



The most important results achieved in solving projects

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Successfully completed VEGA/KEGA projects solved at the department

VEGA 1/0333/20:

Tear fluid and saliva in preventive, predictive and personalized medicine – responsible researcher: doc. RNDr. Vladimíra Tomečková, PhD.

Grant **VEGA 1/0333/20** study tears and saliva as non-traditional collection materials that they provide us the diagnostic potential for detecting various inflammatory diseases. The correct collection of materials is important for further measurement, and therefore in this project we monitored the influence of several collection methods on the quality of the collected tear fluid. The spectrum of each material is unique, personalized and characteristic like a fingerprint. There are no two substances in the world that have the same spectrum. If substances have a similar spectrum, it is possible to find more differences, for example, by mathematically derivation of spectrum. Infrared spectra, synchronous fluorescence fingerprints, circular dichroism spectra, and MALDI-TOF mass spectra of tear fluid were different in patients with various diseases compared to healthy subjects. Atomic force microscopy (AFM) is a fast, complex experimental method that observes the surface of the tear fluid as a whole without separation. The AFM results showed aesthetic value in addition to scientific value. This method, in conjunction with other spectral methods, showed the differences between the tear fluid of healthy people and patients with selected eye diseases, e.g. glaucoma, but she also revealed a systemic disease from the tear, e.g. psychiatric illness like major depression of patients. Every disease is accompanied with inflammation. All psychiatrically treated patients have dry eye syndrome (DED). This inflammatory eye disease was also studied in dogs in cooperation with UVLF in Košice. A new adjunctive anti-inflammatory therapy, namely the application of fisetin antioxidant in eye drops, was proposed as a pilot treatment of dry eye in dogs treated with cyclosporine. We designed these experimental, freshly prepared eye drops ourselves but these drops were made by an experienced pharmacist in cooperation with UVLF in Košice. The results of experimental work funded by VEGA 1/0333/20 were showed in ADC publications (9), conference contributions (7), invited lectures (3) and the exhibition "Tears of Psychiatric Patients" at XI. Czech-Slovak Neuropsychiatric Symposium in May 2022. The obtained results are from the collection of a small volume of tear fluid (2-5 μ l) with the great potential for further goals of personalized research of the tear fluid of patients with various inflammatory diseases, e.g. to develop a sensor or develop smart software that would detect diseases from the tear fluid.



The complete list of publication outputs is at: <https://www.upjs.sk/pracoviska/univerzitna-kniznica/evidencia-publikacnej-cinnosti/>.

VEGA 1/0540/20:

Study of selected biomarkers of the origin and progression of demyelinating CNS diseases – responsible researcher: doc. Mgr. Peter Urban, PhD.

As part of the project **1/0540/20**, pathological changes in the biological material of patients with multiple sclerosis were monitored in several phenotypic forms, using the methods of real-time PCR, western blot, ELISA, zymography and fluorescence microscopy. The use of whole blood in the determination of specific protein markers of the progression of selected demyelinating diseases is limited mainly due to the presence of the blood-brain barrier, preventing the free passage of molecules between the central nervous system and the blood. The advantage of exosomes as extracellular vesicles, which occur in large quantities in biological fluids, is the ability to pass through the blood-brain barrier, which provides a unique opportunity to obtain information from direct internal intercellular communication. In the first stage of the project, in cooperation between the UPJŠ FM and UNLP clinics, whole blood was collected from patients with various multiple sclerosis phenotypes as well as healthy controls. In the next phase of the project, a molecular analysis of selected biomarkers was performed (serum and exosomal proteins and miRNA) that are involved in the process of de/remyelination, e.g. NfL, CHI3L1, CXCL13 and MCP-1, or are specific for a given type of exosomes, e.g. ALIX, and necessary for their identification, whether they are involved in ECM degradation, e.g. metalloproteinases 2 and 9. Based on the results of all mentioned techniques and measurements, a complex algorithm was created to detect the transition from the relapsing-remitting form of MS to the secondary progressive form with a less successful possibility of treatment and subsequent regression of the disease. This non-invasive analysis of patients' serum will contribute to the improvement of diagnostics and subsequently improve the prognosis of patients' survival. Overall, the results of the project were published in **4** ADC papers, **1** ADN paper, and **2** ADF papers. The obtained results were presented at two conferences and published in the form of abstracts in a collection. The results were also used in for the preparation of one diploma thesis as well as one rigorous and dissertation thesis of a doctoral student.

