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ABSTRACT OF DOCTORAL THESIS

Assessment of gut biomarkers and cognitive behavioural therapy in patients with depression and inflammatory bowel disease

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CUPRINS

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INTRODUCTION

Depression is a global problem. Major depressive disorder (depression) is a mood disorder that affects the way a person thinks, feels and acts. The key elements of the diagnosis are depressed mood and loss of interest/pleasure, for more than two weeks. Depressive symptoms cause dysfunction at individual, family, professional and social levels.

So far, the neurotransmitter hypothesis has provided support in the treatment of depression but has important limitations. These are related to the reduced efficacy of antidepressant medication and the delay in the onset of symptom relief. On the other hand, the psychological and social aspects that have an impact on the onset and progression of the illness are not integrated.

Recently the gut-brain axis has started to be explored and to bring new elements to knowledge. Data show that patients with inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are affected by depression at a higher rate than the general population. However, there is limited research on the treatment of depression in these patients. There is also a lack of an integrated approach, rapid screening and early treatment.

Based on these premises, I set out to assess depression in patients with IBD aiming to find a rapid diagnosis and early treatment, respectively, in relation to gut biomarkers and cognitive behavioral therapy (CBT).

OBJECTIVE

Given the primary research aim of assessing depression in patients with IBD in relation to gut biomarkers and TBI, we had the following research questions:

Question 1. What is the prevalence and cost of depression in Romania?

Question 2. Can gut biomarkers help us in the early diagnosis of depression?

Question 3. Can CBT improve depression in patients with IBD?

With this in mind, the research objectives were as follows:

Objective 1. To assess the prevalence and cost of depression in Romania.

Objective 2. To identify a biomarker correlated with depression that can support early diagnosis.

Objective 3. To implement a CBT treatment program for depression in patients with IBD.

To do this, we investigated depression in Romania, specifically its prevalence and cost (Study 1). We explored finding new biomarkers to facilitate rapid diagnosis, from the category of intestinal permeability biomarkers (study 2 and study 3). And we applied an early treatment, mixed CBT programme in study 4.

For the first study, the main objectives were to assess the prevalence and costs of depression in Romania in order to provide additional data for the country and the Central and Eastern Europe region and to identify arguments for a more efficient use of funds. Specifically, we carried out prevalence calculations, direct costs, indirect costs and total costs of depression.

For the second study, the main objectives were to assess correlations between calprotectin and depression; to assess correlations between calprotectin levels and depression severity; to assess correlations between calprotectin and depression symptoms; to identify a cut-off value of calprotectin that allows fast screening for depression. The secondary objective was to assess the relationship between calprotectin and anxiety and quality of life in patients with IBD.

For the third study, the main objective was to investigate the role of the intestinal permeability biomarkers - calprotectin, zonulin, lipopolysaccharide-binding protein (LBP) and intestinal fatty acid-binding protein (I-FAB) - in relation to depression in patients with IBD. The secondary objective was to assess the relationship between depression and quality of life in patients with IBD.

For the fourth study, the main objective was to apply a mixed CBT program to patients with depression and IBD and to assess depression and quality of life after applying the CBT program to patients with IBD.

The research results could have significance in the care of patients with IBD and depression. If CBT relieves depressive symptoms in patients with IBD, this is an argument in support of using CBT as the first stage of treatment for these patients. A protocol

can be developed to introduce this early treatment into the care management of these patients. In terms of early diagnosis, the research will make progress into the analysis of biomarkers associated with depression. The thesis will also provide a detailed analysis of the prevalence, direct and indirect costs of depression care in the last 7 years in Romania.

In conducting the research, limitations are related to the analysis of a restricted population, patients with IBD mono-centrically allocated. The small sample size limits the generalizability of the results. The short period allocated to the study does not allow identification of long-term effects of CBT.

