parameters: total cholesterol, triglycerides, uric acid and serum creatinine.

In Chapter X, a clinical evaluation of oxidative stress parameters and serum concentrations of total cholesterol, triglycerides, creatinine in smoking and non-smoking patients diagnosed with MADD is performed.

Chapter XI includes an extensive analysis of the impact of smoking on patients diagnosed with MADD.

Chapter XII presents the tools for determining depression and anxiety scores, HAM-D and HAM-A, respectively, in smokers and non-smokers, with the evaluation of the variability of the concentrations of the MDA, vitamin C and uric acid parameters, according to the scores HAM-A and HAM-D.

In Chapter XIII a brief study was performed regarding passive smoking in the context of oxidative stress in patients with MADD.

CHAPTER VIII. DEMOGRAPHIC ANALYSIS OF PATIENTS DIAGNOSED WITH MADD

VIII.1. Introduction

The purpose of this study was to identify possible vulnerability factors and possible sources of exposure that could have a significant impact on the development of mixed anxious and depressive disorder (MADD). Thus, smoking and non-smoking subjects diagnosed with MADD were evaluated, taking into account age distribution, gender distribution, aria of residence, marital status, as well as exposure to tobacco smoke.

VIII.2. Results

Demographic study of the investigated patients

This study comprised 31 patients diagnosed with mixed anxious and depressive disorder. Patients included in the study were between the ages of 28-76 with a mean of 57.61 ± 13.41, a landmark close to the median value (60 years), which shows a normal distribution of the study group. The gender distribution of the studied group of patients is 68% women and 32% men. Within the studied group, the patients in the urban area represent 61% while the patients in the rural area 39%. The classification by age group shows that there is a predominance of patients in the urban area, in the age groups 45-58 years respectively 60-76 years, while in the group of patients 28-31 years, the number of patients in the rural area is equal with the urban residency.

Smoking patients accounted for 68% and non-smokers 32%. The classification of smoking and non-smoking patients according to age shows a predominance of smoking for patients aged between 44-58 years and among very young patients 28-31 years. The linear regression curve between the values for the quantification of HAM-D and the age of the patients diagnosed with TMAD obtained a moderate correlation between age and the HAM-D score ($r = 0.35$, $p<0.05$) in the smoking and non-smoking patients diagnosed with MADD. The association between the demographic parameters and the risk of depression is shown in Table I.

Table I. The risk of depression associated with demographic factors - statistical parameters

Parameter	Relative risk	Confidence interval CI 95%
Gender		
Female	2,5	1,5-2,8
Male	1,1	1,5-2,4
Social statute		
Household	1,3	0,7-1,9
Retired	1,1	0,8-2,0
Employee	0,5	0,3-1,0
Marital status		
Married	0,8	0,5-1,2
Divorced	1,7	1,1-2,0
Unmarried	1,9	1,2-2,5
Widowe	1,3	1,0-1,9
Residence		
Urban	1,5	0,8-2,1
Rural	0,9	0,2-1,3
Age		
28-31 years	0,9	0,3-1,1
44-58 years	1,3	0,8-1,5
60-76 years	1,9	0,7-2,5

VIII.3. DISCUSSIONS

Assessing the risk of depression and anixety by demographic factors

This study approaches as a novelty the quality of life analysis for smokers as well as non-smokers diagnosed with anxious and depressive disorder. The scales used to assess anxiety and depression were HAM-A, respectively HAM-D. These scales quantify anxiety and depression,

respectively, when questioning each subject investigated. We describe a statistical correlation ($r = 0.35$, $p<0.05$) between age and the scale for depression evaluation, HAM-D.

The assessment of the relative risk of depression associated with the different demographic factors showed a 2-fold higher prevalence of the risk of depression for females compared to males. The incidence of depression and anxiety according to the marital status of the subjects investigated in the present study does not differ significantly from other countries, and the data obtained are in accordance with the literature (Fluharty M, 2017). At the same time, there was a significant statistical correlation between the number of packs-years and the marital status in smokers.

VIII.4. Conclusions

- From the analyzed data it was found that the female gender implies a higher risk of depression and anxiety compared to the male one;
- With regard to the age of the investigated patients, a higher prevalence of depression was observed for the patients between 60-76 years;
- The risk of depression associated with demographic factors shows that unmarried people have a higher incidence in the development of this condition compared to married people.