The complete list of publication outputs is at: <https://www.upjs.sk/pracoviska/univerzitna-kniznica/evidencia-publikacnej-cinnosti/>.

VEGA 1/0620/19:

The use of innovative molecular biochemical methods in the diagnosis of non- of the perceptive endometrium in the process of in vitro fertilization – responsible researcher: doc. RNDr. Miroslava Rabajdová, Ph.D.

The **VEGA 1/0620/19** project focused on the use of innovative molecular biochemical methods in the diagnosis of non-perceptual endometrium in the process of in vitro fertilization. One of the most important results is the identification of specific miRNA molecules identified in a woman's plasma and in the embryo's culture medium. Specific canonical miRNA/iso-miRNA molecules showed significantly different representations and distributions in the plasma of women in the group of women with a successful IVF process compared to the group of women with a failed IVF process. The distribution of specific canonical miRNA/iso-miRNA molecules (miRNA profile) in plasma can be used for diagnostics on the day of the IVF process, to evaluate biological competence - readiness, resp. maternal unpreparedness for the IVF process. The secretion of specific canonical miRNA/iso-miRNA molecules (miRNA profile) into the embryo culture medium can be used for diagnostics on the day of the IVF process and to



evaluate the competence, quality of the embryo. The achieved scientific-research results were published in **9** scientific-research works *in extenso* in peer-reviewed domestic and foreign journals. **Two** papers are registered in the Web of Science database and **4** papers have been published in foreign journals (IF: 1.216, IF: 3.196, IF: 4.155, IF: 4.856). The results were presented at domestic and foreign conferences and published in the form of abstracts (5) as well as published papers (7). Partial results were published in the form of scientific papers in other domestic journals (3), respectively in the form of presentations of doctoral students at selected events, e.g. 45th Brno Oncology Days (under the auspices of Masaryk University in Brno, Doctoral Students' Seminar dedicated to the memory of Academician Bod'a, under the auspices of UVMP, SAS and UPJŠ in Košice.

The works are registered in UL UPJŠ and the complete list of publications is also on the UL UPJŠ website: [EPC BIB - Výsledky vyhľadávania \(upjs.sk\)](http://EPC%20BIB%20-%20V%C5%9Bledky%20vyh%C4%81ad%C3%A1van%C3%ADa%20(upjs.sk)) or [EPC - Kod pracoviska= UPS? and Projekt\(číslo\)= |1/0620/19| \(upjs.sk\)](http://EPC%20-%20Kod%20pracoviska%3D%20UPS%3F%20and%20Projekt%28%25cislo%29%3D%20%2F%201%2F%200620%2F%2019%20(upjs.sk)), respectively [EPC - Kod pracoviska= UPS51010 and Projekt\(číslo\)= |1/0620/19| \(upjs.sk\)](http://EPC%20-%20Kod%20pracoviska%3D%20UPS51010%20and%20Projekt%28%25cislo%29%3D%20%2F%201%2F%200620%2F%2019%20(upjs.sk))

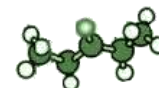
VEGA 1/0559/18:

Study of the onset and progression of periodontide using the latest molecular-biochemical methods – principal investigator: doc. RNDr. Janka Vašková, PhD.

The **VEGA 1/0559/18** project was aimed to analyse the saliva of patients with parodontal disease by studying of selected clinical biochemical biomarkers. Samples were collected from patients with gingivitis, chronic and aggressive form of parodontitis and subsequently compared with a group of healthy individuals. In the context of the observed antioxidant parameters, it is a significant finding that superoxid dismutase activity is significantly reduced in patients with gingivitis, but in more advanced disease stage, is increased in aggressive and chronic parodontitis. Differences between individual groups of patients are significant.

