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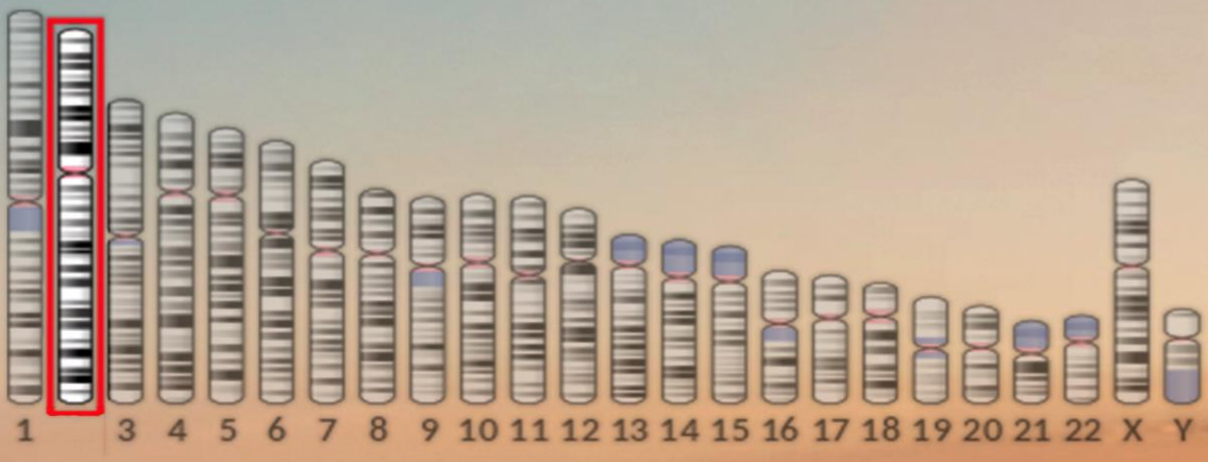

2nd Congress of Geneticists in BiH with International Participation

special edition

BOOK OF ABSTRACTS

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Welcoming address,

Dear colleagues and friends,

We proudly invite you to join us on the occasion of the 2nd Congress of Geneticists with International Participation, organized by Genetic Association in Bosnia and Herzegovina. This year, although we celebrate our 10th anniversary, with COVID-19 threat still looming, we give precedence to the safety; therefore, the Congress will be held online.

The circumstances aside, we hope to maintain the standard established at the 1st Congress of Geneticists with International Participation in 2019. We gather international authors, bh diaspora representatives, and local scientists that pursue research in various fields of genetics.

This year, we again stand together with University of Sarajevo - Institute for Genetic Engineering and Biotechnology, the publisher of Genetics and Application. With special edition of the journal dedicated to the Congress we also contribute to the popularization of the journal.

We invite the presenters and authors to provide *in extenso* manuscripts for the following issues of the journal.

We want to draw your attention to the "Workshop on Advanced Biotechnology in Bosnia and Herzegovina – novel tools, safety measures, ethical issues in genetic manipulation", that we co-organize with the United States Department of Agriculture and The Department of State. Our intention is to facilitate the introduction of the most advanced method of genetic editing (i.e. CRISPR Cas9) in Bosnia and Herzegovina and develop platform for its safe and responsible utilization, efficient control and monitoring.

I use the opportunity to express my gratitude to all my colleagues from the Organizing and Scientific Committees as well to all of our young colleagues that incorporated their knowledge, experience and efforts into this Congress. In hope that we will make this event into a tradition of *Sharing, Learning and Innovating Genetics* I wish you all pleasant and fruitful work.

Prof. dr. Kasim Bajrović

SCIENTIFIC PROGRAMME

2nd Congress of Geneticists in Bosnia and Herzegovina with International Participation,
September, 13th - 17th 2021.
Online Edition

MONDAY 13th September CET 16:00 – 19:45

Human and Medical Genetics

Chaired by ***Lejla Alic*** *(University of Sarajevo, Faculty of Medicine, Bosnia and Herzegovina)* and ***Marija Vukovic*** *(Clinical Centre Banja Luka, Bosnia and Herzegovina)*

16.00 – 16.15	Opening address
16.15 – 16.50	Genomics integrated health systems: envision the future (*Borut Peterlin, Slovenia*)
16.50 – 17.20	The effectiveness of postnatal genomic diseases diagnostics by array CGH (*Tatjana Damnjanovic, Serbia*)
17.20 – 17.50	Genetics of male infertility (*Maja Barbalic, Croatia*)
17.50 – 18.10	Poster viewing / Coffee break
18.10 – 18.40	The role of genetics polymorphisms in COVID-19 disease course and outcome – what do we know so far? (*Biljana Jekic, Serbia*)
18.40 – 19.10	Population genetic analyses implicate biogenesis of translation machinery in human ageing (*Nazif Alic, United Kingdom*)
19.10 – 19.30	Challenges in optimization of Ion GeneStudio S5 NGS protocol for SARS-CoV-19 genome sequencing (*Dino Pecar, Bosnia and Herzegovina*)
19.30 – 19.45	Sex-dependent influence of glutathione-s transferase gene polymorphism on asthma control in children observed at the age of 8 to 10 years (*Amina Asceric, Bosnia and Herzegovina*)

TUESDAY 14th September CET 16:00 – 18:00

Genetics of Natural Resources

Chaired by ***Fuad Gasi*** *(University of Sarajevo, Faculty of Agriculture, Bosnia and Herzegovina)*

16:00 – 16:40	Genetic data are crucial to conserve and manage wildlife in Southeast Europe (*Elena Buzan, Slovenia*)
16:40 – 17:00	Two-decade's experience of genetic monitoring of broodstock from several fish farms in Bosnia and Herzegovina (*Belma Kalamujic Stroil, Bosnia and Herzegovina*)
17:00 – 17:20	Poster viewing / Coffee break
17:20 – 17:40	The role of gene bank in conservation of biodiversity (*Toni Eterovic, Bosnia and Herzegovina*)

17:40 – 18:00 Genetic diversity and differentiation of alpine salamanders from the Dinarides – an evolutionary perspective with insights for species conservation (*Emina Sunje, Bosnia and Herzegovina*)

WEDNESDAY 15th September CET 16:00 – 19:05

Bioengineering, Biotechnology and Bioinformatics

Chaired by ***Adaleta Durmic Pasic*** *(University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Bosnia and Herzegovina)*

16:00 – 16:40 Cutting-Edge Crop Breeding with CRISPR-Cas Technologies (*Yiping Qi, USA*)

16:40 – 17:20 From genome to molecules: practical cases of structural elucidation of siderophores by using genome mining (*Carlos Jiménez, Spain*)

17:20 – 17:50 Genome mining strategies in natural products identification (*Lada Lukic Bilela, Bosnia and Herzegovina*)

17:50 – 18:10 Poster viewing / Coffee break

18:10 – 18:30 Experience the future of drug discovery and disease modeling research with genetic engineering solutions and organoids from Merck (*Igor Pongrac, Croatia*)

18:30 – 18:50 Corticosteroid-induced expression of microbial virulence can enhance development of host infectious disease (*Kamelija Madacki-Todorovic, Bosnia and Herzegovina*)

18:50 – 19:05 Allelopathic effect of cedarwood and geranium essential oils on seed germination of selected plant species (*Amina Hadziemric, Bosnia and Herzegovina*)

THURSDAY 16th September CET 16:00 – 18:15

Biomonitoring and Genetic Toxicology

Chaired by ***Nurşen Başaran*** (*Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkey*) and ***Goran Gajski*** *(Institute for Medical Research and Occupational Health, Zagreb, Croatia)*

16:00 – 16:35 Herbal products: are they safe because they are natural? (*Nurşen Başaran, Turkey*)

16:35 – 17:10 Brazil nut consumption reduces DNA damage in patients with type 2 diabetes mellitus probably through changes in oxidative status (*Vanessa Moraes de Andrade, Brazil*)

17:10 – 17:25 Poster viewing / Coffee break

17:25 – 18:00 Application of blood and buccal micronucleus assay in monitoring children¸ exposed to diagnostic radiation in clinical settings (*Goran Gajski, Croatia*)

18:00 – 18:15 Chromosome aberrations in medical personnel occupationally exposed to low-dose ionising radiation (*Anja Haveric, Bosnia and Herzegovina*)

FRIDAY 17th September CET 16:00 – 19:30

Forensic Genetics

Chaired by ***Jasmina Cakar*** *(University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Bosnia and Herzegovina)*

16:00 – 16:35 DNA identification of the victims of war conflicts and various mass disasters: overview of the Western Balkan experience (*Damir Marjanovic, Bosnia and Herzegovina*)

16:35 – 17:10 Private forensic practice in Serbia: experience and perspectives (*Ana Banjac Canak, Serbia*)

17:10 – 17:45 Benefits of X and Y-STR analysis in sexual assault cases and kinship relatedness (*Zlatko Jakjovski, Republic of North Macedoia*)

17:45 – 18:00 EZ2 connect FX: applications in human identification and forensics (*Ondrej Krsicka, Qiagen, Germany*)

18:00 – 18:15 Poster viewing / Coffee break

18:20 – 18:35 NGS in forensics: current practices, possibilities and obstacles (*Rijad Konjhodzic, Bosnia and Herzegovina*)

18:35 – 18:50 Laboratory for biological expertise and dna analysis of the agency for forensic and expert examinations, overview of previous activities (*Una Morankic, Bosnia and Herzegovina)*

18:50 – 19:05 The significance of determininig the abo blood groups from dried blood traces found at crime scene (*Amira Kekić, Bosnia and Herzegovina*)

19:05 – 19:20 Determening the forensically significant SNP-s of the mitochondrial HVIII region in population of Bosnia and Herzegovina (*Nejira Handzic, Bosnia and Herzegovina)*

19:20 – 19:30 Closing remarks (Rifat Hadziselimovic, *President of the Scientific Board*)

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Human and Medical Genetics

Marija Dusanovic Pjevic (University of Belgrade, Faculty of Medicine, Institute of Human Genetics, Belgrade, Serbia): ANALYSIS OF THE ASSOCIATION BETWEEN TUMOR NECROSIS FACTOR-A -308 G/A POLYMORPHISM AND THROMBOLYTIC THERAPY SIDE EFFECTS IN ACUTE ISCHEMIC STROKE PATIENTS

Milka Grk (University of Belgrade, Faculty of Medicine, Institute of Human Genetics, Belgrade, Serbia): ANALYSIS OF IL10RB GENE HAPLOTYPES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

Lucija Zunic (Genom Ltd, Zagreb, Croatia) PANEL ANALYSIS OF EVIDENCE-BASED INFERTILITY GENES IN SERTOLI CELL-ONLY SYNDROME PATIENTS

Azra Licina Sinanovic (Association for Nutrition and Dietetics "Food for Health", Tuzla, Bosnia and Herzegovina): GENETIC DIVERSITY OF THE MALE POPULATION OF THE BIJELO POLJE MUNICIPALITY THROUGH THE PRISM OF THE Y CHROMOSOME

Alma Mudrov (University of Tuzla, Faculty of Pharmacy, Tuzla, Bosnia and Herzegovina): KAUFMAN OCULOCEREBROFACIAL SYNDROME: PHENOTYPIC LANDSCAPE AND LITERATURE REVIEW

Aldijana Avdic (University of Tuzla, Faculty of Natural Sciences and Mathematics, Department of Biology, Tuzla, Bosnia and Herzegovina): MOLECULAR-GENETIC CHARACTERIZATION OF ApoE GENE POLYMORPHISM AND VNTR POLYMORPHISM OF eNOS GENE IN HUMAN POPULATION OF TUZLA CANTON

Maja Milosevic Markovic (University of Belgrade, School of Dental Medicine, Department of Human Genetics, Belgrade, Serbia): THYMUS SERPYLLUM AND MENTHA PIPERITA ESSENTIAL OILS AFFECT SHH AND NOTCH SIGNALING PATHWAYS IN BASAL CELL CARCINOMA IN VITRO

Nora Puseljic (Institute of Emergency medicine OBŽ, Osijek, Croatia): CLINICAL MANIFESTATIONS OF A PATIENT WITH MICRODUPLICATION 12q23 AND DELETION Xp22

Maja Zekavica (University Medical Center “Zvezdara“, Department of Microbiology and Genetics, Belgrade, Serbia): PRESENCE OF HLA-Cw*06 ALLELE IN PATIENTS WITH PSORIASIS VULGARIS - THE FIRST REPORT FROM OUR CENTER

Lejla Caluk Klacar (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): ASSOCIATION OF SELECTED THROMBOPHILIA GENETIC MARKERS WITH RECURRENT PREGNANCY LOSS IN BOSNIA AND HERZEGOVINA

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Dalila Destanovic (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): IS DNA BARCODING A FEASIBLE TOOL FOR BIODIVERSITY ASSESSMENT IN THE PROTECTED AREAS OF CANTON SARAJEVO?

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Belmina Saric (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): GLU288 AND LYS137 OF SARS-CoV-2 MAIN PROTEASE AS TARGETS FOR INHIBITION BY SELECTED THYME PHYTOCONSTITUENTS

Tarik Corbo (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): IMPORTANCY OF MACHINE LEARNING IN BIOLOGICAL STUDIES

Mirsada Salihovic (University of Sarajevo, Faculty of Science, Department of Biology): INVESTIGATION OF THE ANTIOXIDANT ACTIVITY OF PHYSALIS ALKEKENGI L. VARIOUS EXTRACTS

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Ana Djuranovic (University of Belgrade, Faculty of Medicine, Institute of Human Genetics, Belgrade, Serbia): STUDY OF IL1B AND iNOS GENES POLYMORPHISMS AS A RISK FACTOR FOR THE DEVELOPMENT OF CEREBRAL PALSY

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Daniel Sosiński (Daniel's Green Technology, Szczecin, Poland): CO-INHERITANCE OF GENETIC VARIANTS LINKED TO THE RISK OF PREGNANCY LOSS IN BOSNIAN WOMEN- OWN STUDIES REVIEW

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Aleksandra Markovic (University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Kragujevac, Serbia): MICRONUCLEI FREQUENCY IN PATIENTS WITH ENDOMETRIAL CANCER IN RELATION TO STAGE OF DISEASE, AGE AND SMOKING HABITS

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Svetlana Janceva (University Clinic for Rheumatology, Skopje, Republic of North Macedonia): THE IMPORTANCE OF ANTI-dsDNA ANTIBODIES SCREENING AS A USEFUL TOOL FOR EVALUATION AND TREATMENT OF PATIENTS WITH SYSTEMIC RHEUMATIC DISEASE

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Mirela Dzehverovic (University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina): DNA ANALYSIS OF HUMAN SKELETAL REMAINS FROM THE MEDIEVAL NECROPOLES IN BOSNIA AND HERZEGOVINA – A GENETIC STRUCTURE OF OUR ANCESTORS AND PREDICTION OF THEIR MIGRATIONS

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INVITED PRESENTATIONS

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GENOMICS INTEGRATED HEALTH SYSTEMS: ENVISION THE FUTURE

Borut Peterlin

University Medical Center Ljubljana, Clinical Institute of Genomic Medicine, Ljubljana, Slovenia

Genetic predisposition contributes to significant morbidity and mortality worldwide. Due to the available technology, genetic risk factors can be efficiently recognised and health measures developed. Timely discussion on reshaping of health systems is especialy important not only in developed countries but even more in developing ones. Current genomic technologies can already deliver information for significant improvement of public health and quality of life in terms of identification, prevention and treatment of genetic disorders. Genomic diagnosis and screening for rare diseases has already an important impact on public health. Morever, genomic information can significantly contribute to the prevention and treatment of common, complex disorders.