CHAPTER IX. CLINICAL QUANTIFICATION OF THE EVALUATION PARAMETERS OF OXIDATIVE STRESS FOR SMOKERS AND NON-SMOKERS DIAGNOSED WITH MADD

IX.1. Introduction

In this chapter, the clinical methodology of quantification and validation of serum MDA as well as the quantification and clinical validation of the method of determining plasma vitamin C in smoking and non-smoking patients diagnosed with MADD were performed.

IX.2. Clinical determination of serum MDA in smokers and non-smokers diagnosed with MADD

MDA is a strongly reactive 3-carbon dialdehyde, which can be present in many biological samples (serum, plasma, urine) and has become one of the most used indicators to estimate the effects of oxidative stress on lipids. The method of quantification of MDA from human serum was performed by UV-VIS spectrophotometry (Dobrin I 2010). Concentrations for malondialdehyde were expressed in nmol / mL. The experimental values are presented in Table VII.

Table VII. Experimental values determined for MDA

	P1	P2	P3
Malondialdehyde			
% from the target concentration-SR1	40	80	100
nmol alondialdehyde / mL	0.4	0.8	1.0
Series 1	0.0709	0.1846	0.2347
Series 2	0.0709	0.1843	0.2313
Series 3	0.0709	0.1835	0.2306

IX.3. Clinical determination of serum Vitamine C in smokers and non-smokers diagnosed with MADD

Determination of vitamin C in human serum by the method of High Pressure Liquid Chromatography

Ascorbic acid, a reference substance obtained from Sigma-Aldrich that had a purity of at least 99%, as well as plasma certified for the control samples, were used to carry out the study. The values obtained are shown in Table IX.

Table IX. Standard concentrations determined in the calibration curve of vitamin C for normal and pathological level

	Physiological field		Pathological field			
Concentration mg/L	3	5	10	15	20	25
Concentration µg / mL sample analyzed	0.75	1.25	2.5	3.75	5	6.25
Series 1 (areas)	39.9	50.2	85.26	136.25	168.52	199.25
Series 2 (areas)	40.4	51.93	85.98	135.93	170.63	195.23
Series 3 (areas)	38.9	51.02	84.26	134.42	164.25	197.25
Average (areas)	39.73	51.05	85.16	135.53	167.8	197.24

IX.4. Determination of uric acid in MADD patients smokers and non-smokers

In this study, serum uric acid determination was performed spectrophotometrically using the Cobas Integra 400 plus automatic analyzer, Roche Diagnostics (Roche, USA). Serum uric acid normal values are based on sex, so we have a reference range of 2.6-5.7 mg / dL in women and 3.5-8.2 mg / dL in men.

IX.5. Determination of total cholesterol in smokers and non-smokers diagnosed with MADD

The determination of total cholesterol was performed by the colorimetric enzymatic method, using the Cobas Integra 400 plus analyzer. The baseline values for total cholesterol in venous blood vary by sex, age, diet and geographical region and range from 100-200 mg / dL.

IX.6. Determination of triglycerides in MADD patients, smokers and non-smokers

Serum triglyceride determination was performed spectrophotometrically using the Cobas Integra 400 plus automatic analyzer, Roche Diagnostics (Roche, USA). The reference values for serum triglycerides are 35-160 mg / dL.

IX.7. Determination of serum creatinine in MADD patients, smokers and non-smokers

Serum creatinine determination was performed spectrophotometrically using the Cobas Integra 400 plus automatic analyzer, Roche Diagnostics (Roche, USA). The reference values for serum creatinine in women are in the range of 0.5-0.9 mg / dL, and in men they are between 0.6-1.3 mg / dL.

CHAPTER X. CLINICAL EVALUATION OF OXIDATIVE STRESS PARAMETERS IN SMOKERS AND NON-SMOKERS DIAGNOSED WITH MADD

X.1. Introduction

In this chapter, an evaluation of serum MDA, the most important marker of lipid peroxidation studied in the evaluation of oxidative stress was performed, as well as for two non-enzymatic markers: vitamin C and serum uric acid.

X.2. Results

X.2.1 Profile of the biochemical and of the evaluation parameters for oxidative stress

Within this chapter we analyzed the distribution of concentrations of MDA, Vitamin C and uric acid related to the interval of biological reference (IBR). Serum concentrations of total cholesterol, triglycerides, serum creatinine were also evaluated, with a concentration distribution

in smoking and non-smoking patients diagnosed with MADD. The results of the descriptive statistics of the investigated subjects are presented in Table XVI.