The opposite character has glutathione peroxidase activity. Previous studies have indicated that increased SIRT1 levels can be considered as a parameter of parodontitis progression, and in accordance with this, it has been found that SIRT-1 levels are not different in all three groups of patients. However, SIRT-2 levels, which is involved in regulating the expression of antioxidant enzymes, were significantly increased. mRNA of selected specific matrix metalloproteinases (eg MMP2, MMP9) and IL1-beta expression were also monitored. The most significant result achieved in solving the VEGA 1/0559/18 is a detection of up-regulation of IL1-beta gene in aggressive and chronic parodontitis. The analyzed results indicate that IL1-beta, both for inflammatory cytokine, plays a key role in pro-inflammatory protein activation. Research results achieved were published in **4** scientific and research papers *in extenso* in reviewed domestic and foreign journal. **Two** papers are registered in the Web of Science database and two were published in current content journals (IF: 1.216 and IF 3,024), registered in WOS/SCOPUS databases. The results were presented on both domestic and foreign conferences and published in the form of abstracts (3) as well as full paper published contributions (7).

Published papers are registered in UK UPJŠ and a complete list of published outputs is accessible from:
https://aleph.upjs.sk/F/7MCUFCUA1PPU88Y2FRQTTHU74T9REDC8AE879GSQTCJ5F7159Q-40009?func=find-c-0&local_base=epc01



VVGS-2018-747:

New diagnostic options for severe eye diseases – principal investigator: doc. RNDr. Vladimíra Tomečková, PhD.

The main output of the project **VVGS-2018-747** was the exhibition "**Human Tear as Art**", which presented to the general public 27 interesting artistic and scientific images of the tear fluid of sick people, imaged using an atomic force microscope. The presented images are the result of the cooperation of the ophthalmologist MUDr. Gabriela Glinská and three research teams united around doc. RNDr. Vladimíra Tomečková, PhD. The pilot science and art project aims to present the results of several years of research to the general public through images, which show the interesting diagnostic potential of original images of human tear fluid in various diseases, as tears of patients with different diagnoses differ in structure from tears of healthy people. Part of the grand opening of the exhibition was a lecture and screening of a short film with unique depictions of various eye and systemic diseases in the tear fluid of sick people associated with a short lecture. The artistic enrichment of the opening was a sample from the performance *The Little Prince* of the theater ANIMA MEA, as reminiscent of Saint-Exupéry's idea that the most important thing is invisible to the eyes. The exhibition presented the results of research focused on microscopic observation of tear fluid and is an example of successful cooperation between the Faculty of Medicine and the Faculty of Science of Pavol Jozef Šafárik University and the Slovak Academy of Sciences. The achieved scientific-research results were published in two journals with impact factor and one paper was published in a domestic journal. The results were presented at domestic and foreign conferences in the form of invited lectures (3/2).

Published papers are registered in UK UPJŠ and a complete list of published outputs is accessible from:

https://aleph.upjs.sk/F/7MCUFUA1PPU88Y2FRQTTHU74T9REDC8AE879GSQTCJ5F7159Q-40009?func=find-c-0&local_base=epc01

VEGA 1/0372/17:

The use miRNA and fluorescence techniques in diagnosis of bladder tumors – principal investigator: prof. Ing. Mária Mareková, CSc.

The urinary bladder tumor diagnosis is based on the changes of vital cell functions on the gene and protein level. Response to processes ongoing in tumor environment are molecules and metabolites that can be detected in urine. Revealing the causes of tumor is an extremely challenging process and requires both standard clinical trials and genomic, transcriptomic and metabolomic access. The presence of various urine molecules (e.g. miRNA) and metabolites that represent natural fluorophores, are characterized by physiological condition and allow pathological conditions to be distinguished. The most significant results from the solution of **VEGA 1/0372/17** includes the formation of a group of patients with histologically defined tumors in which the 3D fluorescence metabolomic analysis (so-called fingerprints) isolated specific miRNA from urine. Based on the combination of individual analyzes, which will continue even after the end of the project, we plan to create an algorithm characterizing the micro environment of the tumor, which could be used in the diagnosis of the urinary bladder tumor. Creating a database of fingerprints of patients from different urological diagnosis and their subsequent computer validation could contribute to early diagnosis of the diseases. Implementation of these modern and still highly current molecular methods contributes to improving laboratory diagnostics and moves it towards personalized medicine. The scientific and research results were published in **three** scientific and research papers *in extenso* in reviewed domestic journal and one paper was published in a foreign current content journal (IF: 2.943; 5-year Impact Factor: 3.321), registered also in the Web of Science