Keywords: genomics, prevention, health systems
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THE EFFECTIVENESS OF POSTNATAL GENOMIC DISEASES DIAGNOSTICS BY ARRAY CGH

Tatjana Damnjanovic

University of Belgrade, Faculty of Medicine, Institute of Human Genetics, Belgrade, Serbia

Array-based comparative genomic hybridization (aCGH) is a proven method broadly implemented in clinical practice to detect gains and losses of DNA and the absence of heterozygosity across the genome. In the postnatal setting aCGH is a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, dysmorphic features, congenital anomalies, epilepsy, and autism. In the Institute of Human Genetics, Faculty of Medicine, University of Belgrade we implemented aCGH as a clinical diagnostic tool in 2018 and become the referent diagnostic Center in Serbia. We have analyzed over 500 patents reffered to our laboratory from January 2018 to July 2021. The causative variants detection rate reached approximately 17%. Highest detection rate of 37,5% has been observed in patients with combined phenotype which included developmental delay/intellectual disability, dysmorphic features with microcephaly, and congenital anomalies. Detected pathogenic variants vary in size from 240 kb to 9.726 Mb and are mostly small rearrangements below the resolution of classical karyotype. Some very rare pathogenic variants have been detected, such as a deletion of first two exons of ERBB4 gene that caused severe behavioral disorder, intragenic duplication of 404kb in MYTIL gene resulted in a developmental delay with microcephaly, and VEGFC gene deletions expressed in phenotype as lymphedema and mild intellectual disability. Unexpectedly, we have detected a deletion of notably size (3p26.3-p26.1; 3.77 Mb) in three members of the same family without any clinical significance. Regardless of the evolving ability of the next-generation sequencing technology to detect copy number variations, aCGH is still of great importance for postnatal genomic diseases diagnostics.

Keywords: array-CGH, congenital malformation, intellectual disability, dysmorphic features, autism

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GENETICS OF MALE INFERTILITY

Maja Barbalic, Monika Klaric Logara, Lucija Zunic, Tihana Maric, Filip Rokic, Lovro Trgovec-Greif, Ana Vicic, Oliver Vugrek, Feodora Stipoljev, Ana Katusic Bojanac, Robert Beluzic

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Male infertility affects 7% of men worldwide. Sertoli cell-only syndrome (SCOS) is the most severe form of male infertility characterized by a complete lack of spermatogenic cells in almost all seminiferous tubules. Y chromosome microdeletions, Klinefelter syndrome and *CFTR* mutations are the leading known genetic causes of male infertility and are routinely screened in standard clinical procedures. However, more than half of infertile men remain with unknown etiology that could be accounted for yet undiscovered genetic factors. As part of our male infertility project, we performed: 1. whole exome sequencing on two small families with a SCOS proband (2 infertile brothers and a SCOS patient and his healthy father) 2. ran an NGS panel consisting of high evidence genes associated with male infertility on 6 SCOS patients and 3. developed an NGS amplicon-based panel that simultaneously analyzes Y chromosome microdeletion, Klinefelter syndrome, *CFTR* and 11 candidate genes associated with infertility and ran the panel on 32 infertile men. All samples were collected in two medical centres in Zagreb, Croatia. Whole exome sequencing revealed likely pathogenic variants in *FANCM* and *DMRT1* genes. Evidence based NGS panel analysis of 6 SCOS patients revealed likely causative variants in *CHD7* and *SCYP3* gens. Finally, by running our NGS amplicon based panel, we detected pathogenic variants in 5 individuals (2 Y chromosome microdeletions and pathogenic variants in *CFTR* and *AR* genes). Our work confirmed the heterogeneous nature of male infertility. On the other hand, we showed that genetic variants associated with male infertility could be detected by running only one assay, with possibility of adding novel candidate genes. However, introduction of novel candidate genes in male infertility panel should be done with great caution.

Funding: The European Regional Development Fund (KK.01.2.1.01.0113)

Keywords: male infertility, SCOS, genetics, NGS panel, whole exome sequencing
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THE ROLE OF GENETICS POLYMORPHISMS IN COVID-19 DISEASE COURSE AND OUTCOME – WHAT DO WE KNOW SO FAR?

Biljana Jekic

University of Belgrade, Faculty of Medicine, Institute of Human Genetics, Belgrade, Serbia

Although mass vaccination has already begun to yield results, the emergence of new strains of the virus that could be resistant to vaccines, insufficient response of the population to vaccination and the lack of immunity in a certain percentage of vaccinated, still keeps us away from collective immunity against the Sars-Cov-2 virus. The most severe COVID-19 symptoms are acute respiratory distress syndrome accompanied by hypoxia and overreaction of the patient`s immune system presented as cytokine storm. Although a number of predictive factors for the development of severe disease and its lethal outcome have been described (obesity, diabetes, elevated d-dimmer, level of virus exposure, etc.,) frequent deviations from expected predictions result in need for intensive monitoring of a large number of patients or more reliable predictive markers. Polymorphisms in genes for the proteins involved in the route of Sras-Cov-2 virus infection of the cells and mechanisms of organisms' response to the infection could play a significant role in the course and outcome of the COVID-19 diseases. Several groups of researchers, including ours, have analyzed the association of polymorphisms within these genes and the outcome of the COVID-19 disease. Sars-Cov-2 enters the cell through ACE2 (angiotensin converting enzyme 2) receptor after priming of viral spike protein by TMPRSS2 (transmembrane protease serine 2). ADAM17 (ADAM Metallopeptidase Domain 17) cleaves ACE2 from the alveolocyte surface and transforms it into a soluble form, thus preventing Sars-Cov-2 from entering the cell. *HIF-1A* gene encodes for the hypoxia inducible factor-1 alpha, a major regulator of adaptive response to hypoxia. HIF-1 alpha regulates transcription of numerous genes, including down regulation of *ACE2* and *TMPRSS2* and up regulation of *ADAM17*. HIF-1 alpha also regulates the development of cytokine storm. Our group analyzed association between *HIF-1A* rs11549465 and rs2057482, and *ACE I/D* polymorphisms with clinical parameters and disease outcome in 129 COVID-19 patients. We have observed lower mean platelet counts in carriers of *HIF-1A* rs11549465CC genotype (p=0.05). Additionally, in group of patients under the age of 40 we have observed an association of thrombocytopenia with rs11549465CC/rs2057482CT *HIF-1A* genotypes combination (p=0.037) and oxygen therapy admission with *ACE* II genotype (p=0.036).

Keywords: COVID-19, polymorphisms, gene, *HIF-1A, ACE,* predictive marker
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GENETIC DATA ARE CRUCIAL TO CONSERVE AND MANAGE WILDLIFE IN SOUTHEAST EUROPE

Elena Buzan

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The entire Balkan Peninsula is a hotspot of mammal diversity. Habitat loss and fragmentation are major threats to the survival and persistence of mammal populations, including in this area, as connectivity between populations has declined dramatically. Anthropogenic activities impede the daily and seasonal movements of wildlife, disrupt ecological processes, and can act as physiological stressors. Therefore, one of the most important tasks of current wildlife conservation and management is to avoid habitat fragmentation, restore connectivity between populations in formerly connected habitats, and adequately manage populations with unique genetic diversity. It is generally accepted that genetic diversity is one of the most important factors enabling populations to respond and adapt to environmental change. Since neutral genetic variation often provides an incomplete picture of the evolutionary potential of populations, it has been proposed that adaptive genetic diversity in natural populations should also be monitored. In the contribution, I will focus on our research of five mammal species with different populations' abundance, spatial behaviour, movement ecology, diet preferences, and life-history traits: three wild ungulates (roe deer – *Capreolus capreolus*, chamois – *Rupicapra rupicapra*, wild boar – *Sus scrofa*), and two mesopredators (red fox – *Vulpes vulpes*, wildcat – *Felix sylvestris*) inhabiting the area between Alps and Scardo-Pindic mountains. I will present how we used neutral and adaptive genetic markers, in order to investigate: (i) the spatial distribution of neutral and immunogenetic variation, (ii) the relationship between neutral and adaptive diversity, (iii) the effect of genetic variation on body mass and reproductive ability, and also to obtain (iv) robust insight into the spatial behaviour. Although current approaches to biodiversity conservation focus largely on species and pay less attention to genetic diversity and intraspecific variation, it is noted that conservation managers increasingly recognize the value of explicitly considering genetic information in management actions and priority settings, which is particularly urgent in biodiversity hotspot areas such as Southeast Europe.

Keywords: genetic diversity, molecular markers, connectivity, ungulates, mesopredators.
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CUTTING-EDGE CROP BREEDING WITH CRISPR-CAS TECHNOLOGIES

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Genome editing technologies based on CRISPR-Cas systems are revolutionizing plant research and agriculture. This talk will first provide a general overview of the landscape of the cutting-edge CRISPR genome editing tools, including CRISPR-Cas9, CRISPR-Cas12a, CRISPR-Cas12b, C-to-T base editors, A-to-G base editors, and prime editors. Then, I will present a few case studies for the application of these tools different plant species, including rice, maize, wheat, carrot, tomato, and poplar. Next, I will introduce highly efficient multiplexed genome editing systems based on CRISPR-Cas9 and Cas12a. Their powerful applications will be showcased in rice, such as simultaneous editing of multiple crop traits, targeted protein evolution for herbicide resistance, and creation of quantitative traits by editing cis-regulatory elements. Finally, I will talk about an exciting gene activation technology, CRISPR-Act3.0, and its use for accelerating crop breeding and metabolic engineering. Collectively, these CRISPR technologies will greatly aid the development of high-yield, nutritious and resilient crops to feed the growing population worldwide.

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FROM GENOME TO MOLECULES: PRACTICAL CASES OF STRUCTURAL ELUCIDATION OF SIDEROPHORES BY USING GENOME MINING

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Although iron, an essential nutrient in virtually all living organisms, is the fourth most abundant element in the Earth's crust, its bioavailability to microorganisms is limited because it is oxidized to insoluble ferric iron (Fe^{3+}). To overcome this problem, most microorganisms use low molecular weight organic compounds named siderophores. They have specific and high affinity towards this metal and they are considered virulence factors because they are critical for survival. We are studying the siderophores from fish pathogenic bacteria and its application against the infectious diseases which causes considerable economic losses in aquaculture. In this communication, I will show the results from our studies on the siderophores produced by Gram negative bacteria involved in the main fish infection diseases in aquaculture, namely vibriosis by *Vibrio anguillarum,* photobacteriosis by *Photobacterium damselae* subsp. *piscicida*, and furunculosis by *Aeromonas salmonicida* subsp. *salmonicida*. Their biosynthesis is directed by a large family of modular ribosomal peptide synthetase-polyketide synthetase (NRPS-PKS) hybrid multienzymes which facilitate their identification using genome mining. The key role of genome mining for the identification and elucidation of the structures of these siderophores and the prediction of the structure of cryptic ones will be display too.

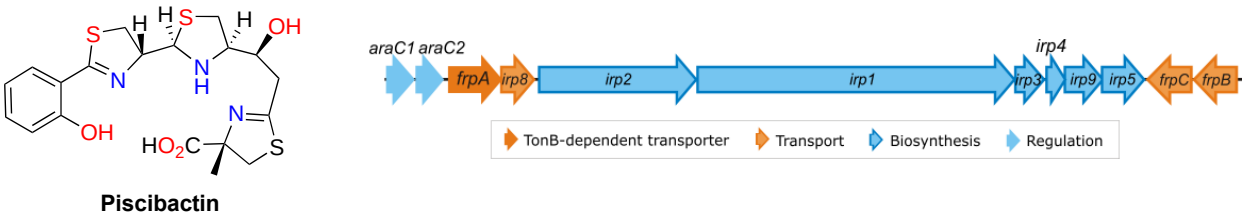

Fig. Structure of piscibactin (Pcb) and genetic map of *irp* gene cluster encoding its biosynthesis and transport.

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Keywords: Genome mining, siderophore, iron uptake, Gram-negative bacteria.
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GENOME MINING STRATEGIES IN NATURAL PRODUCTS IDENTIFICATION

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The genomic era has opened up a host of new opportunities for the development of novel natural products (NP), which are encoded by biosynthetic gene clusters (BGC) that regulate their synthesis through complex enzymatic reactions and regulatory switches. Genome mining (GM), a set of computer methods for detecting and annotating BGCs in genomic data, has emerged as a key technology for exploiting and exploring NP diversity. Furthermore, because the identification of NP biosynthetic pathways leads to the elucidation of their possible functional and chemical interactions, machine learning (ML) genome mining approaches play an important role in understanding NP chemical diversity by analyzing the architecture and structure of microbial, fungal, and plant genomes, or their "BGC genomic language." A multidisciplinary approach is emphasized here since carefully designed strategies (target or behavior based, e.g.) and well-chosen tools can greatly facilitate the process of NP identification, especially in exploring of extreme habitats. Thus, the elucidation of complex intra- and interactions between species and their amazing communication directs the discovery of untapped sources of natural products. The importance of an integrative approach that combines genome mining (GM), comparative genomics and functional genetics/genomics will be discuss on the example on marine and underground habitats in Dinaric karst and the possible biotechnological potential of microbial communities.

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Keywords: Natural products (NP), biosynthetic gene clusters (BGC), genome mining (GM), natural resources, microbial communities
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HERBAL PRODUCTS: ARE THEY SAFE BECAUSE THEY ARE NATURAL?