Table XVI. Statistical analysis of the investigated biochemical parameters for the group of smoker patients and non-smokers diagnosed with TMAD

Statistical data	MDA	VIT C	AU	CHOL	TLG	CREAT
Reference ranges (RR)	**0-1 nmol/mL**	**4,6-14,9 mg/L**	**2,5-8,1 mg/dL**	**100-200 mg/dL**	**40-165 mg/dL**	**0,5-1,1 mg/dL**
GROUP OF SMOKERS						
Number of cases	19	19	19	19	19	19
Minimum	0,29	0,02	3,40	149	76,39	0,49
Maximum	2,43	7,01	7,05	289	186	1,35
Average	1,51	1,32	5,08	199,28	131,60	0,80
Standard deviation	0,67	1,47	2,75	41,06	58,5	0,23
Median	1,73	1,08	5,30	190	190	0,78
Values >RR	14	0	4	6	2	4
Values < RR	0	17	0	0	0	2
Values in RR	5	2	15	13	17	13
GROUP OF NON SMOKERS						
Number of cases	12	12	12	12	12	12
Minimum	0,27	0,02	3,2	111	44,56	0,47
Maximum	2,38	7,01	6	290	181,30	0,82
Average	1,11	1,10	6,00	197,2	101,51	0,65
Standard deviation	0,82	1,56	1,73	45,12	40,96	0,11
Median	0,97	1,00	4,5	190	91,70	0,65
Values >RR	6	0	0	4	1	0
Values < RR	0	11	0	0	0	4
Values in RR	6	1	12	12	11	8

X.3. Discussions

In the non-smoking patients diagnosed with MADD, 6 of them had the mean concentration value for serum MDA higher than the biological reference range and 6 patients had the value in the biological reference range. Similar values for serum MDA were determined by Ashutosh Bajpai et al. (Bajpai A, 2014), as they reported a value of 1.95 ± 1.04 nmol / mL in patients diagnosed with major depression. The very low values of the median concentration for vitamin C determined in human plasma in smokers (median = 1.08 mg / L) as well as in non-smokers (1 mg / L), represent values that are in accordance with the data reported in the literature and confirm the presence of oxidative stress in depressive and anxiety disorders.

The low levels of serum uric acid in smokers with MADD may be due to decreased endogenous uric acid production as a result of chronic exposure to cigarette smoke, which is a

significant cause of oxidative stress. Creatinine levels were significantly increased in active smokers ($p < 0.01$). Smoking determines an increased risk for increased serum total cholesterol, as well as for triglyceride levels. The literature findings reported statistically significant differences ($p<0.001$) between the concentrations of total serum cholesterol in smokers (173.44 ± 78.64 mg / dL) compared to non-smokers (115.9 ± 47.67 mg / dL) (Joshi N, 2013).

X.4 Conclusions

- The values of serum MDA concentrations determined in 73% of smokers diagnosed with MADD were higher than the established biological reference interval;
- Plasmatic vitamin C levels were very low for 90% of smokers and non-smokers diagnosed with MADD;
- The values of the serum uric acid concentrations for smoking patients were lower than for non smoking patients, due mainly to the decrease of the endogenous production;

CHAPTER XI. THE IMPORTANCE AND PREVALENCE OF SMOKING IN ANXIOUS AND DEPRESSIVE PATIENTS. POSSIBLE COMPLICATIONS IN THE PROGNOSIS AND CLINICAL EVOLUTION OF THESE DISORDERS. AN EXTENDED ANALYSIS OVER THE IMPACT OF SMOKING IN PATIENTS WITH MADD.

XI.1. Introduction

Epidemiological studies indicate a higher frequency of smoking in patients with depression and anxiety than in the general population. A history of psychiatric illness may increase the risk of early onset of smoking and may contribute to the faster onset of nicotine dependence (Brown RA 1996).

XI.2. Objectives

In this chapter, we aimed to evaluate the oxidative stress parameters investigated according to the Fagerström nicotine dependence score in smoking patients diagnosed with MADD, as a new element of the research in this field. Another objective that supports the original part of the study will be represented by analyzing the parameters of assessment of oxidative stress in smokers and non-smokers with MADD, according to the depression score (HAM-D) and to the anxiety score (HAM-A).

XI.3. Hypotheses and working tools - clinical and laboratory assessment tools for smoking status, depression and anxiety assessment scales

The clinical diagnosis of tobacco consumption and dependence is based on: determining the smoking status (occasional smoker, non-smoker, current smoker, former smoker), the type of tobacco product used, as well as assessing the intensity of tobacco consumption.

