Project summary

In 2021, there was a 72% annual increase in suicides in the age group 15-19 years in Slovakia. This observation should be a memento to society and to the scientific community to start seriously dealing with this growing problem. In an observational, exploratory, and prospective study, we will focus on pediatric psychiatric patients to determine the relationship between the incidence of suicidal ideation and attempts and psychiatric diagnosis, examining neurobiological correlates, focusing on both direct and indirect inflammatory markers, an anti-inflammatory cytokine, lipid profile, markers of oxidative and nitrosylation stress, rate-limiting enzyme of GSH synthesis, and Nrf2/Keap1/ARE signaling pathway in comparison to a control group. Based on our findings, we will propose possible predictors of suicidal behavior in children. We will also focus on the potential relationship of overcoming a COVID19 infection with the incidence of suicidal ideation and suicide attempts and with neurobiological markers.

General information

Suicidality in children and adolescents, neurobiological parameters, and their mutual association

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Rationale & background information

Suicide is a serious global problem and is one of the leading causes of death worldwide. According to the WHO, it is the fourth leading cause of death in young people aged 15-19 years (1). In the USA, the number of suicides has increased significantly in the last two decades -1(2), and the presence of a psychiatric disorder is a major risk factor. Approximately 40% of patients with depressive disorder are reported to have a history of repeated suicide attempts (3,4). According to the CDC (Center for Disease Control and Prevention), suicide rate among children aged 10-14 years increased by 100% between 2010 and 2019, compared to a 40% increase among adolescents aged 15-19 years (5). According to Eurostat, suicide was the second most common cause of death in the 15-19 age group in 2016 (6). In the Slovak Republic, according to the National Health Information Center, suicide and suicide attempts saw the largest year-on-year increase in the same age group in 2021. The number of reported suicide attempts more than doubled in a year in adolescents under 14 years of age and increased by almost one-third in the 15-19 age group (7). Suicide is associated with multiple psychosocial risk factors. Understanding suicidal behavior and its possible causes from a neurobiological perspective is also important. A growing body of evidence points to the involvement of inflammation in suicide vulnerability. Studies have found elevated plasma levels of C reactive protein in individuals after suicide attempts (8). In adolescents after suicide, increased cytokine levels, increased mRNA (in brain tissue) and protein levels (in cortical areas) of IL1ß, IL6, and TNF have been found (9, 10). In patients with suicidal behavior, decreased plasma levels of IL2 and IL4 and higher plasma levels of TGFß have been found. A review by Marini et al (2016) reported the results of 15 studies in adults (11), and only one in adolescents (12), suggesting the

need to monitor the association between suicidal attempts and inflammation in adolescents. In recent years, a preference for tryptophan metabolism via the kynurenine-tryptophan pathway, resulting in serotonin depletion and consequently melatonin depletion, has emerged as being important. The level of quinolinic acid, one of the end products of the kynurenine pathway, has been shown to increase in plasma and cerebrospinal fluid in those who have made suicide attempts and in the anterior cingulate cortex in depressed patients who have died by suicide. (13). The neutrophil/lymphocyte ratio (NLR), which is a marker of peripheral inflammation, is considered a marker of suicide vulnerability in adult patients with depression and bipolar disorder (14,15). Our recent findings (VEGA 1/0703/13, "Molecular foundations of psychiatric disorders in childhood (depression, anxiety states), involvement of oxidative stress and the possibility of using omega3 fatty acids in therapy" and subsequently APVV150063 "Molecular foundations of depressive disorder in children and adolescents, the impact of omega3 fatty acids and oxidative stress") suggest that HDL and the omega6/omega3 fatty acid ratio have a role in the pathophysiology of depression (16). In a recent paper, Serna Rodriquez et al. (17) found that apolipoprotein E is a possible biomarker for suicide risk. Our previous results indicate the involvement of oxidative stress in the pathophysiology of depression (18). We have found increased lipoperoxidation and reduced activity of glutathione peroxidase in depressed patients. Increased nitrotyrosine has positively correlated with the severity of depression and points to a disorder in nitroxide metabolism in depressed children, which was also confirmed in the work of Ilavská et al. (19) by the negative correlation between nitrotyrosine and serotonin levels. The activation of nitrosative stress was also confirmed by the results of a metaanalysis in adult patients with suicidal thoughts and attempts (20,21).