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Herbal products are being increasingly used all over the world for preventive and therapeutic purposes because of the belief of their safety. They have become an important part of health care system in many countries across the world. They can easily be purchased in the health food stores or online. However, as the contents of herbal medicines have many bioactive components, the lack of sufficient study on their efficacy and toxicity, inadequate controls of their availability reduce their safety. Unlike conventional drugs, herbal products are not regulated for purity and potency. Herbal products contain substances which can induce or inhibit enzymes that can take part in drug metabolism. Interactions between herbal products and drugs may increase or decrease the pharmacological or toxicological effects of the active component. Therefore the concurrent use of drugs wih some medicinal plants can cause serious adverse effects and also decrease the efficacy of the therapy. Particularly, drugs with narrow therapeutic index (warfarin, digoxin, etc.), and many plants which affect drug metabolizing enzymes (Hypericum perforatum, Panax ginseng, Ginkgo biloba,Ephedra etc.) when used together, may lead to unpredictable adverse reactions. Patients with chronic diseases who also use herbal medicines must consider the adverse effects and interactions of these substances. Some of the adverse effects reported for herbal products could be caused by impurities. On the other hand, herbal adulteration, which is described as intentional substition with another plant or a drug to increase the potency of the product, is one of the common malpractises in herbal raw-material trade. In the presentation the unpredictable adverse effects of herbal products due to their possible interactions with drugs and also due to the adulteration and contamination of herbal products with prohibited chemicals, will be discussed in detail.

Keywords: herbal products, drug interactions, adulteration, toxicity
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BRAZIL NUT CONSUMPTION REDUCES DNA DAMAGE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS PROBABLY THROUGH CHANGES IN OXIDATIVE STATUS

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Type 2 diabetes mellitus (T2DM) is a metabolic disease, occurring largely due to lifestyle changes. There is a strong link between T2DM and oxidative stress, that leads to damage in lipids, proteins and DNA. Dietary interventions are essential for the treatment of T2DM-related complications. Knowing that Brazil nuts are the richest source of selenium in nature, and that this mineral presents several health benefits, the aim of this study was to assess the effects of consumption of selenium through Brazil nut on biochemical and oxidative stress parameters, as well as genomic instability in T2DM patients. We evaluated 74 patients with T2DM. Participants consumed one Brazil nut a day (that provides 210 µg of selenium) for six months. Blood and exfoliated buccal cells samples were collected at the beginning and at the end of treatment. The glycemic profile, lipid profile, oxidative stress and DNA damage were evaluated. The data relative to biochemical parameters presents an increased in fasting glucose levels, HDL- and LDL-cholesterol. On the other hand, insulin levels and triglycerides/HDL-cholesterol ratio were decreased. Also we observed an increase in GSH levels, and GPx and CAT activity. In relation to oxidant production, DCF and nitrites levels were decreased. Besides, were observed an increase in total thiols, and a decrease in protein carbonyl and MDA levels. Relative to genomic instability, the levels of DNA damage in T2DM were significantly decreased, as well as the frequency of micronuclei and nuclear buds. Taken together, our results indicate that Brazil nut consumption could be an ally to module the genomic instability in T2DM patients, probably through changes in redox balance.

Keywords: Brazil nut, selenium, type 2 diabetes mellitus, DNA damage, oxidative stress
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APPLICATION OF BLOOD AND BUCCAL MICRONUCLEUS ASSAY IN MONITORING CHILDREN EXPOSED TO DIAGNOSTIC RADIATION IN CLINICAL SETTINGS

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It has been postulated that micronuclei frequency is related to the increasing presence of carcinogens. The lymphocyte micronucleus cytome (L-MN Cyt) assay has become one of the best-validated methods for measuring chromosome damage; however, the buccal micronucleus cytome (B-MN Cyt) assay has been gaining more attention in recent years, especially as it is minimally invasive, hence more appropriate for children biomonitoring. As children might be more sensitive to radiation, there is a need for constant biomonitoring of young populations receiving X-ray diagnostic examinations. Therefore, we aimed to evaluate the effects of diagnostic chest and sinus X-ray exposure on lymphocytes and buccal cells using both MN Cyt assays. Additionally, doses were measured using thermoluminescence and radiophotoluminescent dosimetry systems and were in satisfactory agreement. Using L-MN Cyt assay, it was shown that the mean number of micronuclei, nucleoplasmic bridges and nuclear buds were significantly higher after the diagnostic procedure. Furthermore, the B-MN Cyt assay was done in order to evaluate DNA damaging, replicative, cytostatic, and cell death effects. Micronuclei as well as other biomarkers of DNA damage (nuclear buds and so-called "broken eggs") and genomic instability (normal basal cells, normal differentiated cells, binucleated cells, cells with condensed chromatin, pyknotic cells, cells with karyorrhectic chromatin and karyolitic cells) were analysed. The only significant increase was noted in cells with condensed chromatin, indicating more cells undergoing early stages of apoptosis. It should be pointed out that interindividual differences existed for each monitored child in both assays. Based on our results, MN Cyt assay could be very useful in acute events where children are exposed to genotoxic agents from physical sources. Besides, B-MN Cyt assay could be used for monitoring genetic damage in children who are often exposed to diagnostic procedures, as it is a minimally invasive method of sample collection.

Keywords: micronucleus cytome assay, pediatric X-ray diagnostics, human biomonitoring
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DNA IDENTIFICATION OF THE VICTIMS OF THE WAR CONFLICTS AND VARIOUS MASS DISASTERS: OVERVIEW OF THE WESTERN BALKAN EXPERIENCE

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The primary value of DNA profiling significantly increased over the last two decades due to the introduction of short tandem repeat (STR) loci in routine paternity testing, as well as forensic and mass disaster human identification. Data obtained by DNA typing are highly reliable and can be used as a very powerful tool that produces valuable results. Identification of human remains found in single and mass graves employs different methods: identification of remains by a living person who knew the decedent by direct facial recognition, fingerprint analysis, dentition analysis, identification of special features such as individual scars or tattoos, recognition of clothing and belongings, autopsy findings, the analysis of skeletal remains by forensic anthropologists to estimate the species of the remains, sex, age, race etc. For years now, different methods of forensic DNA testing (known as *DNA fingerprinting*) have been widely established and accepted as the standard procedure in various investigations. This lecture presents that experience gathered over the last, almost, twenty years through the projects of identification of the human skeletal remains in Bosnia, Slovenia and Croatia, It shows that current protocols and the procedures optimized for relatively fresh bones and teeth can be used, without significant modifications, in the analysis of much older samples from the WWII and even older. Also, introducing of new technologies can help recover information from degraded DNA samples that typically result in partial profiles and total loss of information from regular STR amplicons. This approach has already been used in the analysis of highly degraded samples like those processed within the identification of victims from the World Trade Center terrorist attacks, WWII victims and now usage of this scientific approach is proved even for the several hundred years old ancient bone samples.

Keywords: human identification, skeletal remains, short tandem reoeats
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PRIVATE FORENSIC PRACTICE IN SERBIA: EXPERIENCE AND PERSPECTIVES

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DNA Center for Genetics is a private genetic laboratory in Belgrade founded in 2008. The centre is divided into two departments: medical genetics and DNA expertise. The Department of Medical Genetics deals with molecular diagnostics of various hereditary diseases, as well as prenatal diagnostics. The DNA Expertise Department perform forensic DNA analysis, and paternity and kinship testing. The Centre is entered in the register of institutions of the Republic of Serbia that are involved in DNA expertise. Two permanent court experts work in the centre. Expert examinations are performed on the orders of courts and prosecutor's offices on the territory of the Republic of Serbia, including cooperation with certain legal entities in the region as well. The DNA Centre performed about 1,000 expertises annually. In addition to expertise performed by orders of legal authorities, the DNA Centre also performs expertise at the personal requests which are mostly related to paternity, motherhood and kinship testing. Since the establishment of the laboratory, GEDNAP testing is performed annually. There are many everyday challenges in our expertise such are legislation and strategy of DNA laboratory work, availability and prices of equipment, consumables and biostatistical programs, continuous education of parties in the process with the aim to know exactly what answers DNA expertise can provide. Still, there is enough experience, knowledge and professional integrity in our regional forensic community to offer a concept for solving at least part of the everyday problems we all face. That was the initiative launched in 2017 at our last gathering in Sarajevo.

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BENEFITS OF X AND Y-STR ANALYSIS IN SEXUAL ASSAULT CASES AND KINSHIP RELATEDNESS

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With large number of criminal acts, such as rape and murder, identification of the perpetrator is very difficult to determine. The cause is the lack of sufficient quantity of biological traces provided as material evidence, or the biological material is decomposed and no result can be obtained by using other identification methods. Thus the case remains unsolved. In daily routine practice, autosomal STRs are very well established because they give high probability match of identification of missing person or biological trace. Besides using autosomal STRs, we recommend Y-STRs to be used in all rape cases too, thus separating the male from female profile, and also the male kinship relatedness in cases of incest could be followed, the rape performed by several blood-related men or similar. In kinship relatedness cases, especially in paternity cases where biological father is deceased, by analyzing X and Y STRs from the living relatives high probability positive match identification is confirmed. In this paper we will present one rape case and one kinship (paternity) case with deceased father, where positive identification was confirmed with very high probability match. We can conclude that X and Y–STR loci are very useful in identification procedures of a murder, sexual assault cases and paternity and kinship cases, thus exhumation and using more expensive and hazardous methods of DNA extraction are avoided, so the benefits are protection of the health of the employees, saving money and time.

Keywords: Identification, kinship, X-STRs, Y-STRs, sexual assault, crime case, paternity

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EZ2 CONNECT FX: APPLICATIONS IN HUMAN IDENTIFICATION AND FORENSICS

Ondrej Krsicka

QIAGEN GmbH

Get the most out of every sample with ultra-efficient nucleic acid extraction on EZ2® Connect Fx. The newly developed purification instrument of QIAGEN is building on the proven and worldwide trusted technology of the EZ1® Advanced XL. It purifies high-quality DNA and RNA in under 20 minutes and so increases lab throughput by processing up to 24 samples in parallel and 288 samples per day with just one instrument! Users of the EZ2® Connect Fx can achieve reproducibility and convenience with prefilled, ready-to-use forensic grade (ISO18385) reagent cartridges, track their samples at every step with an internal camera for load checks and an external barcode reader. They will benefit from the EZ1® Advanced XL magnetic-bead purification technology they know and trust and can easily access a variety of dedicated human ID and forensics protocols at the push of a button. A wide range of sample types for various downstream forensics applications can be prepared. The EZ2® Connect Fx features: Easily accessible and visible work deck with a flexible throughput for up to 24 samples per batch, easy access to work deck for optimized functionality and with illumination that indicates instrument status. Efficient decontamination with UV-LED enabling so a long instrument lifespan and the powerful. Colored LCD touch display with up-to-date graphical user interface for ease of use, guided instrument setup and maintenance facilitates process safety, options to display reports support traceability and pre-programmed protocols to enable convenient startup. Recovery protocol for securing the most valuable forensic samples. The optional integration into QIAsphere provides an instrument overview, real-time run status monitoring, a maintenance viewer, push notifications, run reports, automatic software patch management, media center, instrument scheduler and usage monitor. Internal memory with pre-programmed protocols enable convenient startup. The traceability is supported by storage of reports, log files, maintenance reports, etc. and with its 4 MB storage capacity approximately 100 reports can be stored. With its small footprint it is a benchtop instrument – suitable also for labs with limited bench space - 71% increase in samples with only 45% increase in footprint.

Keywords: Forensic DNA extraction, magnetic-beads technoliogy, fast pre-programmed protocols, traceability
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NGS IN FORENSICS: CURRENT PRACTICIES, POSSIBILITIES AND OBSTACLES

Rijad Konjhodzic

Alea Genetički Centar

Next generation sequencing has established itself as a leading molecular method of analysis for large scale DNA analysis. NGS applications have enabled whole genes to be sequenced in single sequencing reaction, weather individually, or any number simultaneously, going as far as whole clinical exome sequencing. This has, naturally, proved to be a valuable diagnostic as well as research tool, as genetic conformation for a number od diseases, for which this is necessary, is much easier to obtain. In addition to this, molecular analysis can now assume leading diagnostic role, rather then confirmatory, as has been so far the case. Forensic DNA analysis has generally been concantrated on sex and autosomal chromosome STR's, and mitochondrial DNA hypervariable region SNPs. Autosomal SNP analysis has seen very limited use in forensics in pre NGS period, due to the number of SNP analysis required for statistically significant analysis. With the introduction of NGS, this field has opened up, as NGS scale enables acquiring of of large number of SNPs in hotspot panels in a single reaction. However, forensic DNA analysis differs greatly in application of standard operating procedures than Research Use Only, or even IVD anlyses. Fact that findings of the DNA forensic analyses have to be presented and defended in the court of law means that every aspect of the procedure can be an issure during the precedings. Having in mind that procedures in obtaining results in NGS and capillary electrophoresis differ greatly, especially problematic being NGS library creation, a number of potentially problematic points may be raised, being somewhat similiar to the time when automatic capillary electrophoresis was introduced over staining. This presentation will deal with some of the issues involving forensic DNA analysis on NGS platforms.

Keywords: Next Generation Sequencing, autosomal DNA, mitochondrial DNA, SNP
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ORAL PRESENTATIONS

2nd Congress of Geneticists in BiH with International Participation

The Official Publication of the
Institut for Genetic Engineering and Biotechnology
University of Sarajevo

Genetic Association in Bosnia and Herzegovina

POPULATION GENETIC ANALYSES IMPLICATE BIOGENESIS OF TRANSLATION MACHINERY IN HUMAN AGEING

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Reduced provision of protein translation machinery promotes healthy ageing in a number of animal models. In humans, however, inborn impairments in translation machinery are a known cause of several developmental disorders, collectively termed ribosomopathies. Here, we employ population genetic approaches to investigate whether adult, tissue-specific biogenesis of translation machinery drives human ageing. We assess naturally occurring variation in the expression of genes encoding subunits specific to the two RNA polymerases (Pols) that transcribe ribosomal and transfer RNAs, namely Pol I and III, and the variation in expression of ribosomal protein (RP) genes, using Mendelian Randomisation. We find each causally associated with human longevity (ß=-0.15±0.047, $p=9.6x10^{-4}$; ß=-0.13±0.040, $p=1.4x10^{-3}$; ß=-0.048±0.016, $p=3.5x10^{-3}$, respectively) and this does not appear to be mediated by altered susceptibility to a single disease. Interestingly, we find that reduced expression of Pol III, RPs or Pol I promote longevity from different organs, namely visceral adipose, liver and skeletal muscle, echoing the tissue-specificity of ribosomopathies, and we provide evidence that Pol I and RPs may act from organs where their expression is limiting. Our study demonstrates the utility of leveraging genetic variation in expression to elucidate how essential cellular processes impact human ageing. The findings extend the evolutionary conservation of protein synthesis as a critical process that drives animal ageing to include humans.