The severity of depressive symptoms is assessed using the Hamilton Scale of Depression scoring, which contains questions about the patient's mental state, sleep disturbances, daily activities and cognition, especially attention and focus. The final score is divided into three levels of severity: mild, medium and severe.

Anxiety disorders are assessed using the Hamilton Anxiety Measurement Scale, which contains questions about phobias, fears, a history of panic attacks, if any, as well as questions about their influence on daily life. Like the depression scale, there are 3 degrees of severity: mild, medium and severe.

XI.4. Results

Table XVII. Statistical analysis of the evaluation parameters of MADD and tobacco dependence for the group of patients investigated

Statistical data	HAM-D	HAM-A	CO in exhaled air	Fagerström Test	Number of packs years - PY	Number of cigarettes per day
Reference ranges (RR)	**0-7**	**0-13**	**0-4 ppm**	**0-3**	-	-
GROUP OF SMOKERS						
Number of cases	19	19	19	19	19	19
Minimum	12	17	10	2	6	5
Maximum	26	41	21	8	40	20
Average	17,47	29,63	14,21	5,16	17,72	11,56
Standard deviation	4,25	5,20	3,60	1,61	11,15	5,71
Median	16	29	14	6	13,50	10
GROUP OF NON SMOKERS						
Number of cases	12	12	0	0	0	0
Minimum	14	17				
Maximum	25	41				
Average	18,75	30,67				
Standard deviation	3,08	5,53				
Median	18,50	31				

Table XVII shows very high values of the HAM-A anxiety assessment score, determining a minimum of 17 and a maximum of 41, with an average value of 29.63. The HAM-D depression evaluation score recorded values in the range of 12-26, much higher than the biological reference range (0-7), with an average value of 17.47. From the analysis of the presented data, we also observe that for smoking patients an average carbon monixide (CO) concentration in the exhaled air of 14.21 ppm was determined, with values between 10-21 ppm,

values much higher than the biological reference interval (0-4 ppm).), and the value of the PA number was between 6-40.

Factor analysis applied to subjects diagnosed with MADD

The aim was to apply the factor analysis in order to explain the variation of the parameters used in the assessment of oxidative stress. The results of the Spearman correlations between the clinical parameters investigated for the 31 subjects diagnosed with MADD are presented in Table XXI. By applying the correlation matrix between the investigated clinical parameters, relatively good correlations were obtained: r = 0.39-0.84; p <0.05.

Table XXI. The correlation matrix between the investigated clinical parameters in the patients with MADD (p <0.05).

Variable	CO	MDA	VIT C	TGL	CHOL	UA	CREAT
CO	1,000						
MDA	r=0,21 p=0,281	1,000					
VIT C	r=0,27 p=0,14	r=0,28 p=0,121	1,000				
TGL	r=0,53 p=0,005	r=0,28 p=0,124	r=0,18 p=0,330	1,000			
CHOL	r=0,65 p=0,000	r=0,57 p=0,000	r=0,39 p=0,028	r=0,55 p=0,028	1,000		
UA	r=0,70 p=0,002	r=0,59 p=0,000	r=0,42 p=0,018	r=0,70 p=0,000	r=0,83 p=0,000	1,000	
CREAT	r=0,55 p=0,000	r=0,59 p=0,000	r=0,39 p=0,025	r=0,66 p=0,000	r=0,77 p=0,000	r=0,84 p=0,000	1,000

XI.5. Discussions

The results of the Shapiro-Wilk´s W-test normality test show that the data analyzed in the study are not normally distributed, p>0.05. Regarding the analysis performed in Table XXI, we tried to generate the most important variables that are statistically correlated, for a p <0.05. As noted, the most important statistical correlations are the CO concentration with the following biochemical parameters: serum triglyceride concentration (r = 0.53; p = 0.005), total serum cholesterol concentration (r = 0.65; p = 0.000) , serum uric acid concentration (r = 0.70; p = 0.002), and respectively serum creatinine concentration (r = 0.55; p = 0.000). MDA was statistically correlated with the following biochemical parameters: value of total serum cholesterol (r = 0.57, p = 0.000), value of serum uric acid (r = 0.59: p = 0.000), value of serum creatinine (r = 0.59 ; p = 0.000) (Table XXI). The presence of lipid peroxidation in major depression has important implications. Thus, by analyzing the statistical correlations obtained in

the case of smokers and non-smokers with MADD from the present study, a direct linear relationship between the evaluated enzymatic and non-enzymatic markers can be identified. These results suggest that lipid peroxidation may be particularly relevant in the management of patients with depression and anxiety. Increased lipid peroxidation in peripheric blood system may indicate a systemic increase in oxidative stress characterized largely by the deficiency of the anti-oxidant system evaluated in this study by determining uric acid and vitamin C.