Recently, more attention is being paid to signaling pathways that control oxidative stress. Among these is the Nrf2/Keap1/ARE pathway (nuclear factor erythroid 2/ Kelchlike ECH associated protein 1/antioxidant response element), which under the conditions of oxidative stress activates the transcription of protective genes antioxidant enzymes, thus reducing inflammation and neurodegeneration, protecting mitochondria against the oxidative stress and increasing the resistance to stress (22). COVID19 Corona virus induced hyperinflammation is also suggested to increase suicide risk. Several studies have confirmed an increase in psychiatric disorders in patients with COVID19, including suicidal ideation and behavior (23, 24).

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Study goals and objectives

1. Determination of the prevalence of suicidal ideation and suicide attempts in children and adolescents hospitalized in pedopsychiatric inpatient wards concerning psychiatric diagnoses.
2. To determine neurobiological correlates with the focus on inflammatory markers (CRP, the neutrophil-to-lymphocyte ratio, fibrinogen, orosomucoid, neopterin), lipid profile (total cholesterol, LDL and HDL cholesterol, subfractions of HDL lipoproteins, cortisol in hair, markers of

oxidative and nitrosylation stresses (nitrotyrosine, lipoperoxides, 8isoprostanes, AOPP, activity of glutathione peroxidase, catalase, SOD, glutamyl cysteinyl ligase (GCL), proteins of Nrf2/Keap1 /ARE signaling pathway and total plasma antioxidant capacity) in the child and adolescent patients with suicidal ideations and after suicide attempts compared to a control group of healthy children.
3. Determine the correlations between established neurobiological markers and psychopathological factors (incidence of suicidal ideation/suicide attempts, severity of depressive symptoms, presence of adverse life event) concerning age, gender, and diagnosis, and identify a potential predictive marker for use in clinical practice based on the results.
4. Investigate a potential relationship between overcoming a COVID-19 infection and the incidence of suicidal ideation and suicide attempts and neurobiological markers.

Study design

In patients hospitalized at the Department of Pediatric Psychiatry of the Comenius University Medical Faculty (LF UK) and the National Institute of Children's Diseases (NÚDCH) aged 10-17 years, we will screen for suicidal ideation and history of suicide attempts (in the last 7 days) as part of the initial pedopsychiatric examination. Selected patients with detected suicidal ideations and suicide attempts, after informed consent is signed by their legal guardian, will be enrolled in the project, a standard pedopsychiatric examination will be performed to establish a diagnosis based on the diagnostic criteria according to ICD10.
Inclusion criteria: patients hospitalized at the Department of Pediatric Psychiatry of the Comenius University Medical Faculty and the National Institute of Children's Diseases (NÚDCH), age 10-17 years, signed informed consent by the legal guardian, verbal consent of the patient to be included in the project, presence of suicidal ideation and/or suicide attempts.
Exclusion criteria: chronic somatic inflammatory and oncological disease.
The research group will consist of 60 patients who will be divided into two groups: patients with suicidal ideations (SI) and patients after suicide attempts (TS tentamen suicidii). The control group will consist of 20 healthy volunteers who are not under the care of a child psychiatrist or psychologist, in collaboration with primary care pediatricians (Children's Health Centre Juvenalia, s.r.o., Dunajská Streda).