Keywords: ageing, human genetics, ribosome biogenesis
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CHALLENGES IN OPTIMIZATION OF ION GENESTUDIO S5 NGS PROTOCOL FOR SARS-CoV-19 GENOME SEQUENCING

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Ion AmpliSeq Library kit plus is robust and straight forward protocol for library preparation but certain optimization for better coverage were done. NGS system used for SARS-CoV-19 genome sequencing in this report is Thermo Fisher Ion GeneStudio S5. In this report we will note optimizations of protocol we experimented with clinical SARS-CoV-19 samples. We isolated viral RNA with an automatic extractor using magnetic beads protocol. Viral loads were checked with Q-PCR kit and samples which passed the detection with a Ct value less than 25 were considerate for next step of protocol. We tested tree cDNA synthesis kits and results are reported here. In this report we will mention results of experimenting with different cycles of first PCR amplification.In this report samples used for library preparation will be noted: type of medium swabs was stored, condition of storage of samples and cDNA concentration of used samples. We will also report the correlation between cDNA concentration, first PCR cycle and quantified library. In this report we will mention correlation between given concentration of the library with generated coverage for each sample. Results of this report will be useful for applicative scientists who work with SARS-CoV-19 samples for whole genome sequencing so they can see and apply good laboratory practice for optimal NGS library preparation protocol.

Keywords: NGS, SARS-CoV-19, genome, sequencing
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SEX-DEPENDENT INFLUENCE OF GLUTATHIONE-S TRANSFERASE GENE POLYMORPHISM ON ASTHMA CONTROL IN CHILDREN OBSERVED AT THE AGE OF 8 TO 10 YEARS

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Asthma is a chronic inflammatory disease that occurs as a result of a complex interaction between many environmental factors and genetic predisposition. Some studies demonstrate a close association between the glutathione S transferase gene and clinical asthma expression. Glutathione S transferase has a number of polymorphisms, including GSTM1 and GSTT1, which are thought to have a direct impact on the pathophysiology of asthma. The aim of this study was to examine the association between sex and GST gene polymorphisms in children with allergies (dust and mites) and asthma, aged 8-10 years. Blood samples were taken from 49 kids (46 born in term and 3 preterm) which, due to respiratory difficulties, are being monitored through the Pulmonary Counselling Center of the Pediatric Clinic KCUS. DNA was extracted using EXTRACT*ME* GENOMIC DNA KIT. Multiplex PCR was used to determine the presence or absence of *GSTT1* and *GSTM1* genes in the presence of the control β-*globin* gene. Genetic testing was performed at the Centre for Genetics at the Medical Faculty of the University of Sarajevo in Bosnia and Herzegovina. 32 boys and 14 girls born in term, along with 3 preterm kids were analyzed. GSTT1 was detected in 56.25% (18) of males, while GSTM1 was observed in 50% (7) of females. A positive correlation between allergies and GSTT1 was found as strong in boys and medium in girls. GSTM1 and allergies were negatively correlated in males, while the same polymorphism was positively correlated with asthma in females. GSTT1 polymorphism appears to play a role in asthma development in children, regardless of their sex. However, GSTM1 seems to have a protective effect against allergy development in males, while it potentially increases the risk of childhood asthma in females.

Keywords: GSTM1, GSTT1, asthma
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TWO-DECADE'S EXPERIENCE OF GENETIC MONITORING OF BROODSTOCK FROM SEVERAL FISH FARMS IN BOSNIA AND HERZEGOVINA

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Genetic information from individuals, populations, broodstocks, and species has various applications in fisheries and aquaculture. Decades-worth of studies demonstrated that genetically heterogeneous individuals appear to be more resistant to environmental perturbations during development, have better survival rates and higher relative growth rates. Therefore, monitoring aquatic genetic resources, including fish, is necessary to trace alterations in the status and trends in their utilization and conservation. Notwithstanding acknowledgment that this approach can provide significant backing to the development and implementation of management and conservation strategies, the effective integration of genetic and genomic methods into fisheries and aquaculture management is inconsistent and underdeveloped in many countries worldwide. Both the Law on Freshwater Fisheries of the Federation of Bosnia and Herzegovina and the Law on Fisheries of the Republic of Srpska require stocking of wild waters with healthy fish, fingerlings, fry, and fertilized eggs, after health control and determination of the quality of fish. However, neither law specifically prescribes mandatory genetic testing of fish stocks before stocking. The Laboratory for Molecular Genetics of Natural Resources of the Institute for Genetic Engineering and Biotechnology, University of Sarajevo, has been providing the expertise of molecular-genetic characterization of fish broodstocks and fry for the Bosnian-Herzegovinian fisheries and aquaculture sector for more than two decades. Over this timeline, close to 2000 samples, mostly *Salmo trutta*, *S. marmoratus* and *S. obtusirostris*, were analyzed. Results revealed the managers' tendency to continually select broodstock from a small number of closely related individuals, resulting in reduced genetic variability and increased inbreeding. Our studies of various rivers in Bosnia and Herzegovina showed *S. trutta* individuals of allochthonous genetic lineages, indicating the incidences of escapes from the fish farms and/or uncontrolled stocking with such fish. Although genetic control for fisheries and aquaculture is sometimes perceived as costly, the absence of such activities can be even more damaging in financial, socioeconomic, and environmental terms that can arise through unaccounted for illegal and unreported fishing and the uncontrolled release of farmed fish. Consequently, the governments, academia/science sector, and private industry must see the benefits of genetic resource management and commit to a strong partnership.

Keywords: monitoring, fish, fisheries, aquaculture
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THE ROLE OF GENE BANK IN CONSERVATION OF BIODIVERSITY

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Globally, the number of domestic animal breeds has been steadily declining in recent decades, some breeds have become endangered and some have become extinct. Therefore, the importance and need to preserve the genetic resources of domestic animals is of local, regional and global importance. The storage of gametes, embryos and somatic tissues enables the transfer of genetic material between populations and many years after the donor animal is not alive, and thus the genetic diversity of a particular species or breed can be preserved forever. Population growth leads to increased demand and consumption of animal products, which forces livestock producers to increase productivity per animal, and as a consequence we have changes in the genotypes of traditional breeds. As a result, many indigenous breeds are endangered and can be lost in the absence of care for their preservation. Indigenous breeds have unique genetic traits that allow them to adapt to harsh climatic conditions, the ability to exploit poor quality food, and resistance to endemic diseases. National populations of commercial breeds of domestic animals are also endangered due to large imports of genetic material of highly productive individuals, thus suppressing the characteristics of domestic breeding. For now, the only known way to preserve the genetic fund is the preservation of ex situ in-vitro or so-called**.** "Gene banks", whose role is the permanent preservation of genetic material from rare and endangered breeds or individuals of the population "domestic and wild". There is a growing worldwide awareness that an international approach is needed to conserve biodiversity in many of its forms, including animal genetic resources. Establishing systemic storage of samples in the gene bank to preserve as much biodiversity as possible is crucial and should be used whenever possible. The storage of biological material needs to be started before the reduction of biodiversity leads to the extinction of certain breeds.

Keywords: genetic, gene banks, animal
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EXPERIENCE THE FUTURE OF DRUG DISCOVERY AND DISEASE MODELING RESEARCH WITH GENETIC ENGINEERING SOLUTIONS AND ORGANOIDS FROM MERCK

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Merck Life Science | Research Solutions

Recently, the power of genetic engineering allowed creation of a wide range of genetically modified mammalian cell lines that model patient-specific genome alterations. In parallel, the advances in cell culture led to 3D cell culture approaches like stem cell organoids that have been implemented to more closely model in vivo cellular responses. These advanced tools allow scientists the creation of more predictive models that can accurately replicate the complex physiology and genomic features of various tissues or organs in therapeutic research and drug screening. Merck combines the expertise in genome editing technologies using CRIPSRs and ZFNs and the expertise in stem cell biology to provide the researchers worldwide with customized solutions for disease modeling, target identification, compound screening, as well as more predictable toxicity and efficacy testing compared to traditional animal models. In addition, we simplified the access for scientific community to organoids which due to their reproducibility and scalability represent valuable tools for translational studies including the development of personalized medicines. Altogether, these novel translational in vitro cellular research models may allow us to study nearly any human disease in a dish and permit the development of therapeutics tailored to individual patients in the future.

Keywords:
drug discovery, disease modeling, organoids, CRISPR, 3D cell culture, in vitro models
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CORTICOSTEROID-INDUCED EXPRESSION OF MICROBIAL VIRULENCE CAN ENHANCE DEVELOPMENT OF HOST INFECTIOUS DISEASE

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Corticosteroids are non-inflamatory drugs, which are commonly used in treatment of different forms of arthritis, kidney, skin or thyroid disorders, as well as for the treatment and relief of symptoms of allergies and some gastrointestinal disorders. Even the application of corticosteroids is of wide range, however, it is little known about role of corticosteroid-based drug administration on dissemination of infections diseases and development of microbial patogenicity. Our study included the clinicaly isolated strains of *Escherichia coli (E. coli)* as well as referent *E. coli* strain as control. Metabolic activity and levels of virulence of tested *E. coli* strains were investigated *in vitro* conditions under presence of coticosteroid-based drugs at different incubation time points. Administration of glucocorticoid drug dexamethasone in defined concentration, resulted in significant increase in expression of *E. coli* virulent factor enzyme aspartyl proteinase, even for 2.6 fold for some *E. coli* strains. This study indicates posibility of important role of coriticoid drug dexamethasone as trigger molecule for expression of enzyme aspartate proteinase, known as one of the *E.coli* virulent factors.

Keywords: Corticosteroid, *E.coli*, aspartate proteinase, virulence
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CHROMOSOME ABERRATIONS IN MEDICAL PERSONNEL OCCUPATIONALLY EXPOSED TO LOW-DOSE IONISING RADIATION

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With the modernization of protection measures and significant reduction of radiation exposure in medicine, chromosome aberration (CA) analysis has been considered as a gold standard for genetic evaluation recommended only when effective dose is estimated to be 200 mSv or higher. However, because of the scarce data on the genotoxic effects of medical radiation exposure in population of Bosnia and Herzegovina, we have summarized results of conducted cytogenetic analysis in peripheral blood lymphocytes of 66 medical workers from Clinical Centre of University of Sarajevo and 89 non-exposed volunteers. In 6 medical workers (8.6% of all tasted in a group) and in one person from the control group (1.1%), frequencies of structural CAs were above established limits. Dicentric chromosomes and a chromosome rearrangement were found in 3 samples of medical workers. Nevertheless, CA frequencies in a group of occupationally exposed subjects were not significantly increased compared to the control group. Cytogenetic surveillance has a major role in evaluation of accidental unplanned exposures and is still a standard in biological dosimetry as a method for estimation of received radiation. However, all other possible CA inducing factors should be inevitably considered. The normal values from this study contribute to the background data for the general population of Bosnia and Herzegovina for future biomonitoring or comparative studies.

Keywords: occupational exposure biomonitoring, cytogenetic surveillance, peripheral blood lymphocytes
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LABORATORY FOR BIOLOGICAL EXPERTISE AND DNA ANALYSIS OF THE AGENCY FOR FORENSIC AND EXPERT EXAMINATIONS, OVERVIEW OF PREVIOUS ACTIVITIES

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The Agency for Forensic and Expert Examinations is an administrative organization within the Ministry of Security of B&H with operational autonomy established in 2009 on the basis of the Law on the Directorate for Coordination of Police Bodies and Agencies for Support of the Police Structure of Bosnia and Herzegovina. The Laboratory for Biological Expertise and DNA Analysis of the Agency has begun working in July 2016 and since then it has done a series of expertise to the orders of prosecutors and courts at all levels of government in Bosnia and Herzegovina, in both criminal and civil proceedings. We are currently associate members of the European Network of Forensic Science Institutes DNA Working Group, and our staff are also members of the *International Society for Forensic Genetics.* The Laboratory is taking active role in advocating for legislation of a DNA database, and therefore simultaneously conducts activities on accreditation in accordance with the standard BAS EN ISO/IEC 17025:2018.

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THE SIGNIFICANCE OF DETERMININIG THE ABO BLOOD GROUPS FROM DRIED BLOOD TRACES FOUND AT CRIME SCENE

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Every individual has unique blood group. ABO blood typing has several applications in forensic sciences and is also a major part of routine biological investigations. Dried blood samples are more often found at a crime scene than wet form. In our study, ABO blood typing was performed on the dry blood samples obtained from 200 subjects using the modified absorption-inhibition technique. The results were compared with those obtained using whole blood extraction. In conducted study, blood samples were collected from 8 different materials and substrates, in 20 different volumes and after 7 days, 6 months and one year. Different techniques of sampling were used depending on the type of substrate from which traces were taken. Our results indicate the reliability of the use of properly dried blood traces to determine the exact blood group of ABO system regardless volume of blood sample, type of substrate, sampling time and techniques of sample collection. Thus, the results of this study indicate the reliability of the use of ABO blood typing, which is a very important method of excluding suspects in many criminal cases.

Keywords: forensic science, dried blood stain, ABO blood group
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DETERMENING THE FORENSICALLY SIGNIFICANT SNP-s OF THE MITOCHONDRIAL HVIII REGION IN POPULATION OF BOSNIA AND HERZEGOVINA

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Mitochondrial DNA analysis (mtDNA) is a widely used method of detecting genetic material in the field of human identification. Each cell contains many copies of mtDNA molecules exclusively inherited from the mother. The lack of a recombination process and the high rate of mutations were found in the mtDNA control region which ensures a high level of polymorphism mainly found in the hypervariable regions HVI, HVII and HVIII. When the HVI and HVII regions are not informative enough to discriminate forensic samples then the HVIII region is sequenced to find discriminating alleles. This study aimed to find and determine the frequency of the 'hotspots' where SNPs with forensic potential are located thanks to which the HVIII region alleles are discriminated over the alleles presented in the HVI and HVII region. The results obtained by the method of single base extension were compared with the CRS (Cambridge Reference Sequence) of the human genome and based on that we determined forensically significant SNPs, by statistical analysis, the frequency of their occurrence in BIH population was determined. The primary purpose of conducting this study was to improve forensic practice in a way that the obtained results are a reference of which it will be easier to interpret the results in everyday forensic practice. In this study, HVIII region was proven to be an 'axillary' region to analyzing when HVII and HVI regions are insufficiently informative and when there is an insufficient number of SNPs generated in both regions.