XI.6. Conclusions

In this study we cand find the following conclusions:

- The profile of the investigated biological parameters was characterized by very high values of the Fagerström dependency score for smokers, as well as by high concentrations of CO in the exhaled air;

- The results of the study show for smokers and non-smokers very high values of the HAM-A anxiety evaluation score (17-41) as well as for the HAM-D depression evaluation score (12-26), the investigated subjects being diagnosed with severe anxiety and moderate depression;

- The statistical results of the Man-Whitney U test for smokers and non-smokers diagnosed with MADD show a significant statistical difference ($p<0.05$), especially for CO compounds, uric acid, total cholesterol, triglycerides and creatinine, which means that these compounds have variations in the assessment of oxidative stress;

- The correlation matrix between the biochemical and clinical parameters for the assessment of oxidative stress in smokers and non-smokers diagnosed with MADD have statistically significant correlation coefficients ($r = 0.39\text{-}0.84$; $p<0.05$), which could explain the variation in serum MDA and Vitamin C concentrations depending on nicotine dependence;

- The discriminant analysis performed in case of smokers shows a direct correspondence between the level of the MDA concentrations and the intensity of tobacco consumption evaluated in packs years (PY). These results could explain a possible production of chemical oxidative stress and implicitly depression and anxiety disorders due to free radicals resulting from tobacco smoke;

CHAPTER XII. THE VARIABILITY OF OXIDATIVE STRESS PARAMETERS DEPPENDING ON THE DEPRESSION EVALUATION SCORE (HAM-D) AND THE ANXIETY EVALUATION SCORE (HAM-A)

XII.1. Introduction

The depression mechanism is not yet fully known, but it is assumed that it is largely due to genetic, environmental, and also neurobiological factors.

XII.2. Objectives

The present study aims to evaluate the concentrations of oxidative stress parameters, respectively MDA, Vitamin C and uric acid, depending on HAM-D and HAM-A scores in smoking and non-smoking patients diagnosed with MADD, but also a complex statistical analysis to observe if there is variability and a correlation of the oxidative stress assessment parameters depending on the severity of depression and anxiety.

XII.3. Results

Smoking patients diagnosed with severe depression had low serum uric acid and vitamin C levels compared to patients diagnosed with moderate depression (Figure 31).

Smoking patients included in the study were classified with mild and severe anxiety according to the assessed scores. In smokers with severe anxiety, low levels of uric acid and vitamin C can be observed, as well as high values of MDA depending on the HAM-A anxiety scale (Figure 32)

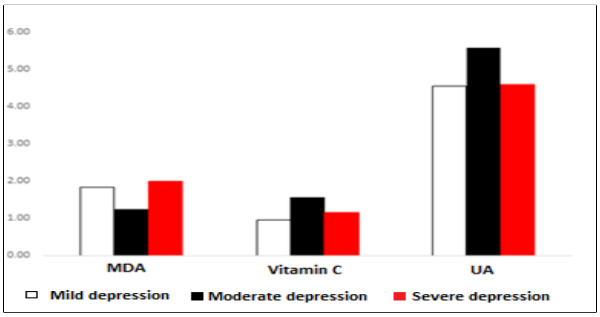

Figure 31. Mean concentration of oxidative stress parameters depending on HAM-D depression score in smokers with MADD

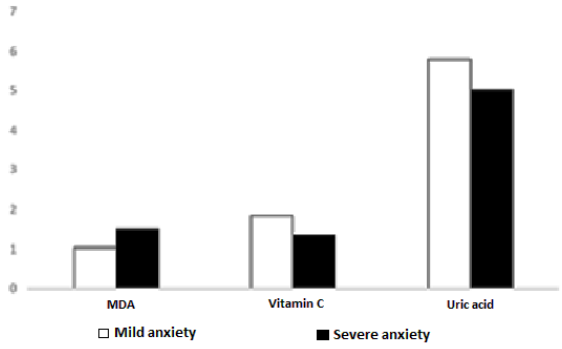

Figure 32. Mean concentration of oxidative stress parameters depending on HAM-A anxiety score in smokers with TMAD

Non-smoking patients were diagnosed with severe anxiety (low serum UA levels) and moderate depression. Vitamin C values were

very low relatively to the reference range, with insignificant differences between the two groups, and the MDA values were of the same order of magnitude.