database/Scopus. The results were presented on both domestic and foreign conferences and published in the form of abstracts (8) as well as full paper contributions (1).

Published papers are registered in UK UPJŠ and a complete list of published outputs is accessible from:

https://aleph.upjs.sk/F/7MCUFUA1PPU88Y2FRQTTTHU74T9REDC8AE879GSQTCJ5F7159Q-40009?func=find-c-0&local_base=epc01

KEGA 013UPJŠ-4/2016:

Clinical biochemistry – *principal investigator*: prof. Ing. **Mária Mareková**, CSc.

Determined objectives of the **KEGA 013UPJŠ-4/2016** - E-version of teaching materials (teaching texts, methods, causeries, tests, videos) and a nationwide university textbook were met. The electronic version of the textbook is already available at (<https://portal.lf.upjs.sk/clanky.php?aid=222>), its book form is ready to print. From a global point of view, results achieved unambiguous benefit. As electronic teaching materials and the print textbook contains the latest recommendations and trends in clinical-biochemical diagnostics. Modern interdisciplinary approaches that will contribute to a higher level intermediation and student motivation were used in their preparation.

The main outputs of the project were: e-learning materials - teaching texts, methodologies, case reports, tests that are available for students of medical faculties on the portal of UPŠ LF in Košice under the name Clinical Biochemistry and Laboratory Medicine. These can be continuously updated and supplemented according to the needs and suggestions of teachers as well as students. The forthcoming nationwide university textbook "Clinical Biochemistry" fills a gap in the market, as despite the teaching of the subject Clinical Biochemistry at all Faculty of Medicine in the Slovak Republic, the textbook has been absent so far. Its use should be in both undergraduate and postgraduate education. As part of the project, partial results were also presented at scientific and professional events (Olomouc, Prague).

<https://portal.lf.upjs.sk/articles.php?aid=114> and <https://portal.lf.upjs.sk/clanky.php?aid=222>

VEGA 1/0873/16:

Characterization of microenvironment of endometrial carcinoma – *principal investigator*: doc. RNDr. **Miroslava Rabajdová**, PhD.

During the solution of the **VEGA 1/0873/16** project, pathological changes in the biological material of patients with endometriosis and endometrial carcinoma were monitored by scanning microscopy and molecular biochemical methods. In the first part of the project, morphological changes of DNA and chromatin, related to high-intensity transcription of specific oncogenes, were monitored and evaluated in comparison with a healthy control. A high-sensitivity atomic force microscopy (AFM) technique was used to investigate the surface properties of samples with high spatial resolution. A greater width of ssDNA as well as dsDNA was detected in the blood of patients with endometriosis and endometrial adenocarcinoma when compared to the control group. Similarly, the width of nucleosomes was greater, suggesting increased activity of nuclear histone deacetylases associated with epigenetic histone modification processes. The obtained results point to the possible use of the AFM method as a unique detection technique, which can verify the occurrence and differentiate endometriosis from uterine endometrial carcinoma based on the interconnection of relationships between temporal and spatial dynamics of molecular mechanisms. In the second part of the project, changes in expression of mRNA of specific pro- (PLGF) and anti-angiogenic factors (endogline) in the blood of patients with a precancerous form of endometriosis method by real-time PCR were