Keywords forensic science, mitochondrial DNA, molecular biology, hypervariable regions, third hypervariable region
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STUDENTS ORAL PRESENTATIONS

2nd Congress of Geneticists in BiH with International Participation

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University of Sarajevo

Genetic Association in Bosnia and Herzegovina

GENETIC DIVERSITY AND DIFFERENTIATION OF ALPINE SALAMANDERS FROM THE DINARIDES – AN EVOLUTIONARY PERSPECTIVE WITH INSIGHTS FOR SPECIES CONSERVATION

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The fragmented population of alpine salamanders from the Dinaric Alps belongs to a distinct evolutionary lineage known as *Salamandra atra prenjensis*. However, the phylogenetic relationships within this lineage are unknown since previous studies did not comprehensively include Dinaric population fragments. In this study, we use six microsatellite loci and two mtDNA markers to examine the evolutionary relationships and genetic structure in several isolated fragments of alpine salamanders that are well spread along the Dinarides. We discovered that during Pleistocene glaciations, *S. atra* persisted in at least two Dinaric refugia: an old one in the Prenj mountain, and a more recent one in Gorski Kotar, whereas there is evidence of colonization for the populations of the Čvrsnica and Prokletije mountains. The results revealed that mountain Prenj was probably the main diversification center of alpine salamanders in the Dinarides. In addition, to provide a state-of-the-art review of the evolutionary history of the species, in this study, we also included available sequences of *S. atra* from the entire distribution range; we discuss the results from a conservation perspective.

Keywords: Amphibia, Caudata, Salamandridae, Pleistocene, Balkan Peninsula, dispersal routes, refugia, lineage conservation
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ALLELOPATHIC EFFECT OF CEDARWOOD AND GERANIUM ESSENTIAL OILS ON SEED GERMINATION OF SELECTED PLANT SPECIES

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Herbicides are a class of phytotoxins used for inhibiting the growth of unwanted weeds. Even though they are widely used certain types of herbicides demonstrate harmful effects on soil, underground water and passive consumers. In order to avoid these harmful effects, we must try to find alternatives. In search of new options with herbicidal properties presented study investigated growth suppression of two weed species (*Taraxacum officinale, Chenopodium album*) and a carrot (*Daucus carota*) by cedarwood (*Cedrus atlantica)* and geranium (*Pelargonium graveolens*) essential oils. The seeds were treated with three different concentrations of essential oils (10, 20 and 30 µg/mL), with water used as control. The number of germinated seeds was recorded during the period of 10 days and the seedling length was measured on the 10^{th} day. Both essential oils, cedarwood and geranium, showed significant inhibition of *T. officinale* germination at the 20 µg/mL concentration, with 100% germination inhibition at 30 µg/mL concentration of used oil. Cedarwood oil had, however, no significant effect on the seed germination rate of *D. carota* and *C. album* seeds. Geranium oil partially inhibited the germination of *C. album* at 30 µg/mL, while it had insignificant effect on germination of *D. carota* seeds. Both essential oils had influenced the seedling length of all species tested, with the seedlings length progressively decreasing as the essential oil concentration increased.

Keywords: bioherbicides, essential oil, *Cedrus atlantica, Pelargonium graveolens*, allelopathy
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e-POSTER PRESENTATIONS

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University of Sarajevo

Genetic Association in Bosnia and Herzegovina

ANALYSIS OF THE ASSOCIATION BETWEEN TUMOR NECROSIS FACTOR-A -308 G/A POLYMORPHISM AND THROMBOLYTIC THERAPY SIDE EFFECTS IN ACUTE ISCHEMIC STROKE PATIENTS

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Thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) is the gold standard therapy for the treatment of acute ischemic stroke (AIS). However, due to the potential destructive effect of the rtPA on the extracellular matrix, it may lead to the blood-brain barrier (BBB) breakdown and hemorrhagic complications after this therapy. Tumor necrosis factor-α (TNF-*α*), as one of the inflammatory mediators, is associated with BBB breakdown and increased risk of hemorrhagic complications after AIS treated with rtPA. Polymorphism -308 G/A within the *TNF-α* gene affects *TNF-α* gene expression and consequently AIS patients' recovery and the occurrence of rtPA-induce side effects, especially hemorrhagic. To explore the association between genotypes of the -308 G/A *TNF-α* gene polymorphism (rs1800629) and the occurrence of the rtPA therapy-induced side effects in the AIS patients. From 2016 to 2018, a total of 166 consecutive patients with AIS treated with rtPA at the St. Sava Hospital in Belgrade were enrolled in the study. Patients' outcome was determined using the Modified Rankin Scale (mRS) 3 months after the AIS commencement. At hospital admission, all patients underwent neurological and laboratory assessments. The favorable outcome has been defined with scores 0-1 and unfavorable with scores 2-6. Additionally, rtPA-induced side effects were followed during the hospitalization. Genotypisation was performed using the polymerase chain reaction in real time (Real-Time PCR) method. Statistical analysis was performed by SPSS software version 22.0 (SPSS Inc, Chicago, Illinois, USA). The GG genotype was the most frequent among our patients (76.5%), whereas only one (0.6%) patient had an AA genotype. We have observed no association between any genotypes with thrombolytic therapy outcome and rtPA-induced side effects. However, after grouping genotypes by the recessive model (GG VS. GA+AA), the GG genotype showed borderline evidence of association with occurrence of the hemorrhagic transformation (p=0.054). Polymorphism -308 G/A within *TNF-α* gene might have an influence on hemorrhagic complications after rtPA therapy.

Keywords: acute **i**schemic stroke, thrombolytic therapy, rtPA, hemorrhagic transformation, *TNF-α* -308 G/A polymorphism
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ANALYSIS OF IL10RB GENE HAPLOTYPES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

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Interleukin 10 receptor (IL10R) is a tetrameric receptor composed of two IL10RA subunits and two IL10RB subunits. This receptor is part of the Interleukin 10 (IL10) pathway and might be important for maintaining immune homeostasis. As IL10 exerts its action via IL10 receptors the *IL10RB* gene haplotypes could potentially affect this interaction. We will access the frequency of *IL10RB* haplotypes in patients with Systemic Lupus Erythematosus (SLE) and with Rheumatoid Arthritis (RA). This is the beginning of a larger study in which we want to analyse associations between selected polymorphisms/haplotypes and susceptibility to SLE and RA. Genotipisation for 63 patients with SLE and 48 patients with RA was performed using TaqMan assays, and amplification reaction was performed in ABI7500 RealTime PCR machine (Applied Biosystems, Foster, CA) according to manufacturer's instructions. For this study, we selected three polymorphisms within the *IL10RB* gene (rs999788, rs2834167, and rs1058867). Haplotype analysis was performed using Haploview software. Haplotype block was defined between rs999788 and rs2834167 *IL10RB* gene polymorphisms (r2=0.65, D'=0.96) after use of the Confidence intervals LD method. Patients with Systemic Lupus Erythematosus were harbouring *IL10RB* gene CAA, CAG, TGA, CGA and TGG haplotypes (41.9%; 27.8%; 12.9%; 7.9%; 7.7%, respectively), while patients with Rheumatoid Arthritis had CAA, CAG, TGG, TGA and CGG haplotypes (40.6%; 32.3%; 12.5%; 9.4%; 5.2%, respectively). *There was no statistically significant difference between frequencies of IL10RB haplotypes in SLE and RA patients.* According to our study, *IL10RB* gene haplotype frequencies do not differ between SLE and RA patients. It is necessary to compare these two groups of patients with the healthy control group.

Keywords: IL10RB, systemic lupus erythematosus, rheumatoid arthritis
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PANEL ANALYSIS OF EVIDENCE-BASED INFERTILITY GENES IN SERTOLI CELL-ONLY SYNDROME PATIENTS

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Sertoli cell-only syndrome (SCOS) is the most severe form of azoospermia that is characterized by a complete lack of spermatogenic cells in almost all seminiferous tubules. Apart from well-established genetic determinants that are responsible for infertility such as Klinefelter syndrome, *CFTR* variants and Y-chromosome microdeletions, it is hard to clearly define the causative role of several hundred candidate genes that were reported as a possible cause of infertility. We selected 92 evidence-based genes associated with infertility and examined data derived from whole-exome sequencing in 6 SCOS individuals. Likely causative variants that passed our filtering criteria for functional impact were detected in 3 patients. Two patients had likely causative variant in the *PKD1* gene that were only 37 bp apart. Apart from the *PKD1* variants, causative variants were detected in *CHD7* and *SCYP3* genes. All those genes have an autosomal dominant effect on male infertility. However, only *CHD7* and *SCYP3* have likely causative role in isolated forms of male infertility. Our findings suggest that panel testing of infertile men could add to the diagnostic yield, however, the construction of a panel consisting of only real causative genes is crucial.

Keywords: male infertility, SCOS, genetics, NGS panel, gene disease association
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GENETIC DIVERSITY OF THE MALE POPULATION OF THE BIJELO POLJE MUNICIPALITY THROUGH THE PRISM OF THE Y CHROMOSOME

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Genetic genealogy becomes more popular in recent years. Several types of commercial tests are available for testing certain genetic markers which could use in genealogy researches. For genealogists it is especially interesting Y chromosome test, which test the diversity of selected molecular genetic markers located on the Y chromosome, which is only exists in males. The aim of this paper is to collect, summarize and analyze data on the genetic diversity of the male population of the four largest national confessions, classified in two groups based on religion, of Bijelo Polje municipality, Montenegro. The paper presents the results of data were obtained by testing 83 samples of DNA materials taken from the male inhabitants of Bijelo Polje (53 Bosniak and Muslim confessions - group 1 and 30 Montenegrin and Serbian confessions - group 2). The results of the research point out that the two national confessions of Bijelo Polje municipality can be found within seven haplogroups: I2, I1, J2, E2, R1b, R1a and G2, while T1 haplogroup is found only in one sample of group 1. The most common haplogroup is I2 identified in 31.33% of samples. At the second place is I1 identified in 18.28%, while in the third place is haplogroup E2 identified in 13.25% of samples. J2 and R1b are in the four and fifth place, while the other three haplogroups are present in a lower percent. According to the national confession, the most common haplogroup for group 1 is I2 identified in 35.85%, while I1, J2 and E2 identified per 13.21% in each. R1a is identified in 11.32% while the other three haplogroups are present in a lower percent. For the group 2, on the first place is I1 identified in 30%, while on the second place is I2 identified in 23.33%. R1b and E2 are in therd and fourt place with 13.33% while other three haplogroups are present in a lower percent. T1 haplogroup is not identified in this population. These results will contribute to popularization of the genetic genealogy as a new tool in genealogical research.

Keywords: genetic genealogy, haplogroup, Bijelo Polje, Y chromosome
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KAUFMAN OCULOCEREBROFACIAL SYNDROME: PHENOTYPIC LANDSCAPE AND LITERATURE REVIEW

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Kaufman oculocerebrofacial syndrome is a distinct condition characterized by intellectual disability, distinctive pattern of craniofacial features and eye abnormalities. It is inherited in an autosomal recessive manner and caused by biallelic mutations in the *UBE3B* gene. Kaufman oculocerebrofacial syndrome is a rare disorder, and the knowledge on its phenotypic and molecular characteristics is still expanding. We performed an extensive literature review in order to pinpoint all of the phenotype/genotype patterns, evaluate relevant functional studies and expand on the existing phenotype of this condition.

Keywords: Kaufman oculocerebrofacial syndrome, *UBE3B*
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MOLECULAR-GENETIC CHARACTERIZATION OF *ApoE* GENE POLYMORPHISM AND VNTR POLYMORPHISM OF *eNOS* GENE IN HUMAN POPULATION OF TUZLA CANTON

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Multifactorial diseases such as cardiovascular diseases, caused by genetic and external factors, represents a source of research at the molecular level. In multifactorial diseases, genetic polymorphisms represent genetic markers that can potentially have predictive significance for modulating the effect of genetic predisposition. The aim of the study was to determine the allelic and genotypic frequencies of *ApoE* and *eNOS* genes and to accurately assess the association of polymorphic alleles of *ApoE* and *eNOS* genes with cardiovascular diseases in the human population of Tuzla Canton. Based on the distribution analysis of *ApoE* gene genotypes in the group of subjects with cardiovascular diseases, the highest frequency of 32.0% was determined for the genotype E3/E4. In the control subjects group, the genotype E3/E3 had the highest frequency, which was determined in 34.5% of subjects. By analyzing the distribution of eNOS gene genotypes in the group of subjects with cardiovascular diseases, the highest frequency was recorded for genotype bb and amounted to 30.5%, and for the same genotype in the control group, a frequency of 36.5% was determined. Based on the correlation between the observed polymorphisms of the *ApoE* gene and the *eNOS* gene and cardiovascular diseases, the association of the combined genotypes of the *ApoE* and *eNOS* genes with cardiovascular diseases has also been analyzed. In the group of subjects with cardiovascular diseases, the highest frequency of 37.0% was recorded for the combined genotype E3/E4 of *ApoE* and genotype bb of *eNOS* gene. The research indicated the association of *ApoE* gene genotypes and cardiovascular diseases, as well as the association of combined *ApoE* and *eNOS* gene genotypes with cardiovascular diseases. *eNOS* gene polymorphisms can certainly be observed in correlation with other risk factors for development of cardiovascular diseases.

Keywords: polymorphism, *ApoE*, *eNOS*, cardiovascular diseases, Tuzla Canton
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THYMUS SERPYLLUM AND *MENTHA PIPERITA* ESSENTIAL OILS AFFECT SHH AND NOTCH SIGNALING PATHWAYS IN BASAL CELL CARCINOMA *IN VITRO*

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Basal cell carcinoma (BCC) is the most common skin cancer and the most frequently occurring form of all cancers. Although it rarely metastizes, BCC may be locally aggressive and recurrent. Among other treatment modalities, phytotherapy has also been considered in the management of BCC. *Thymus serpyllum* and *Mentha piperita* have shown significant antitumor effects on several different types of cancers. Hence, the aim of the present study was to examine whether essential oils of these two plants affect Sonic Hedgehog and Notch signaling pathways, known to play key roles in BCC pathogenesis. Primary cultures were generated from five BCC tumor tissues and their distant resection margins (>5 mm) which served as controls. The cells were cultivated in humidified atmosphere under standard conditions until achieving 80% of confluence, when passaging was done. After reaching the 5th passage tumor cells were treated with 262 μg/ml essential oil of *Thymus serpyllum* and 556 μg/ml essential oil of *Mentha piperita*. RNA isolation was performed from treated and un-treated tumor cells and from healthy control cells by standard procedure followed by cDNA synthesis with reverse transcriptase. The relative expression of Sonic Hedgehog signaling cascade (*SHH, PTCH1, SMO and* GLI1) and Notch signaling pathway (Notch 1 and Jagged 1) molecules were determined by real-time PCR. After the treatment with *Thymus serpyllum* there was a significant decrease of SMO and GLI1 expression, and a slight decrease of SHH gene expression in treated compared to un-treated cells. After exposure to *Mentha piperita* essential oil there was a significant increase of PTCH1, Notch 1 and Jagged 1 expression. This study showed that *Thymus serpyllum* acts through downstream cascade of Sonic Hedgehog signal pathway while *Mentha piperita* acts through upstream parts of Sonic Hedgehog pathway and Notch signaling which can be useful in the therapy of basal cell carcinoma.