Statistical tests were used to evaluate the concentrations of MDA, vitamin C and uric acid in terms of depression (HAM-D) and anxiety (HAM-A) scores. Thus, with the help of the Statistics10 program, statistically moderate correlations were obtained between the HAM-A anxiety scale and the MDA ($r = 0.23$, $p = 0.34$), HAM-D and MDA ($r = 0.16$, $p = 0.51$), HAM-A and vitamin C ($r = -0.07$, $p<0.75$), as well as between HAM-D and Vitamin C ($r = -0.11$, $p = 0.62$).

XII.4. Discussions

In the present study, we noticed for smoking patients high values of MDA as well as low values of Vitamin C and serum uric acid, especially in subjects with severe depression and those with severe anxiety. For smokers, high serum MDA values were obtained for 63% of the investigated subjects; the highest value was 2.4 nmol / mL, being much higher compared to the maximum permissible limit (1 nmol / mL). The results obtained are consistent with the results published in the literature (Moylan, 2013).

Statistical tests applied to evaluate the concentrations of MDA, vitamin C and uric acid in terms of depression (HAM-D) and anxiety scores (HAM-A) revealed statistically moderate correlations between HAM-A and MDA, respectively, vitamin C, as well as between HAM-D and MDA, respectively, vitamin C. Mann-Whitney U-test results in smokers and non-smokers diagnosed with severe anxiety (HAM-A >25) show a statistically significant difference between smokers and non-smokers for uric acid ($p = 0.039$). More and more studies point out that this non-enzymatic antioxidant has low serum levels in smokers due to decreased endogenous production (Tomito M, 2008).

XII.5. Conclusions

- Smoking patients diagnosed with severe depression have low values of serum uric acid and plasma vitamin C, and the values for serum MDA were higher than for patients diagnosed with moderate depression;
- Smoking patients diagnosed with severe anxiety have low levels of serum uric acid and plasma vitamin C, as well as high concentrations of serum MDA;
- Non-smoking patients who were diagnosed with severe anxiety and moderate depression had oxidative stress assessment parameters characterized by low concentrations of uric acid (in patients with severe anxiety) while vitamin C values were very low relative to the interval of reference, with insignificant differences between the two groups, and the MDA values were of the same order of magnitude for both conditions investigated;
- The data analysis showed a statistically significant difference ($p<0.05$) for oxidative stress parameters between smokers and non-smokers diagnosed with MADD.

CHAPTER XIII. OXIDATIVE STRESS RELATED TO SECOND HAND SMOKING IN MADD PATIENTS

XIII.1. Introduction

Within this chapter, the variability of the biological parameters was performed for patients with MADD, both actively and passively exposed to tobacco smoke.

XIII.2. Method

We analysed the results of the biochemical (MDA, vitamin C, uric acid, total cholesterol, triglyceride, creatinine) and hematological parameters (WBC-white blood cells, RBC-red blood cells, HB-hemoglobin, HCT-hematocrit, PLT-platelet, VSH- sedimentation rate of red blood cells), after which the descriptive statistics of the data obtained was made. Attempts have been made to establish correlations between oxidative stress parameters and nicotine dependence assessment parameters. A factorial analysis was also performed to statistically quantify possible factors of oxidative stress production in passive and active smokers diagnosed with MADD.

XIII.3. Results

The statistical results for the investigated biochemical and hematological parameters are presented in Table XXV and respectively in Table XXVI.

Table XXV. Descriptive statistics of the biochemical parameters investigated in the group of active and passive smokers patients diagnosed with MADD

Statistical data	MDA	VIT C	UA	ChOL	TLG	CREAT
Reference ranges (RR)	**0-1 nmol/mL**	**4,6-14,9 mg/L**	**2,5-8,1 mg/dL**	**100-200 mg/dL**	**40-165 mg/dL**	**0,5-1,1 mg/dL**
GROUP OF ACTIVE AND PASIVE SMOKERS						
Number of cases	10	10	10	10	10	10
Minimum	0,29	0,47	3,45	182	66,10	0,49
Maximum	2,40	1,33	7,45	289	186	1,07
Average	1,65	0,98	5,51	195,90	121,18	0,81
Standard deviation	0,64	0,32	1,44	48,28	49,97	0,20
Median	1,80	1,0	5,40	187,40	114,35	0,88
Values >RR	9	0	1	3	3	1
Values < RR	0	10	0	0	0	1
Values in RR	1	0	11	7	7	8

Table XXVI. Descriptive statistics of hematological parameters in the group of active and passive smokers patients diagnosed with MADD