The paper is structured in five chapters. Chapter 1 presents the current state of knowledge on depression. Chapter 2 examines the specificity of depression in patients with IBD. Chapter 3 details the prevalence and cost of depression care in Romania. Chapter 4 evaluates calprotectin as a screening test for depression. Chapter 5 examines the relationship between depression and gut biomarkers. Chapter 6 presents the effectiveness of a mixed CBT program for patients with depression and IBD. The paper is followed by conclusions, presentation of personal contribution, originality of research and references.

GENERAL METHODOLOGY

For study 1, a retrospective analysis was conducted using anonimous electronic data on health insurance claims to the National Health Insurance House (CNAS). The data used in the study are depression care services and their related costs and the number of depression-related deaths.

Studies 2, 3 and 4 were carried out between 01.01.2020-10.01.2023. The patients participating in the studies were referred to the Patient Centres of the Emergency County Hospital "Sfântul Apostol Andrei" in Constanța and Colentina Hospital "Bucharest". Patients aged >18 years with a diagnosis of BC and or UC who signed an informed consent were included in the studies. Exclusion criteria are detailed in each study. In studies 2 and 3, 60 patients were included of which 30 patients completed all stages of the study. Structured clinical interviews were conducted and severity of depression, anxiety and quality of life were assessed. Serological samples for zonulin, IFABP, LBP and faecal calprotectin samples were collected according to the protocol for collection and transport of potentially infectious biological samples. The studies were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Faculty of Medicine, Ovidius

University of Constanta. "Sf. Apostol Andrei" Emergency County Hospital no. 33/03.11.2020

Study 4 was a randomised control group trial. We applied a mixed CBT programme adapted to BII. The Living Life To The Full (LTTF) CBT program is a CBT program that addresses depression and anxiety. It has eight weekly sessions. Participants were assessed for BII activity indices- BHI and SCCAI, severity of depression, anxiety and quality of life, and fecal calprotectin, .in week 1 and at program completion, in week 9 .The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Faculty of Medicine, Ovidius University of Constanta with no. UOC 15910/19.10.2022.

Statistical analysis. Data collection and organization was performed in Office Excel (Microsoft, USA) and SPSS v 23 (IBM, USA) was used for statistical analysis. Descriptive statistics were evaluated for all continuous variables. The Shapiro-Wilk normality test verified the normality of the data. Pearson's test and Spearman's rho correlation coefficient (ρ) and associated probability (*p*) were used for parametric and non-parametric data, respectively. The Mann-Whitney (U) test assessed the difference between classes of depression scores. ROC analysis for calprotectin was calculated in R Studio version Rbase 4.2.3. Principal component analysis (PCA) was used to analyze cost variables and identify year clusters. It was also used to analyze the relationship between calprotectin, depression and anxiety symptoms and to allow identification of clusters of depression and anxiety symptoms. A value of $p < 0.05$ was considered statistically significant.

Study 1. Prevalence and cost of depression in Romania

Although depression is a global problem, data for Romania and Eastern Europe are scarce. An analysis has become relevant to identify the state of play and to identify mechanisms at health system level.

Purpose. The objective of the study was to assess the prevalence and public cost of depression in Romania over a 7-year period.

Material and method. The study was a retrospective analysis using electronic health insurance claims data at National Health Insurance House (CNAS). A total of 2,504,792 anonimous electronic data of patients diagnosed and settled in the public health insurance system between 2015 and 2021 were used. The data used in the study are the services and

their related costs, reimbursed for hospital care, outpatient care, psychotherapy, family medicine, sick leave costs and the number of depression-related deaths. Additional information was provided: age and gender of insured persons.