Keywords: basal cell carcinoma, Sonic Hedgehog signaling pathway, Notch signaling pathway
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CLINICAL MANIFESTATIONS OF A PATIENT WITH MICRODUPLICATION 12q23 AND DELETION Xp22

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Microdeletion and microduplication syndromes are disorders caused by submicroscopic deletions or duplications of contiguous genes on particular parts of chromosomes. The aim was to present the clinical manifestations of microduplication 12q23 and deletion Xp22 present in one patient, which have not been described together so far. A 10-month-old girl, born from the first pregnancy, of healthy non-consanguine parents. There were no perinatal risk factors but several verified developmental defects were present - microcornea of the left eye with bilateral corneal dystrophy and cataracts, cardiomyopathy, congenital deafness, and germinolysis of the brain. Elements of phenotypic dysmorphia: soft formation on the left parietal-occipital part, gothic palate, microretrognathia, cutaneous chin hemangioma, rectal diastase 1 cm, minor umbilical hernia, furrows four fingers of both palms. Neurological status: hypertonic, hyperreflexia, enlarged reflexogenic zone, feeding difficulties with poor weight progression. Ultrasound of the brain described asymmetry of the corpus callosum and possible dysgerminolysis. An ultrasound of the heart shows a thickened septum of the left ventricle, a trabeculated posterior wall with numerous recessions indicating a “spongy” myocardium. Magnetic resonance imaging of the brain shows hypoplasia of the corpus calosum, and a suspected extradural venous anomaly corresponding to the atretic parietal meningocele. Excluding TORCH infection and mitochondrial disorder, Chromosomal Microarray Analysis was performed and revealed microduplication 12q23 and deletion Xp22. A regular diet of B1 milk formula and antirachitic therapy is carried out. This presentation expanded the range of clinical manifestations and phenotypic features for microduplication 12q23 and deletion Xp22. In the literature, both mutations have been described separately whose clinical picture differs from the manifestations present when both mutations coexist in a single patient.

Key words: microduplication 12q23, deletion Xp22, chromosomal microarray analysis
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PRESENCE OF HLA-Cw*06 ALLELE IN PATIENTS WITH PSORIASIS VULGARIS - THE FIRST REPORT FROM OUR CENTER

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Psoriasis is a chronic, recurrent, inflammatory, systemic skin disease associated with a number of comorbidities. Psoriasis occurs worldwide with prevalence ranging between 0.09% and 11.4%. Estimated prevalence of about 2% of the Serbian population means that up to 140.000 individuals are affected. Both environmental and genetic factors contribute to its pathogenesis, its appearance and severity. Candidate genes have been identified in the PSORS1 region of chromosome 6, most significantly the HLA-C gene in the region of the major histocompatibility complex. The HLA-C allele HLA-Cw*06 is strongly associated with the development of psoriasis in world’s population and with a more severe form presenting at a younger age. HLA-Cw*06 has also been shown to influence the response of psoriasis patients to biological treatments. To ascertain the association between the presence of HLA-Cw*06 allele in psoriasis vulgaris patients and the occurrence of the disease. Genomic DNA was isolated from peripheral blood cells of 100 participants (33 patients and 67 controls, age and gender matched) and genotyping for HLA-Cw*06 allele was performed using both PCR-SSP and PCR-RFLP methods: PCR-SSP was used to specifically match HLA-Cw*06, and PCR-RFLP was used to distinguish between homozygotes and heterozygotes. We found 9 heterozygotes and 3 homozygotes in cases (12 affected, or 36%) and 8 hetero- and 2 homozygotes in control group (10 affected or 14.92%). Albeit no significant difference between groups was found, association between allele and disease approached significance (P=0.06). Also, our analysis showed a nonsignificant 2.4-fold increase in risk for development of psoriasis in HLA-Cw*06 allele carriers (OR=2.4, 95%CI=0.95-6.22). Although the association between risk allele and disease is considered to be not quite statistically significant, our results showed that there is a risk for psoriasis development in HLA-Cw*06 carriers and that larger study group is needed to establish the true relationship between this allele and the disease.

Keywords: psoriasis vulgaris, HLA-Cw*06, genetic analysis, Serbian population

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ASSOCIATION OF SELECTED THROMBOPHILIA GENETIC MARKERS WITH RECURRENT PREGNANCY LOSS IN BOSNIA AND HERZEGOVINA

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This study aimed to analyze the proportion of selected gene polymorphisms in the risk assessment for thrombophilia in women with RPL compared to the control group of women with other complications in pregnancy, since hereditary thrombophilia is a risk factor for reproductive disorders, including infertility, recurrent pregnancy loss, and labor complications. The study included 97 women tested in the Laboratory of Human Genetics of the Institute for Genetic Engineering and Biotechnology, University of Sarajevo, in the period from 2012 to 2020. Materials used were 3 ml peripheral blood collected with EDTA coated tubes. All subjects were classified into two groups: women with RPL (N=45), and a group of women with other clinically significant complications in pregnancy (N=52). This study was approved by the Ethics Committee of the Institute for Genetic Engineering and Biotechnology, number 579/20. Genotyping of prothrombin (FII) G20210A, factor V Leiden (FVL, FV), MTHFR (C677T), PAI-1 (4G/5G), and factor XIII (FXIII) Val34Leu polymorphisms was performed by ASA-PCR reaction. ACE I/D polymorphism was genotyped using a single end-point PCR reaction. The results of the Fisher test and the Chi-square test (for allelic and genotypic associataions) were obtained using software MedCalc Statistical Software, and statistical significances were set at a p-value of $p < 0.05$. A statistically significant association (allele and genotype) between two examined groups was found at two gene loci, loci FII and FXIII. The p-value of the allele association for FII G20210A polymorphism is 0.019, while the p-value of the genotype association is 0.009. Notably, the p-value of allele association for FXIII Val34Leu polymorphism is 0.025, and genotype is 0.015. We did not find an association between FVL, MTHFR C677T, PAI-1 4G/5G, and ACE I/D polymorphisms with RPLs in the Bosnian population. The presence of thrombophilia polymorphisms may predispose women to recurrent pregnancy loss. This finding is significant but should be validated in larger dataset before translation into medical practice.

Keywords: thrombophilia, gene polymorphism, miscarriage, complications in pregnancy, risk factors

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GENETIC STRUCTURE AND DIVERSITY OF INTERNATIONAL CULTIVARS OF ALMONDS (*PRUNUS AMYGDALUS* L.) IN HERZEGOVINA

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The research of genetic characterization of international almond varieties in the territory of Herzegovina includes seven international almond cultivars. The international group of almonds consisted of selections from Italy (Tuono, Genco, Supernova), two selections from France (Fereagnes and Ferraduel) and two originating from the USA (Texas and Nonpareil). Genetic characterization was performed using 10 microsatellite markers, of which 9 microsatellite markers were derived from *Prunus persicae* and 1 from *Prunus armeniaca*. Microsatellite primers, used in the preparation of this paper, showed high polymorphism in previous studies by a group of authors who analyzed almond populations. The results of genetic characterization show that the total number of alleles detected by 10 pairs of primers in seven international almond cultivars was 5.4 alleles per locus. The average number of effective alleles for the ten SSR loci of international cultivars was 3.924. The Shannon Information Index averaged 1.413. The observed heterozygosity (Ho) averaged 0.529 and the expected heterozygosity (He) was 0.686. The results of these studies indicate that in the territory of Herzegovina there are international cultivars of almonds that can be used in breeding programs to improve the represented genotypes of the free population of almonds in the territory of Herzegovina.

Keywords: almond, international group, cultivars, microsatellites, genetic characterization
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IS DNA BARCODING A FEASIBLE TOOL FOR BIODIVERSITY ASSESSMENT IN THE PROTECTED AREAS OF CANTON SARAJEVO?

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There are five protected areas in Canton Sarajevo (Bentbaša, Bijambare, Skakavac, The Spring of Bosna River and Trebević), and each includes a form of a freshwater body. Therefore, special attention should be paid to the preservation of freshwater species. The traditional approach in species identification of benthic macroinvertebrates can be challenging for many reasons and requires efforts of several narrowly specialized taxonomists. Consequently, DNA barcoding has been widely applied as an auxiliary method in assessing and biomonitoring plankton, benthos and nekton. With protected areas of Canton Sarajevo as a model, the goal of our study was to test the applicability of DNA barcoding for species determination using available infrastructure and biodiversity data. The total macrozoobenthic community was sampled at the Natural monument "Skakavac" at four sub-localities. Caddisflies, the most researched group of benthic macroinvertebrates in Bosnia and Herzegovina, were sampled at all five protected areas. Sampling was done according to the AQEM methodology. Genomic DNA from specimens was isolated by a modified salting-out protocol. Both Folmer and JJ-primers were used to amplify the standardized DNA barcoding region of cytochrome c oxidase subunit I (*COI*). A subset of representative specimens was DNA barcoded and determined to the lowest taxonomic level possible. Results were as follows: Ephemeroptera-100% to genus level; Plecoptera-66.66% to species level, 33.33% to genus level, Diptera-83.33% to genus level, 16.66% to family level; Annelida-100% to genus level; Trichoptera- 54.05% to species level, 45.95% to genus level. At the current state of research and present records of species of Bosnia and Herzegovina in the BOLD database, DNA barcoding cannot be used as a sole tool in biomonitoring of protected areas in Canton Sarajevo. Further research in these areas, coupled with morphological species identification in collaboration with specialized taxonomists and genetic characterization, is what academic institutions and non-governmental organizations should strive for.

Keywords: Macrozoobenthos, cytochrome c oxidase subunit I, freshwater bioassessment
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IDENTIFICATION OF ACC DEAMINASE PRODUCING METAL TOLERANT BACTERIA INHABITING *Medicago lupulina* L. RHIZOSPHERE

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In natural environments plants are continuously exposed to different biotic and abiotic stressors which affect their growth. When confronted by various kinds of stressors, plants respond by readjusting levels of ethylene which reduces shoot and root growth, thus re-establishing homeostasis. The ACC (1-aminocyclopropane-1-carboxylic acid) is key intermediate in ethylene synthesis. Some plant-growth-promoting rhizobacteria produce ACC deaminase which cleaves ACC and interrupt ethylene synthesis. Serpentine soils, derived from ultramafic rocks, with its unique physical and chemical properties are inhospitable environment for plant growth. As these soils are expected to be the source of metal tolerant bacteria we collected samples of rhizosphere associated with *Medicago lupulina* L. from five sites in Krivaja – Konjuh ophiolite complex (Bosnia and Herzegovina). Heavy metals and nutrients concentrations were determined by flame atomic absorption spectroscopy. Heavy metal tolerance of isolated rhizobacteria was tested using tryptone yeast agar supplemented with Cu, Ni and Co. Metal-tolerant isolates were screened for ACC deaminase activity by inoculation of bacterial suspensions on DF (Dworkin & Foster) salts solid medium supplemented with 1 mM ACC and medium without ACC (negative control). Identification of metal tolerant isolates with ACC deaminase activity was performed using DNA sequencing of the 16S rRNA gene. Total of 124 tested bacterial isolates showed strong resistance to Cu and Ni and weaker resistance to Co. Isolates that showed multiple heavy metal resistance were screened for ACC deaminase activity. Out of 35 tested metal tolerant bacterial isolates, 24 showed intense growth on DF media supplemented with ACC. This indicates using ACC as a sole nitrogen source and implies ACC deaminase activity. Identification showed that most isolates belong to Phyla Proteobacteria with *Pseudomonas* as most abundant genus. Some representatives of Firmicutes were also identified. ACC utilizing bacterial strains which have the potential to curb stress induced ethylene production in plants will be further screened for other PGP traits and used in plant inoculation experiments in order to test their capacity to mitigate stress in plants.

Keywords: rhizobacteria, ethylene, ACC deaminase, serpentine soil, heavy metals
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GLU288 AND LYS137 OF SARS-CoV-2 MAIN PROTEASE AS TARGETS FOR INHIBITION BY SELECTED THYME PHYTOCONSTITUENTS

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Since the outbreak of the Coronavirus Disease 19 (COVID-19) pandemic, researchers have been trying to investigate various active compounds found in plants that could have potential to inhibit the proliferation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Thymus vulgaris* L., member of Lamiacae family, is an aromatic and medicinal plant that has been used in traditional medicine. It has showed antihelminthic, expectorant, antiseptic, antispasmodic, anti-microbial, antifungal, antioxidative, antivirotic, carminative, sedative, and diaphoretic effects due to the possession of a wide range of secondary metabolites. Therefore, the aim of this study was to evaluate bioactive compounds found in *Thymus vulgaris* L. as potential antiviral components and inhibitors of the SARS-CoV-2 main protease (Mpro), using a molecular docking approach. The main protease (3-chymotrypsin-like protease) plays a key role in viral gene expression and replication and serves as an important target in drug designing against COVID-19. Using *in silico* method we analyzed the binding affinity of thymol, carvacrol, rutin and thymoquinone, as phytoconstituents of Thyme, to SARS-CoV-2 main protease Mpro (PDB ID: 6Y84), using acetoside as a positive control of binding affinity. Obtained results by molecular docking showed highest affinities (rmsd l.b. 0.000; rmsd u.b. 0.000) for rutin (-10.3 kcal/mol), thymol (-6.1 kcal/mol), thymoquinone (-6.1 kcal/mol), carvacrol (-5.9 kcal/mol), and for positive control acetoside binding affinity was -10.0 kcal/mol. Visualization of interaction between SARS-CoV-2 main protease and ligands showed that carvacrol, rutin and thymol had interaction close to positions Glu288. Also, carvacrol and thymol showed similarity for binding modes to main protease close to position of Lys137 just as positive control acetoside. After analyzing the docking modes and docking scores we have found that phytoconstituents of Thyme have potential to be inhibitors of SARS-CoV-2 Mpro and also to be used as adjuvant in treatment of COVID-19, but greater studies and clinical researches are needed to confirm that and to elucidate mechanism of action.