Statistical data	WBC	RBC	HB	HCT	PLT	ERS
Reference ranges (RR)	**4,0-10,0 x103 /µL**	**3,80-5,60 x106 /µL**	**12-17 g/dL**	**36-47 %**	**150-400 /10^3/µL**	**5-15 mm/1h**
GROUP OF ACTIVE AND PASIVE SMOKERS						
Number of cases	10	10	10	10	10	10
Minimum	5,75	3,93	3,93	36,20	134	7
Maximum	9,90	5,88	5,88	50,20	390	33
Average	8,46	4,90	14,02	43,32	279,10	19,20
Standard deviation	1,24	0,55	1,26	4,68	80,40	9,35
Median	8,8	4,87	13,75	45,4	320,5	15
Values >RR	1	1	2	2	0	4
Values < RR	0	0	0	0	1	0
Values in RR	9	9	8	8	9	6

The factorial analysis applied to the investigated data set, corresponding to patients actively and passively exposed to tobacco smoke can be explained by the contribution of 3 factors. The first factor counts for 40% of the total variation of the data set and is represented by the CO concentration in the exhaled air, the Fagerström addiction score and the recent consumption of cigarettes, which means that exposure to tobacco smoke could be of major importance in the induction of chemical oxidative stress, due mainly to the reactive species in the cigarette. The second factor represents 21% of the total variation of the data set and is the serum triglyceride concentration. The third factor represents 14% of the total variation of the data set and is represented by the HAM-A anxiety assessment score.

XIII.4. Discussions

Regarding the factorial analysis carried out in the present chapter, some recent studies have shown that active smoking increases the biomarkers of oxidative stress, and also passive exposure to tobacco smoke can change inflammatory markers (cytokines) in at least 3 hours from exposure (Kostikas K, 2013). Anxiety disorders are the most common problems that affect the population and have been associated with several factors, among which the most important are genetic and environmental factors. Of these, passive smoking represents a very common environmental factor in the epidemiology of depression and anxiety disorders.

Also related to the factorial analysis performed above, numerous studies have associated the increase of serum triglycerides with smokers (Joshi N, 2013). Passive smoking has been associated with increased levels of total cholesterol and triglycerides. There are several studies on the effects of passive smoking on lipid profile (Attard R, 2017), and the results of the present study are consistent with these literature data.

XIII.5. Conclusions

- Passive and active smoking patients included in the study were diagnosed with severe anxiety and moderate depression;

- 90% of the investigated patients have significantly higher serum MDA values than the biological reference range; the concentrations for vitamin C were very low, being much lower than the reference biological range for all patients investigated;

- The highest value of serum MDA was determined for a non-smoking patient, exposed only passively 8 hours a day to tobacco smoke;

- From the statistical analysis of the above data it can be concluded that the anxiety symptoms in smokers exposed also passively to cigarette smoke had a higher intensity (a higher HAM-A score), in relation to a higher level of oxidative stress proved if double active and passive exposure to tobacco smoke.

XIV. FINAL CONCLUSIONS

The researches conducted for this thesis led to the following final conclusions:

- The demographic analysis of the results shows that females have a higher prevalence of depression compared to males. Similarly, unmarried people, as well as those living in urban areas, have a much higher risk of developing depression, but also the possibility of smoking more cigarettes than married people;

- The UV-VIS spectrophotometric method, for the determination of the MDA validated and statistically evaluated, can be successfully applied for blood samples, following the analysis protocol drawn up in this research; Quantitative determination of vitamin C from biological samples by high performance liquid chromatography HPLC, required the validation of the method and subsequently the working protocol. The method used to quantify plasma vitamin C has been shown to be sensitive, selective and very useful in current practice;

- The laboratory data obtained during the research show high concentrations of serum MDA in 73% of smoking patients diagnosed with MADD, and the resulting values were much higher than the biological reference range. Regarding the results obtained in the quantitative determination of plasma vitamin C, very low values are shown for 90% of smoker and non-smoker patients diagnosed with MADD;

- The values obtained in the quantitative determination of serum uric acid in smokers were lower than in non-smokers diagnosed with MADD, these results being mainly due to the decrease of the endogenous production;

- In patients with severe nicotine dependence, low values of serum uric acid concentration were observed, as well as low values of vitamin C concentration compared to patients with low and moderate nicotine dependence;