Results. Of the total cases analysed, 1,607,957 (63.29%) were women and 932,835 (36.71%) were men. Women were predominantly affected in a ratio of 1.7. The average prevalence of depression in the general population was 2.13%, with an average prevalence of 1.35% in women and 0.78% in men. Total costs (TC) of depression include direct costs (DC) (2.83%) and indirect costs (IC) (97.17%) with an average value of 2,015,731,285E/year and a cost of 5553.4E/patient. Direct costs had an average value of 51.068.833 E/year with a cost of 142.3E/patient. The direct cost is composed of the cost for hospital care 24,182,049 E/year (47.35%), the cost of medication 18,622,196 E/year (36.46%), the cost of specialist outpatient services 6,279,011 E/year (12.30%), the cost of psychotherapy services 1,966,803 E/year (3.85%) and the cost of primary health care 18,774 E/year (0.04%).

The indirect cost is 1,964,662,452 E/year and comprises the cost of morbidity and mortality, i.e. the cost of sick leave (CM) and the cost of lost productivity (CPP). The cost of CM is 523,173,605 E/year (27.36%CT) and with a cost of 272.3E/patient. The CM/CD ratio is 10.8. Cost of lost productivity 1,441,488,846 E/year (69.81% TC).

The number of days of hospitalization is negatively correlated with the number of consultations in specialist outpatient care (r=-0.783, p=0.037) and the number of psychotherapy services (r=-0.783, p=0.037). The cost of hospitalisation is negatively correlated with the number of services in primary care (r=-0.799, p=0.03) and there are no other correlations with other variables analyzed. The cost of psychotherapy is positively correlated (r=0.98, p=0.001) with the cost of specialist outpatient services (psychiatry) and negatively correlated with the number of days of hospitalisation (r =-0.762, p=0.03). The number of sick leaves was not correlated with any other variable studied. Cost of medication and sick leave was not correlated with any other variable analysed.

Conclusions. The prevalence of depression in Romania is significant. Indirect costs exceed direct costs. Within direct costs, the cost of hospitalisation and medication is still significant, but decreasing due to the intervention of specialist outpatient services: consultations with psychiatrists and psychotherapists.

Costs can be optimised by reallocating resources between cost components. For example, psychotherapy services can be prescribed to patients when the diagnosis of a

depressive episode is established, in the course of chronic illness when it is diagnosed as comorbid depression, or concurrently with the prescription of sick leave. It can also preventively be included in the primary care system screening for depression at the annual examination in patients with chronic diseases and in the follow-up of patients with depression.

Study 2. Calprotectin and depression in patients with inflammatory bowel disease

Depression requires early and accurate diagnosis. Among several pathophysiological pathways investigated, recent research focuses on the analysis of the brain-gut axis. These suggest a link between inflammation and depression. Calprotectin is a marker for non-specific intestinal inflammation used in the diagnosis and monitoring of patients with IBD.

Purpose. The purpose of the study was to assess correlations between calprotectin and depression, anxiety and quality of life; correlations between calprotectin levels and depression severity; correlation between calprotectin and each symptom of depression; and a cut-off value of calprotectin for depression screening.

Material and method. This cross-sectional study included 30 confirmed patients with BC and UC. Patients were assessed using clinical interviews and scales to determine severity of depression (PHQ-9, anxiety (GAD-7) and quality of life (EQ-5D). Stool samples were collected from all participants for measurement of calprotectin levels.

Results. Calprotectin levels were correlated with PHQ-9 (ρ=0.416, p=0.022), EQ-5D (ρ=-0.304, p=0.033), but not with GAD-7 (ρ=0.059, p=0.379). Calprotectin levels in patients with mild, moderate, and moderately severe depression were significantly higher than in patients with minimal depression (198µg/g vs. 66.9µg/g, p=0.04). Calprotectin level was correlated with the following depressive symptoms: autolytic ideation (ρ=0.557, p=0.001), fatigue (ρ=0.514, p=0.002), sluggishness (ρ=0.490, p=0.003) and sleep disturbance (ρ=0.403, p=0.014). Calprotectin was an independent predictor of depression, with an odds ratio of 1.01 (95%: 1.002-1.03, P<0.01). An ROC analysis showed that a calprotectin level of 131µg/g or higher had a sensitivity of 82%, a specificity of 61% and an accuracy of 70% for predicting depression. In this study, no significant correlations were found between calprotectin level and anxiety.