detected. The expression level of the endogline gene showed a significant increase in mRNA when compared to the control group. Conversely, the PLGF-angiogenic gene was characterized by reduced expression, indicating reduced neoangiogenesis and neoplastic differentiation. The results achieved confirmed that changes in individual angiogenic genes affect not only the process of angiogenesis during the emergence of disease, but also affect the progression of the monitored disease. The determination of gene transcription activity can therefore contribute to the correct selection of high risk patients with endometriosis. Implementation of these modern and still highly current molecular methods contributes to improving laboratory diagnostics and moves it towards personalized medicine. In summary, there have been achieved scientific research results published in 2 ADC - foreign current content journals, 2 ADM papers were published in journals registered in WOS/SCOPUS databases). Two ADE scientific papers were published in other foreign journals. The results achieved were presented on both domestic and foreign conferences and were published in the form of abstracts (7 outputs). ADC, one ade and two ADF papers are in press.

Published papers are registered in UK UPJŠ and a complete list of published outputs is accessible from:

https://aleph.upjs.sk/F/7MCUFUA1PPU88Y2FRQTTHU74T9REDC8AE879GSQTCJ5F7159Q-40009?func=find-c-0&local_base=epc01

VEGA 1/0115/14:

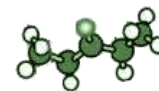
Study of the mechanism of thoracic aorta aneurysm due to regulatory changes of signalling pathway TGF-1 β – *principal investigator*: prof. Ing. **Mária Mareková**, CSc.

In the context of the solution of the project **VEGA 1/0115/14**, pathological changes were monitored in the tissue of the thoracic aorta (TAA) as well as in the blood by fluorescent analysis methods, clinical-biochemical and molecular-biochemical methods. Fluorescent fingerprint, used in blood studies confirmed its diagnostic potential. Changes of the expression of selected inflammatory cytokines (CRP, IL6) and their receptors (TNFR1, TNFR2, IL6R) as well as changes in extracellular matrix damage markers expression (Emilit-1, MMP9, Timp) in the blood and tissue of patients with different degrees of TAA progression. Based on the detection of individual markers, there were already a significantly increased level of mRNA and proteins for CRP and IL6, which indicates the progression of the strings of the vascular wall were shown in early phases of TAA (up to 4.3 cm). Inflammatory changes closely correlated with MMP levels and emilin-1, whose increased activity suggests progressive weakening of the ECM by targeted degradation of fibrin, induction of the TGF- β - β lane, while both processes increase the risk of TAA rupture.

The results of the study of selected parameters confirm the deterioration of the tissue with TAA progression, which could contribute to a better laboratory diagnosis of TAA, respectively to the indications of specific surgical procedures at the molecular level. The results achieved in solving the project are expanding the knowledge of TAA mechanism and can also be used in developing new diagnostic markers that would contribute not only to improve clinical diagnostics, but also monitoring patient therapy. In summary, there have been achieved scientific and research results published in 4 foreign current content journals (ADC), two papers were published in journals registered in WOS/SCOPUS databases (ADM, ADN). Furthermore, seven scientific papers were published in other foreign / domestic journals (ADF). The results achieved were presented on both domestic and foreign conferences and were published in the form of abstracts (14 outputs).

Published papers are registered in UK UPJŠ and a complete list of published outputs is accessible from:

https://aleph.upjs.sk/F/H18E6UM1RL73KCE7FQUK9JASNRG2L8S66S8G79LRHFDM9EIQ4J-33228?func=find-c-0&local_base=epc01



VEGA 1/1236/12:

Effect of humic acids and polyunsaturated fatty acids on animal production health, antioxidant status, the activity of mitochondria, lipid profile, absorption of certain heavy metals and pesticides from feed – *principal investigator*: doc. MVDr. Ladislav Vaško, CSc.