Methodology

The enrolled patients will be asked to fill out the following scales: the self-report Symptom Checklist 90 (SCL90R) (25), the Child Depression Inventory (CDI) (27) self-report questionnaire, and the self-report Yale Vermont Adversity in Childhood Scale (YVACSSR) (29). The examiner will investigate depressive symptoms severity using the Children's Depression Rating Scale Revised (CDRSR) (28) and suicide risk using the Columbia Suicide Severity Rating Scale (CSSRS) (26). Patients who have had a history of COVID19 in the past 6 months will fill out the self-report COVID19 Yorkshire Rehabilitation Scale (C19YRS) (30). Patients and healthy controls will have their biological material (blood, urine, hair) collected, processed usingstandard clinical biochemistry methods, and stored at 80°C. To determine neurobiological correlates: in serum/plasma: basic biochemistry and lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol), markers of acute inflammation (hsCRP, IL6) and anti-inflammatory cytokine (IL2), markers of peripheral inflammation (fibrinogen, orosomucoid) and blood hematology (neutrophil and lymphocyte counts and their ratio) will be determined by standard methods in a commercial clinical laboratory. LDL and HDL subfractions will be determined by the Lipoprint system. BDNF and Nrf2 will be determined by Elisa kit. Spectrophotometry will be used for the determination of oxidative stress markers (lipoperoxides, AOPP) in plasma, antioxidant enzyme activities (catalase and using kits SOD and GPx) in erythrocyte hemolysate and the total antioxidant capacity of plasma. GCL and protein expression of antioxidant enzymes will be determined by western blot (WB). Cortisol in hair, as well as 8-isoprostanes and creatinine in the urine, will be determined using commercial kits. Signaling pathway proteins will be determined by WB and gene expression by RT PCR. For this purpose, sensitive and sophisticated methods of molecular biology are needed to

analyze the expression of selected genes. Gene expression can be examined and analyzed at two levels at the level of protein concentration using the Western Blot method, or at the mRNA level using the qRT¬PCR method. To determine lipoprotein subfractions, we will use a unique method, Lipoprint.

Safety considerations

The only recorded risk is minor bruising from blood sample collection.

Follow-up

Study is prospective and case-control study.

Data management and statistical analysis

Data management will be realised by the responsible investigator Assoc. Prof. Jana Trebatická, MD, PhD. (clinical part and co-operation between researchers) and Prof. Jana Muchová, PhD. (for biochemical part).
For statistical analyses will be responsible biostatistician Prof. Dr. Iveta Waczulíková, PhD.

Quality assurance

Quality assurance will be ensured by the erudition of the scientific team, led by experienced researchers who have extensive experience in conducting clinical studies.

Expected outcomes of the study

As the number of suicidal acts among children and adolescents is increasing significantly, it is necessary to address this problem from both a societal and a scientific perspective. This serious problem requires the validation of individual potential markers in a larger cohort of patients. From an ethical point of view (it is not possible to collect large amounts of biological material blood, and urine from child and adolescent patients), but also from an economic point of view (analyses of potential markers are economically demanding), it is not feasible to evaluate all of the aforementioned markers. Knowledge of the complex molecular-level factors that contribute to suicide in children and adolescents are essential for identifying potential molecular predictors and developing effective prevention strategies. The loss of any young life to suicide is a tragic event that leaves a lasting and devastating impact on their family, friends, and society. The results of the project will enable to:

- Propose potential biological markers for the prediction of suicidal behavior in children and adolescents
- Increase the possibility of prevention programs in potentially at risk children and adolescents
- Reduce the social and economic impact of suicidality in children and adolescents on families and society

Dissemination of results and publication policy

Obtained results will be presented at international scientific events and published in high-impact publications.

Duration of the project

1st year: Obtaining approval from the Ethics Committee of the National Institute of Children's Diseases (NÚDCH). Registration of the project in an international register of studies. Gradual enrolment of

patients in the project depending on their hospitalization at the Department of Paediatric Psychiatry of the Comenius University Medical Faculty and the NÚDCH. Methodological validation of the biochemical methods used.
2nd and 3rd year: Continued enrolment of patients and control group subjects in the project.
Carrying out entrance psychiatric examinations. Administration of clinician-administered and self-assessment scales. Collection of biological material. Continuous execution of biochemical analyses. Ongoing summarization and evaluation of the data collected. Presentation of interim results at scientific conferences
4th year: Summarizing the final results and completing their analysis. Search for correlations between biological markers and psychopathological parameters. Presentation of results at scientific events. Publication of results.