Conclusions. Calprotectin is correlated with depression and low quality of life**.** Calprotectin levels are associated with severity of depression, and a calprotectin level of 131μg/g or higher this may be an accessible potential screening test for depression in patients with IBD.

Study 3. Depression and gut biomarkers in patients with inflammatory bowel disease

A key element in the analysis of the brain-gut axis is the intestinal barrier. Dysfunctions occurring at this level, in particular leaky gut syndrome, have systemic as well as mental health implications.

Purpose. The aim of this study was to examine the role of intestinal permeability biomarkers: zonulin, lipopolysaccharide-binding protein (LBP), intestinal fatty acid-binding protein (I-FAB) and calprotectin in relation to depression in patients with IBD.

Material and method. The initial group included 60 patients of the Gastroenterology Clinic of the Constanta County Clinical Hospital between April and June 2021. Patients were previously diagnosed with IBD; patients with severe depression and severe active IBD were excluded. After giving informed consent, a researcher conducted the structured clinical interview. Eligible patients were administered the socio-demographic assessment, the PHQ-9 on depression severity and the EQ-5D on quality of life . Thirty patients were included in the study who had gone through all stages of the study. Serological samples for zonulin, IFABP/FABP2, LBP and faecal samples for calprotectin were collected according to the protocol for collection and transport of potentially infectious biological samples

Results. The sample analysed numbered 30 participants, 15 (50%) women and 15 (50%) men, of whom 12 (40%) participants with BC and 18 (60%) patients with UC. The age distribution showed a mean of 56.7+/-14.83 years. There was an increased frequency of cases in the age category 30-40 years, 40-50 years, 50-60 years. For the PHQ-9 score, the median value was 6.50 (0-16). A total of two (7%) participants had moderate-severe depression, five (17%) participants had moderate depression and 10 (33%) participants had mild depression. For 13 (43%) participants, depression was absent or minimal.

The mean EQ-5D score was 80.00 (45-100). Quality of life was assessed in the intervals 40-49 by one patient (3.33%), 50-59 by three patients (10%) and 60-69 by one patient (3.33%), while for the interval 70-79 there were eight patients (26.67%), for 80-89 there were six patients (20%) and for 90-99 there were eight patients (26.67%). Only three

patients (10%) assessed their quality of life at a score of 100. Calprotectin had a median value of 149.64 μg/g (2.80-330.31), LBP 42.00 ng/ml (35.14-49.41)(N=5-15 mg/ml), zonulin 32.94 ng/ml (19.23-37.84)(N<38 ng/ml), IFABP 0.87(0.35-3.00)(N<2ng/mL). Lower limit of detection for IFABP kit was 0.156 ng/mL, for LBP 3.125 ng/mL, for calprotectin 3.125 ng/mL.

Calprotectin correlated with PHQ 9 (ρ=0.416, p=0.022) and LBP correlated with PHQ 9 (ρ=0.398, p=0.029). Correlation between PHQ-9 score and zonulin was non-existent (ρ=0.016, p=0.934), similarly correlation between PHQ-9 score and IFABP (ρ=-0.059, p=0.755). For the studied group (n=30), between the two variables, EQ-5D and PHQ-9 depression score, there is a statistically significant negative correlation (ρ=-0.372, p=0.043).

Conclusions. A relationship was found between depression and calprotectin and LBP and its absence in relation to zonulin and IFAB, respectively. The involvement of gut permeability biomarkers in depression remains an open question. Depression and quality of life in IBD patients are correlated, which emphasises the need for access to appropriate treatments.

Study 4 Cognitive behavioural therapy in patients with depression and inflammatory bowel disease

Early treatment measures for depression can reduce the burden at individual, family and societal levels.