Several important and interesting knowledge about the studied substances were identified during the solution of **VEGA 1/1236/12**. It was observed that antioxidant enzymes of mitochondria in various organs as liver, kidney, heart react differently to oral intake of natural substances, e.g. gamma linolenic acid, which is caused by a different preference to nutrients in individual organs. It is apparent from the results achieved that when applying natural or synthetic substances affecting antioxidant status, it is very important to find an effective concentration because higher or lower concentration used can adversely affect the health state of the organism. Completely new knowledge is the finding that oral administration of humic acids change the concentration of microelements not only in different bodies, but also the intracellular redistribution within the body and thus modifies the metabolic activity of the cell. The knowledge in the available literature has not been described and therefore it is desirable continue the research (submitted a new VEGA project) as the effect of only one used huminic acid concentration was studied. The results in the project solution were presented on both domestic and foreign scientific conferences (55) and scientific papers were published in foreign current content journals, respectively. registered in WOS or Scopus databases (9) and domestic scientific (11) and professional (5) journals.

Published papers are registered in UK UPJŠ and a complete list of published outputs is accessible from:

<https://aleph.upjs.sk/cgi-bin/epc2.cgi?set=106532&sestava=7&pocet=93&dotaz=1/1236/12&sesnum=2V3X2XEKAU897XPQSALF6LBC83NKDN2J2U6CJ9138P632XX34U-26396>

VEGA 1/0999/11:

The effect of polyphenols and monoamine oxidase inhibitors on mitochondrial function – *principal investigator*: prof. Ing. Juraj Guzy, CSc.

As part of the solution of **VEGA 1/0999/11**, the effect of selegiline (deprenyl) was monitored in high (0.001 mol/kg) and low doses (0.00001 mol/kg) on Wistar rat's mitochondria. High doses of selegiline are associated with adverse side effects. For rats, their positive effect on the respiratory control ratio of mitochondria was confirmed. It was proved, that tetramethylpyrazine (TMP) caused vasodilation and inhibition of blood plate aggregation, and demonstrated a significant antioxidant effect. Our results point to the protective effect of TMP on small intestine mucosa. Studies provided with the support of this grant can serve as pilot studies for various clinical testing of the therapeutic effect of test substances (eg deprenyl). It would be appropriate to clinically test the therapeutic effect of low doses of deprenyl in people suffering from Parkinson's disease, as their positive effect has been confirmed on the respiratory control ratio of rats bmitochondria. Substituted chalcones showed a protective effect against oxidative stress. The studies with TMP, that causes vasodilation and inhibition of blood platelet aggregation point to the TMP protective effect on the small intestine mucosa, indicating the positive effect of TMP administration in ischemic-reperfusion damage of the small intestine. The results from the project solution were presented to 4 domestic and foreign scientific conferences including conferences organized by FEBS with the publication of the abstract in the current content journal (Mareková, Miria - Tomečková, Vladimír - Revická, Miroslava Doctorand - Guzy, Juraj: Study of Endogenous Fluorescence of Mitochondria by Fluorescence Techniques - no. Project: VEGA 1/0999/11, MŠ SR - APVV 0252-07. In: FEBS JOURNAL. - ISSN 1742-464X. - Vol. 280, SUPPL. 1 (2013), p. 262.) Furthermore, the results were published in one current content journal (Toth, Štefan Jr. - Pekárová, Timea - Varga, Ján - Tóth, Stefan - Tomečková,



Projects – completed successfully

Department of Medical and Clinical Biochemistry



Vladimír - Gál, Peter - Veselá, Jarmila - Guzy, Juraj: Intravenous Administration of Tetramethylpyrazine Reduces Intestinal Ischemia -Reperfusion Injury in Rats - no. Project: VEGA 1/0402/10, VEGA 1/0999/11. In: American Journal of Chinese Medicine. - ISSN 0192-415x. - Vol. 41, No. 4 (2013) p. 817-829) and 3 scientific papers has been published in domestic journals.

Published papers are registered in UK UPJŠ and a complete list of published outputs is accessible from:

<http://aleph.upjs.sk/cgi-bin/epc2.cgi?set=011127&sestava=1&pocet=8&dotaz=Kod%20pracoviska=%20UPS51010%20and%20Rok%20vydania%28985r%29=%202001-%202013%20and%20Projekt%28typ%29=%20VEGA%20and%20Projekt%28%20C4%20D%20C3%20ADslo%29=%201/0999/11%20&sesnum=12SM85PAEHN845NINYYBXBGUFJ11JEB75CG6HLVHKEHDBKBB8G-16648>