- Statistical analysis of the results obtained (Man-Whitney U test) shows a significant statistical difference (p0.05), between smokers and non-smokers diagnosed with MADD, especially for CO compounds, uric acid, total cholesterol, triglycerides and creatinine , which means that these compounds exhibit variations in the assessment of oxidative stress. The correlation matrix between the biochemical parameters and the clinical parameters for the assessment of oxidative stress in smokers and non-smokers diagnosed with MADD has statistically significant correlation coefficients ($r = 0.39\text{-}0.84$; $p<0.05$), which could explain the variation in serum MDA and Vitamin C concentrations depending on tobacco dependence;

- The analysis of the principal components (PCA) applied to the analyzed data set explains the phenomenon of chemical oxidative stress in smoking and non-smoking patients diagnosed with MADD through the variation of the biological parameters determined in order to identify possible causative factors of triggering imbalances between the oxidant and antioxidant systems;

- The discriminant analysis performed for smokers shows a direct correspondence between the level of the MDA concentrations and the intensity of tobacco consumption evaluated in packs-years (PY);

- For smokers diagnosed with severe depression, low values of serum uric acid and plasma vitamin C were determined, and values for serum MDA were higher than for patients diagnosed with moderate depression;

Original contributions:

The studies carried out in the doctoral thesis entitled ***Study of the interaction between the markers of oxidative stress and smoking in mixed anxious and depressive disorder*** have the following original contributions:

- The results presented and interpreted in this paper represent the first study that performs an extensive analysis between tobacco dependence, oxidative stress assessment parameters and mixed anxious and depressive disorder in smokers and non-smokers;
- It is the first comparative study that highlights the decrease of the levels of activity of the antioxidative systems and the increase of the serum MDA concentrations, as a marker of lipid peroxidation in smokers and non-smokers diagnosed with MADD;

- The thesis analyzes simultaniously the two conditions: anxiety and depression in the context of chemical oxidative stress produced by free radicals resulting from chronic exposure to tobacco smoke. It is for the first time that this chemical oxidative stress is quantified in the mixed anxious and depressive disorder depending on the severity of the diagnosis;
- The applied statistical analysis highlights the assessment of the level of oxidative stress in smokers considering several elements: carbon monoxide (CO) from the exhaled air, the number of packs-years (PY), the Fagerström nicotine dependence score, the number of cigarettes consumed per day in the last 12 months (recent consumption). It is for the first time that the parameters of assessment for oxidative stress have been analyzed according to the tobacco consumption quantified in this manner. In the context of this statistical model, it was observed that in patients with medium and high tobacco dependence, serum malondialdehyde (MDA) concentrations were above the reference level, while plasma vitamin C concentrations were very low;
- It is for the first time that an assessment of the level of oxidative stress is performed in patients diagnosed with MADD, both actively and passively exposed to tobacco smoke. This innovative approach in quantifying some biochemical parameters for oxidative stress assessment reveals high concentrations of serum MDA, especially in patients diagnosed with severe anxiety.

Future research perspectives brought to attention by the thesis

The present study is justified by the importance of the diagnosis of mixed anxious and depressive disorder in psychiatric practice, with the posibility of evidentiating potentially modifiable factors, such as oxidative stress and smoking, which can negatively influence the evolution of this medical condition. The routine highlighting and monitoring of these variables in clinical practice could lead to more efficient managment of these patients.

It is for the first time when the assesment of oxidative stress level according to the smoking status is done in patients with MADD. In this context, a first-time evaluation of the level of oxidative stress, based on the severity of nicotine dependence, is also attempted.

The study of oxidative stress markers for the patients with double exposure (active and passive) to tobacco smoke represents a novelty that the present work brings. The subject deserves more attention in the current European socio-legislative context, when the classic exposure to passive smoking (cigarette smoke, pipe, cigar) became prohibited in Romania only at the end of 2016, and only in the public spaces defined by the law. There is still the passive exposure to smoking in homes, personal cars, other spaces, but also the exposure to new tobacco products still allowed in public spaces such as electronic cigarettes, IQOS, smokeless tobacco products etc. Under these circumstances, the impact of passive exposure to tobacco will require continuous careful evaluation, during the next decades, being able to reveal various adverse

effects not yet discovered until now, in all categories of patients, including psychiatric ones, implicitly those with MADD, who represent a vulnerable category.

Showing that there is a relationship between the oxidative stress level and the exposure to tobacco consumption in patients with mixed anxious and depressive disorder could lay the groundwork to new standards for in-depth diagnosis, prevention and monitoring of therapy for this cathegory of patients. Such standards might prove useful to all practitioners who assist mixed anxious and depressive disorder, a psychiatric condition commonly encountered in medical practice.

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