Keywords: Thyme, molecular docking study, SARS-CoV-2, main protease
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IMPORTANCY OF MACHINE LEARNING IN BIOLOGICAL STUDIES

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In the fields of genetics and genomics, machine learning approaches have been used in a variety of applications. Algorithms of this technique use specific data in order to detect certain patterns, build certain models, as well as make predictions based on the best possible model. This method has been used to annotate a wide range of genomic sequence elements and is perhaps most useful for the interpretation of large genomic data sets. Also, this approach is used to predict variants of various biological data, in particular genetic, whether in terms of DNA sequence, the protein structure or other markers. Machine learning systems that can annotate genes can be built using models that each recognize a specific type of genomic element, as well as learnt logic about their relative positions. To better understand the mechanics underlying gene expression, a range of machine learning algorithms have been created. Some methods seek to predict a gene's expression only based on its DNA sequence, while more advanced methods aim to model the expression of all of the genes in a cell by developing a network model. However, in recent years, artificial neural networks have become increasingly popular in the processing and prediction of biological data. These networks, which resemble the central nervous system, are made up of interconnected neural computing elements that can respond to input stimuli and adaptation processes. By using algorithms that imitate real processes in neurons, the network itself can learn to solve a particular problem. The learning process includes recognition and training patterns. Recognition patterns are data classification processes that aim to identify potential correlations between variables. Training patterns aid neural networks in the adaptation process by training the network to recognize input patterns and generate output data linked with the output pattern. The expanding usage of artificial neural networks in biology is clear, with the core purpose being the same: to generate a prediction based on existing data. Because neural networks cannot resolve all problems, a combination of classical and modern bioinformatics analysis, with the addition of an artificial neural network, would be the optimum strategy.

Keywords: machine learning, neural network, prediction, simulation
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INVESTIGATION OF THE ANTIOXIDANT ACTIVITY OF *PHYSALIS ALKEKENGI* L. VARIOUS EXTRACTS

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Physalis alkekengi L. belongs to the genus *Physalis* of the family Solanaceae. Studies have shown that it has good anti-inflammatory, antioxidant, antimicrobial, diuretic, immunomodulatory activities and that some compounds are responsible for inhibiting the proliferation of tumor cells. *P. alkekengi* contains active metabolites such as flavonoids, alkaloids, phenylpropanoids, and physalins, which are responsible for many of the effects of this plant. *P. alkekengi* fruits were dried in an airy room. Dried fruits were used to prepare methanolic extract using the maceration method and ethanolic extract using the Soxhlet method. The antioxidant activity of methanolic and ethanolic extracts of *P. alkekengi* was measured by the DPPH method at the IC_{50}. The obtained IC_{50} values for ethanolic and methanolic extracts are 1.97 ± 0.35 and 3.34 ± 0.26 mg/mL. These values were compared with the obtained IC_{50} values for ascorbic acid, which is used as a commercially available antioxidant and ranges from 0.13 ± 0.03 mg/mL. The concentrations of ethanolic and methanolic extract required to neutralize 50% of DPPH radicals are significantly higher than the required concentration of ascorbic acid. The results indicated that the dry fruits of *P. alkekengi* provide good antioxidants.

Keywords: *Physalis alkekengi* L., ethanol extract, methanol extract, antioxidant activity, DPPH
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SELECTIVE GENOTOXICITY OF HALOGENATED BOROXINE, $K_2(B_3O_3F_4OH)$ IN UT-7 LEUKEMIA CELL LINE AND NORMAL PERIPHERAL BLOOD MONONUCLEAR CELLS

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Halogenated boroxine (HB), K2(B3O3F4OH), is a synthetic derivative of boronic acid with previously proven bioactive potential in different in vitro and in vivo model-systems. Its effects in hematological malignancies have not been tested yet. In this work, we aimed to evaluate the genotoxic potential of HB using alkaline comet assay in human acute myeloid leukemia cell line UT-7 (ACC 137) and normal peripheral blood mononuclear cells (PBMCs), respectively. UT-7 leukemia cells and normal PBMCs isolated from healthy donor were incubated at 37° C for the cultivation period of 72 h. Cultures were treated with HB in concentrations of 0.1, 0.2 and 0.4 mg/mL. Negative and positive controls were set up as well. Cell damage was analyzed using Comet Assay IV software (Instem, UK) and measuring tail intensity (%). Obtained results showed significant increase in tail intensity ($p<0.001$) in all HB treatments of UT-7 cells when compared to negative control. The frequency of hedgehog cells was registered in HB treatments of UT-7 cells for 0.2 mg/mL (49.5%) and 0.4 mg/mL (83.3%), indicating high dose-dependent genotoxic damage. In PBMCs, tail intensity was significantly increased ($p<0.001$) only in the highest tested concentration of HB in comparison to negative control. Hedgehog cells were not found in HB treated PBMCs. These results revealed selective genotoxicity of HB in tumor compared to normal cells *in vitro*.

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Keywords: comet assay, genotoxic damage, UT-7 cell line, PBMCs

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EVALUATION OF DNA DAMAGE IN ORAL LYMPHOCYTES USING COMET ASSAY METHOD IN THE GROUP OF HEALTHY INDIVIDUALS FROM SARAJEVO

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Human biomonitoring studies are used in assessing exposure to environmental genotoxins. Comet assay, used for measuring the level of DNA damage in single cells, has applications in human biomonitoring and genetic ecotoxicology. The assay enables analysis of DNA strand breaks in different types of cells including oral leukocytes which are suitable samples as their collection is non-invasive and convenient, especially for vulnerable populations. The main objective of this study was to initiate comet assay based biomonitoring of the background level of DNA damage in healthy individuals from Sarajevo, analyse inter-individual variation and correlate it with the lifestyle habits and environmental pollution. Oral leukocytes were collected and isolated from 33 healthy individuals during the summer and winter of 2019/2020. The comets were analysed by Comet assay IV (Instem, UK) software and statistical analysis performed over log-transformed values of tail intensity. Overall results revealed no significant differences between two sessions. Comparative analysis of smokers and non-smokers revealed significantly higher damage in smokers compared to non-smokers in the summer period. For the winter period no significant differences were found but the damage was higher in smokers. Independent t-test revealed lower DNA damage in females compared to males. In addition, significant increase in DNA damage was found for the individuals above 49 years of age compared to younger participants that is most likely related to the reduced capacities of the DNA repair system. With the trends of continuous and increasing air pollution, continuous biomonitoring studies are a necessity.

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Keywords: DNA damage, smokers, non-smokers, lifestyle, airpollution
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PATERNITY TESTING WITH TWO AUTOSOMAL STR MISMATCHES: CASES REPORT

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DNA profiling including short tandem repeat (STR) markers is a conventional procedure used in forensic genetics and all kinds of kinship testing, including paternity testing. Paternity testing is based on the comparison of STR profiles of standard trio including child, mother and the alleged father or duo, where mother is not included. General rule is to exclude paternity when three or more mismatches have been observed. With two mismatches detected by autosomal STR profiling, paternity cannot be excluded but has to be confirmed by an additional analysis. Therefore, we report two cases of paternity testing where were detected two autosomal STR mismatches so it required an additional analysis of X-linked STRs to achieve conclusive results. Buccal swab samples were collected from the female child, mother and the alleged father. Genomic DNA was extracted using QiagenDneasy™ Tissue Kit and amplified using PowerPlex® Fusion System and Investigator® Argus X-12. Amplified products were analysed by capillary electrophoresis carried out in ABI PRISM® 310 Genetic Analyzer according to manufacturer's recommendations. STR data were collected using 310 Data Collection Software and analysed using GeneMapper™ v.3.2 software. Comparing the autosomal genetic profiles, in the first case were detected two mismatches among the 22 analysed STR loci, at D16S539 and D18S51 loci. Likewise, in the second case were detected two mismatches, at D8S1179 and FGA loci. In both cases, detected mutations were from paternal source and probabilities of paternity including mutations into account were over 99,999999%. Analysis of 12 X-linked STR loci yielded one mismatch between child's and father's profile at DXS10135 locus in the first case, while in the second case was found a complete match. Single-step mutations observed at autosomal and X-linked STR loci in the first case can be atributted to the age of father at the child's birth. Results of an additional analysis contributed to paternity confirmation in both cases, where the probability of paternity was higher in the case with complete match between child's and father's profile. This case report pointed up the importance of including the analysis of X-chromosome STR markers in cases unsolvable by analysis of autosomal STR markers.

Keywords: kinship testing, STR markers, X-chromosome, mutations
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STUDENTS SESSION – e-POSTER PRESENTATION

2nd Congress of Geneticists in BiH with International Participation

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ANALYSIS OF THE ASSOCIATION BETWEEN I/D POLYMORPHISM WITHIN *ACE* GENE AND COVID-19 OUTCOME IN SERBIAN POPULATION

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Coronavirus disease (COVID-19) is an infectious disease caused by highly infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The clinical picture of the COVID-19 disease varies from asymptomatic to severe one. It was noticed that the patients with a severe form of the COVID-19 are more likely to have a history of hypertension, diabetes, and/or cardiovascular disease and receive Renin-Angiotensin-System (RAS) inhibitor treatment. Hence, it is possible that RAS and Angiotensin-converting enzyme (ACE) has an important role in the pathogenesis of COVID-19. Insertion/deletion (I/D) polymorphism (rs4646994) within the ACE gene is one of the candidates with the potential to affect infection symptoms and mortality. To analyze whether genotypes of the I/D *ACE* gene polymorphism may influence outcome after COVID-19**.** The study included 129 patients with COVID-19 disease treated from May to October 2020 at the Department of Infectious Diseases, Faculty of Medical Sciences, University of Kragujevac, Serbia. Each patient had confirmed PCR SARS-CoV-2 findings. At hospital admission, patients' demographical and clinical data were obtained. To all patients' chest X-rays, and, if required thoracic computed tomography (CT), as well as all significant laboratory analysis, were performed. Molecular-genetic analyses were performed at the Institute of Human Genetics, Faculty of Medicine, University of Belgrade. Genotypisation of the ACE I/D polymorphism was performed by polymerase chain reaction (PCR). Statistical analysis was performed by SPSS software 22.0 (SPSS Inc, Chicago, Illinois, USA). Frequencies of DD, ID, II genotypes were 35.7%, 45.0%, and 19.4%, respectively. We have observed no association between analyzed ACE ID genotypes and the outcome after COVID-19 infection (p=0.662). However, after grouping genotypes (DD VS. II+ID), the patients with at least one I allele had statistically lower thrombocyte levels in serum than patients who were homozygotes for D allele (p=0.006). Additionally, patients with same genotypes (ID+II) had lower leucocyte levels compared to patients with DD genotype, but without reaching significance (p=0.069). I/D

polymorphism within *ACE* gene might have an influence on outcome laboratory parameters after COVID-19 infection.

Keywords: SARS-CoV-2, angiotensin-converting enzyme (ACE), Insertion/deletion (I/D) polymorphism
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STUDY OF *IL1B* AND *iNOS* GENES POLYMORPHISMS AS A RISK FACTOR FOR THE DEVELOPMENT OF CEREBRAL PALSY

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The most common cause of motor disabilities in childhood is cerebral palsy (CP). CP is a group of permanent neurological disorders that affect movement and muscle tone or posture. Hypoxic-ischemic Encephalopathy (HIE) is the most important cause of CP. The immune system and inflammation mediators are activated due to perinatal brain damage. Interleukin 1 beta (IL1B) and *iNOS* are involved in immune reponse and might be involved in neurodegeneration. Polymorphisms in these genes may affect its expression and cause further damage. Aim of this study was to examine possible association between *IL1B* and *iNOS* polymorphisms and CP onset in children with perinatal asphyxia. Study included 152 patients aged 1 to 16 years with anamnesis of perinatal asphyxia. Detailed neurological evaluation and neuroimaging (ultrasound, computed tomography, magnetic resonance imaging) were performed for all subjects. *IL1B* and *iNOS* polymorphisms were genotyped using rs2297518 and rs16944 TaqMan assays. There was no statistical significant difference in genotype distribution of *IL1B* polymorphism between patients who developed CP and patients who did not develop CP. Frequencies of *iNOS* polymorphisms in patients who developed CP were: 64.9% GG, 24.3% AG and 10.8% AA, while in the group of those who did not develop CP were 59.1% GG, 39.4% AG, and 1.5% AA and there is statisticly significant difference between those groups (p=0.025). AA genotype was significantly more frequent in patients with CP (p=0.036, OR=0.1269, 95%CI 0.0154-1.0437). *iNOS* gene polymorphism could be a risk factor for CP in patients with perinatal asphyxia.

Keywords: cerebral palsy, *IL1b* gene, *iNOS* gene, perinatal asphyxia, SNP polymorphisms
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FUNCTIONAL DNMT3B PROMOTER POLYMORPHISM (rs1569686) AND RISK FOR CONGENITAL HEART DEFECTS IN DOWN SYNDROME

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The DNMT3B gene codes for DNA methyltransferase 3b (DNMT3b), a protein required for genome-wide *de novo* methylation during the early stage of embryonic development. Evidence suggested that impaired DNA methylation could be a risk factor for congenital heart defects (CHD). Down syndrome (DS) is one of the most common chromosomal abnormalities associated with congenital heart defects (CHD), with approximately 40 – 60% of cases showing cardiac defects. The most common CHD phenotypes in DS are congenital malformations of cardiac septa, such as atrioventricular septal defects (AVSDs), ventricular septal defects (VSDs), atrial septal defects (ASDs), and tetralogy of Fallot (TOF). The purpose of this study was to analyze the frequency of the phenotype of CHD as well as allele and genotypes association of functional DNMT3B promoter polymorphism −579 G > T (rs1569686) on the development of CHD in DS. The case-control study included 102 CHD+ Down syndrome cases and 88 non-syndromic CHD cases. The median of the ages for CHD+DS cases was 2 years [0 - 27] and for the control group was 7 years [0 - 32]. Demographic data and phenotype of CHD were collected from medical records of participants after parents and guardians gave their written consent. Genomic DNA was isolated from different types of tissue using a commercial kit and quantified by spectrophotometry. Genotype analysis was performed by PCR-RFLP method. Statistically higher frequencies of AVSD and VSD were detected in a group of CHD+DS than controls (P=0.0044; P=0.0225). Study results showed a statistically lower frequency of the DNMT3B rs 1569686 TT genotype (OR = 0.40; 95% CI: 0.18 – 0.86; P=0.020) in CHD+DS cases compared with controls. AVSD and VSD were the most common CHD phenotypes in DS, which were consistent with the literature. Lower frequencies of DNMT3B (rs1569686) TT genotype in-group CHD+DS suggested that those genotype could decreased risk of CHD in DS individuals. Further ongoing studies will better explain those results.