Purpose. The aim of this study was to evaluate the application of a mixed CBT program adapted to BII for patients with depression and BII. The program had face-to-face sessions and online web-based modules.

Material and method. The sample analyzed numbered 16 participants. Eight in the group who received care considered usual medical care (ICO), background treatment of IBD, and additional CBT and 8 in the control group who received only care considered usual medical care, background treatment of IBD.

Results. Of the participants 75% were female and 25% male, of which 37.5% participants with BC and 66.5% patients with UC. Age distribution showed a mean of 43.6 +/- 3.34. There was an equal frequency of cases in the age category 20-30 years, 30-40 years, 40-50 years.

The PHQ-9 score, had a median of 11.12 (8-15). A total of 2 (12.5%) participants had moderate-severe depression, 8 (50%) participants had moderate depression and 6 (37.5%) participants had mild depression. The GAD-7 score was 12.25 (7-20). A total of 2 (12.5%) participants had severe anxiety, 3(18.75%) participants had moderate anxiety, 6 (37.5%) participants had mild anxiety and 3 (18.75%), 5 (31.5%) participants had absent anxiety. The mean EQ-5D score was 61.87 (30-100).

For the 37.5% of patients with BC, the mean Harvey Bradshaw Index score was 7.5 (4-11). Disease activity was assessed in the range <5(in remission) by one patient (17%), between 5-7 by two patients (33%) and between 8-16 by three patients (50%). For the 62.5% UC patients, the mean Simple Clinical Colitis Activity Index (SCCAI) score was 7(4-11). Disease activity was assessed in the range <5 (in remission) by one patient (10%) and by 9 (90%) patients over >5 . For calprotectin, the median value was 401.6 μg/g (50-801).

In the study group, PHQ-9 correlated with GAD-7 at baseline (ρ=1, p=0.000) and endline (ρ=0.760, p=0.029) and with EQ-5D at baseline (ρ=0.894, p=0.003) and endline (ρ=0.808, p=0.015). In the study group, calprotectin correlated with PHQ 9 at baseline (ρ=0.861, p=0.006) and at the end of the study (ρ=0.706, p=0.051).

The study findings showed that cognitive behavioral therapy reduces depression, anxiety and improves quality of life in patients with IBD. Study argues for early integration of psychological treatments in the care of eligible IBD patients.

ORIGINALITY OF THE THESIS

Addressing the biological as well as the psychological and social aspects brings depth to understanding the implications of depression in IBD.

Calculating the prevalence of depression in Romania and conducting a complex health economics analysis of the costs of depression over 7 years is a valuable contribution of this thesis. The results demonstrate that depressive disorders are a burden on Romanian society.

Identifying that the increase in specialist outpatient services and psychotherapy correlates with a decrease in the number of inpatient days and the cost of hospitalisation in the treatment of depression is a useful mechanism to consider in decision-making.

Proposals that depression screening could be introduced in the annual examination and regular examination of patients with chronic illnesses and proposal that psychotherapy services could be recommended at the diagnosis of depressive episodes, in the course of chronic illnesses and concomitent with the prescription of sick leave may be useful in health service planning.

A strong element regarding the originality of the thesis is the identification of a cut-off value for calprotectin with a role in depression screening. Calprotectin has the potential to be a screening test for depression in IBD.

Also, the analysis of the relationship between depression and biomarkers of intestinal permeability is a novel element. This is the first study in Romania, Central and Eastern Europe to analyze the correlation between depression and intestinal permeability syndrome using the set of biomarkers: zonulin, LBP, IFABP and calprotectin.

Given the contradictory and limited data in the recent literature, the results reported in this research provide new arguments for further exploration of the gut-brain axis in mental disorders.

Applying a mixed CBT programme to patients with depression and IBD is a valuable contribution. The research argues that CBT can be a first step in the treatment of depression in IBD and can underpin the integration of psychological care in the treatment of patients with IBD.

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