Problems anticipated

Problem with patient inclusion due to non-compliance of patients' legal representatives

Project management

Assoc. Prof. Trebatická Jana, MD, PhD. Principal investigator, responsible for the clinical part of the project, its organization, planning of examinations, statistical evaluation and interpretation of results, publication of results and conclusions
Vatrál Martin MD, PhD student, responsible for conducting psychiatric examinations, administering self-assessment and clinician-administered scales,
Mgr. Franková Daniela, psychologist, responsible for administering self-assessment
Scales.
Prof. RNDr. Muchová Jana, PhD. Responsible for the biochemical part of the project, its organization, planning of experiments, statistical evaluation and interpretation of results, publication of results and conclusions.
Mgr. Gajdošová Lívia Ph.D. student, conducting analyses of oxidative damage markers in serum.
Ing. Katrenčíková Barbora is responsible for measuring pro- and anti-inflammatory parameters in serum and plasm.
RNDr. Zuzana Paduchová, PhD. Responsible for measurement of LDL and HDL lipoprotein subfractions using the Lipoprint system in serum.
RNDr. Horváthová Martina, PhD. Responsible for the detection of Nrf2/Keap1/ARE signaling pathway proteins using Elisa kit, western blot (WB), and RT PCR.
RNDr. Országhová Zuzana, PhD. Responsible for measurement of activity and expression of antioxidant enzymes (GPx, SOD and catalase), AOPP using commercial kits and WB.
Prof. Ing. Ďuračková Zdeňka, PhD. A consultant, involved in the writing of the project, responsible for coordination between the biochemical and clinical parts of the project.
Mgr. Masarovičová Dominika, PhD student – responsible for the analysis of tryptophan catabolism metabolites in urine by HPLC method.
Eliasi Gomari Zolfa, MD – PhD studentt – responsible for cortisol determination un hair.

Ethics

The condition for patient inclusion in the study is the signed informed consent of the legal representative and the patient's verbal consent.

Approved 23/04/2024, Ethics Committee of the National Institute of Children’s Diseases and the Faculty of Medicine, Comenius University Bratislava (Limbová 1, Bratislava, 83340, Slovakia; +421 259371209; detska.klinika@nudch.eu), ref: EK4/1/2024

2. Approved 17/09/2024, Ethics Committee of the National Institute of Children's Diseases and the Faculty of Medicine, Comenius University Bratislava (Limbová 1, Bratislava, 83340, Slovakia; +421 259371209; detska.klinika@nudch.eu), ref: EK9/2/2024

Informed consent forms

The informed consent was signed by the parent/legal representative and is written in Slovak.

Part 2

Budget

Funds will be provided during the years 2024-2028

Other support for the project

No other support

Collaboration with other scientists or research institutions

The project will be carried out in collaboration with the Faculty of Mathematics, Physics and Informatics of Comenius University (Prof. Šikurová, supervisor of PhD student D. Masarovičová), with Prof. I. Waczulíková, PhD., biostatistician in statistical analyses, with Prof. Ježová from the Slovak Academy of Sciences and the Faculty of Pharmacy of Comenius University (supervisor of PhD student Eliasi Gomari Zolfa, MD)

Curriculum Vitae of investigators

CVs are attached to the registration:
Assoc. Prof. Jana Trebatická, MD, PhD. – responsible investigator
Prof. RNDr. Jana Muchová, PhD. – responsible for the biochemical part
Prof. RNDr. Iveta Waczulíková, PhD. – responsible for statistical analyses
Prof. RNDr. Libuša Šikurová, CSc. – supervisor of PhD. student
Prof. PharmDr. Daniela Ježová, DrSc. – supervisor of PhD student

Other research activities of the investigators

VEGA- 1/0644/23: Coping with psychosocial stressful situations in patients with depressive disorder depending on age and uncovering the mechanisms involved, 01/2023-12/2026, deputy of principal investigator

Financing and insurance

The study is not interventional and is not covered by insurance.