Keywords: Down syndrome, Dnmt3b, congenital heart defects
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CO-INHERITANCE OF GENETIC VARIANTS LINKED TO THE RISK OF PREGNANCY LOSS IN BOSNIAN WOMEN-OWN STUDIES REVIEW

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One of the issues in perinatal medicine is pregnancy loss, which occurs in up to 25% of women with clinically diagnosed pregnancy. The aetiology of pregnancy loss is an extremely complex interplay of: uterine, autoimmune, endocrine, metabolic, abnormal karyotype, antiphospholipid syndrome, thrombophilia, genetic and idiopathic factors. Genetic risk factors in etiopathogenesis of pregnancy loss still remain unclear, and about half of pregnancies loss remains unknown. However, current knowledge indicates that a co-inheritance of risk factors, including multiple thrombophilic variants linked to secondary hypercoagulable states, have an association with adverse pregnancy outcome. For this purpose we determined, whether the co-inheritance of selected SNPs is linked to a higher risk of pregnancy loss. Each time, the same study group consisted of 154 women with pregnancy loss, mean age 33 (±5.4) y.o. and 154 mothers mean age 31.4 (±6.7) y.o. with at least one live-born child, as a control group were investigated. We reviewed SNPs: rs6025 *FV*, rs429358 and rs7412 *ApoE*, rs1799752 *ACE*, rs1799889 *PAI-1*, rs1799963 *PT*, rs1801133 *MTHFR*, rs9468 and rs1800547 INV 17q21.31, rs731236 and rs1544410 *VDR*, and rs10421768 *HAMP*, linked to thrombophilia and the risk of pregnancy loss, published in the years 2015-2019. All statistical analysis for this study were performed using the R CRAN computer software version 3.6.2 (R Core Team (2019). Both in group with and without pregnancy loss, co-inheritance for heterozygotes *FV* and homozygotes of other investigated genes was from 2 to 4 polymorphic alleles, average 2.5. The most common prevalence of co-inheritance was 2, 3 and 4 alleles. In women with pregnancy loss, there were: 27, 13 and 5, and without pregnancy loss: 34, 11 and 6, respectively. In women without co-inheritance of variants predisposing to thrombophilia (n = 107), the mean number of miscarriages was lower (1.3) compare to women with co-inheritance of these variants (n = 47), (1.6). There was no statistically significant difference between co-inheritance of investigated variants predisposing to thrombophilia and miscarriage occurrence in Bosnian women, ($p > 0.05$).

Keywords: thrombophilia, variants of genes, co-inheritance, pregnancy loss
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GENETIC BACKGROUND OF FEMALE INFERTILITY

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Infertility is a huge problem of modern society. According to numerous studies, today 10% of couples in the world population are infertile, and the next 10% have a problem called reduced fertility (subfertility). These changes in fertility of human population are evidently increasing. Recently data showes that infertility affects up to 15% of reproductive-aged couples worldwide. From the aspect of genetics, we can talk about genetic and non-genetic causes of infertility. However, today a large number of studies showes that the occurrence of infertility is very often result of a combination of these two factors, ie. that environmental factors often play the role of "triggers" of certain genetically determined processes, which actually result in development of infertility. When it comes to genetic causes of infertility, we distinguish infertility caused by chromosomal and/or genes abnormalities regarding hereditary material. Even women who do not have defects in chromosomal set, produce more than 20% of chromosomal abnormal eggs, and this percentage increases with aging. Chromosomal disorders related to infertility include abnormalities in number of chromosome and changes in the their normal structure. This article describes the association of chromosomes changes with the manifestation of phenotypic infertility in following cases of sex chromosomes disorders: Turner syndrome, Mixed gonadal dysgenesis, Swyer syndrome, Y-cell line mosaic form, 46,XX testicular disorder of sex development, Triple X syndrome or trisomy X or 47,XXX and autosomal disorders like Robertsonian and reciprocal translocations, chromosome inversions and deletions. Also, article describes the occurrence of infertility in connection with disorders at the gene level, as is the case of: Fragile X chromosome-FRAXA syndrome, Kallman syndrome, Androgen insensitivity syndrome, gene disorders for β-subunit LH and FSH, gene disorders for LH and FSH receptors and XX female gonadal dysgenesis (XX-GD).

Keywords: female infertility, genetic causes, genetic abnormalities, human infertility
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MICRONUCLEI FREQUENCY IN PATIENTS WITH ENDOMETRIAL CANCER IN RELATION TO STAGE OF DISEASE, AGE AND SMOKING HABITS

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Endometrial cancer is the most frequent malignant tumor, which is in the fourth place in terms of frequency, after breast cancer, colon and lung cancer. So, the aim of this study was to evaluate the chromosomal damage in peripheral blood lymphocytes of patients with newly diagnosed endometrial cancer with respect to stage of disease, age and smoking habits. The analyzed sample included 31 persons, 16 patients with endometrial cancer (8 with G1 and 8 with G2 degree of cancer) age from 52 to 79 (65.94±6.97) years and 15 healthy women age from 44 to 69 (53.40±7.15) years. To estimate the frequency of chromosomal damage, the cytokinesis-block micronucleus (CBMN) assay was used. The mean MN frequency was significantly higher in patients with endometrial cancer compared to healthy controls (19.38±2.87/1000 BN cells vs. 9.00±1.69/1000 BN cells, $p<0.001$). There was no significant difference in mean MN frequencies between patients with G1 and G2 degrees (19.13±2.30/1000 BN cells vs. 19.63±2.92/1000 BN cells, $p>0.05$). The analysis of MN distribution per 1000 BN/cells person showed that the cells with 1MN (1.15%) were mostly presented in both patients and controls, while cells with 2MN were less common (0.11%). Cells with 3MN (0.02%) were seen only in a sample of patients. Taking into account the factors that can affect the frequency of MN, using multifactorial linear regression analysis we found that health status (diagnosis) as well as degree of cancer significantly affected the frequency of MN ($p<0.001$),while age, NDI values and cigarette smoking did not affect MN ($p>0.05$). Analysis of nuclear division index (NDI) showed that patients had not significantly lower values compared to healthy controls (1.52±0.17 vs. 1.56±015; $p>0.05$). There was also no significant difference in NDI values between patients with different cancer degrees (1.49±0.16 for G1; 1.56±019 for G2; $p>0.05$). Multifactorial linear regression analysis showed that health status, degree of cancer, MNi, age and cigarette smoking did not affect NDI values. Based on the results we can conclude that the patients with endometrial cancer had an increased frequency of MN frequency in human lymphocytes which is in correlation with the stage of the disease.

Keywords: endometrial cancer, peripheral blood lymphocytes, micronuclei, chromosomal damage.
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OXIDATIVE DNA DAMAGE IN PERIPHERAL BLOOD LYMPHOCYTES OF PATIENTS WITH CARDIOVASCULAR DISEASES

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Cardiovascular diseases are multifactor progressive pathologies that seriously harm human health. Many risk factors for developing these diseases are related to lifestyle as well as to genetic factors. Thus, this study aimed to evaluate the association between potential risk factors for cardiovascular diseases and levels of oxidative DNA damage in peripheral blood lymphocytes of cardiovascular patients, including patients with acute coronary syndrome and heart failure with reduced ejection fraction. The study included 30 persons, 20 cardiovascular patients (57.55 ± 4.65 years) and 10 healthy controls (55.40 ± 5.56 years). The frequency of DNA damage of individual cells expressed as genetic damage index (GDI) was analyzed using the alkaline comet assay. Cells were classified into five classes (0-4) depending on the degree of DNA damage. Increased levels of oxidative DNA damage were observed in cardiovascular patients comparing to the healthy controls (1.36 ± 0.15 vs. 0.37 ± 0.05, $p < 0.001$). However, when we analyzed only cardiovascular patients, we also noticed a difference in GDI values. Patients with health failure with reduced ejection fraction had significantly higher mean GDI value than patients with acute coronary syndrome (1.44 ± 0.16 vs. 1.28 ± 0.10, $p < 0.05$). The number of undamaged cells decreased in cardiovascular patients comparing to healthy persons for about 1.5 times, while number of cells with tail (comet classes from 1 to 4) in cardiovascular patients was increased almost four times comparing to controls. Multiple linear regression analysis showed that health condition, drug therapy, family history of cardiovascular disease and triglyceride were predictors of DNA damage in patients with cardiovascular diseases ($p < 0.05$), while age, gender, blood pressure, cholesterol and cigarette smoking were not significant. We can conclude that increased level of genome instability was observed in cardiovascular patients, among which patients with health failure with reduced ejection fraction have higher level of DNA damage in peripheral blood lymphocytes than patients with acute coronary syndrome. This conclusion is correlated with disease severity and prognosis of treatment.

Keywords: acute coronary syndrome, DNA damage, peripheral blood lymphocytes, comet assay

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DETECTION OF P2RY8/CRLF2 REARRANGEMENT IN PEDIATRIC B-ALL PATIENTS FROM SERBIA

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B-cell acute lymphoblastic leukemia (B-ALL) represents the most common form of ALL in children and the leading cause of pediatric cancer related mortality. Defining its distinct genetic profile is paramount for B-ALL classification, risk stratification and treatment evaluation. Recently, in a group of B-ALL patients with no prognostically significant, recurrent genetic rearrangements (such as BCR/ABL, MLL/AF4, TEL/AML, PBX/E2A, hyper/hypodiploidy and other cytogenetic aberrations), new entity emerged, provisionally defined as BCR/ABL1-*like* ALL, associated with adverse outcome. Fusion of a member of the purine nucleotide G protein-coupled receptor gene family with cytokine receptor-like factor 2 gene (P2RY8/CRLF2) is defined as one of the most common hallmark of this entity, with reccurence of 5-8% in BCP-ALL cases. Aim of this study was to determine the presence of P2RY8/CRLF2 rearrangement in a group of pediatric B-ALL patients with no recurrent molecular and cytogenetic alterations. From november 2009. to june 2021. year in Laboratory of Medical Genetics at Mother and Child Health Care Institute of Serbia 340 B-ALL pediatric patients at day of diagnosis were analyzed for BCR/ABL, MLL/AF4, TEL/AML, PBX/E2A rearrangements and cytogenetics. In total, 59 samples negative for aforementioned aberrations, were tested for P2RY8/CRLF2 fusion. Chromosomal analysis, detection of recurrent aberrations and P2RY8/CRLF2 fusion transcripts was done on bone marrow (BM) samples using GTG banding and RT-PCR protocols. Among 59 patients 24 (41%) were positive for P2RY8/CRLF2 fusion. This rearrangement, acorrding to the current WHO classification, defines these patients as B-ALL subgroup with translocation involving tyrosine kinase/cytokine receptors, which is described as BCR/ABL1-*like*. Aproximative incidence in whole cohort of patients of this rearrangement was 7% (24/340) and is in line with literature. According to current guidelines, P2RY8/CRLF2 rearrangement has potential prognostical and therapy related impact in a significant group of patients. BCR/ABL1-*like* ALL entity has defined genetic signature which is the hallmark for adverse prognosis. High prevalence of this alteration in BCP-ALL patients with no reccurent aberrations found, pose its significance in redefining risk stratification and implementation in routine testing.

Keywords: BCR/ABL1-like, P2RY8/CRLF2, B-ALL
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OSTEOGENIC DIFFERENTIATION POTENTIAL OF ORAL CANCER STEM CELLS

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Cancer stem cells (CSCs) are responsible for cancer aggressiveness, drug resistance, and tumor relapse. CD44 has been identified as a CSC surface marker and has been used for the isolation and enrichment of cultures with CSC population in different types of cancers, including oral squamous cell carcinoma (OSCC). Some CSCs possess the potential to transdifferentiate into different cell lineages, as do normal adult stem cells. Such plasticity of CSCs is considered to be a promising therapeutic target. The aim of this study was to examine osteogenic differentiation potencial of $CD44^+$ cells isolated from comercial cell line SCC-25. $CD44^+$ and $CD44^-$ cells were magnetically separated using magnetic-activated cell sorting system. $CD44^+$ and $CD44^-$ cells were seeded into 24-well culture plates (8×10 4 per well) and cultured in osteogenic differentiation medium for 7 and 14 days. Cells were cultivated under standard conditions in humidified atmosphere with 5% CO 2 at 37°C. After 14 days total RNA was extracted from the culture cells with TRIzol Reagent. The expression levels of CSC markers (Oct-4, Sox2, Nanog) and markers of osteo-differentiation (ALP, BMP4, Runx2) were analyzed by qPCR. Specific staining techniques and cell morphology were used for differentiation confirmation. Concomitantly with differentiation and the increase of osteogenic markers, the levels of cancer stem cell markers decreased in the cultures. Osteogenic markers showed that $CD44^+$ had significantlly higher osteo-differentiation potential in comparison with $CD44^-$ cells. In conclusion, OSCC CD44+ cells exhibit the capacity to differentiate into osteoblastic lineage, a characteristic that may potentially be useful in the development of new strategies for OSCC differentiation therapy.

Keywords: cancer stem cells, oral squamous cell carcinoma, differentiation potential, osteogenic differentiation

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THE IMPORTANCE OF ANTI-dsDNA ANTIBODIES SCREENING AS A USEFUL TOOL FOR EVALUATION AND TREATMENT OF PATIENTS WITH SYSTEMIC RHEUMATIC DISEASE

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Anti-Double Stranded DNA or anti-dsDNA antibodies are anti-nuclear antibodies (ANA) typically produced by the immune system, who target the antigens of the double-stranded DNA. These anti-nuclear antibodies target the essential parts of the cell's nucleus especially the genetic material. In normal condition the antibodies protect the organism from different types of infections and inflammations, contrary to that these anti-antibodies don't differentiate their own cells. In those circumstances the anti-nuclear antibodies attack their own healthy cells, causing pathophysiological changes in the tissue and the organ itself. By direct binding to self-antigens or indirect formation of immune complexes, anti-dsDNA antibodies can be accumulated in the glomerular and tubular basal membranes. Systemic lupus erythematosus (SLE) is characterized by high-titer serological autoantibodies, including double-stranded DNA molecule (dsDNA) antibodies. The main objective of this research is to define the importance of anti-dsDNA antibodies screening as a useful tool for the evaluation and treatment of patients with systemic rheumatic disease. Evaluation and screening of anti-dsDNA antibodies is done and confirmed with enzyme-linked immunosorbent assay (ELISA) on patients with SLE in the period from July to December 2020, monitored at the University Clinic for Rheumatology in Skopje, Republic of North Macedonia. The enzyme-linked immunosorbent assay (ELISA) for anti-dsDNA antibodies shows 98-100% specificity and 40-60% sensitivity for SLE. The prevalence for SLE is around 4/100.000 of the population, but more than 99% of the cases occur in young women, although there is no age range. If a patient has a positive ANA test results plus shows symptoms like SLE, the laboratory diagnostics and anti-dsDNA antibodies screening is very useful tool for evaluation and treatment of patients with systemic rheumatic disease. Early diagnostic of SLE and lupus nephritis is very important for suitable therapy, it could stop the progression of the disease, decrease the mortality, and increase the degree's quality of life on these patients.

Keywords: anti-dsDNA antibodies, anti-nuclear antibodies, systemic lupus erythematosus, enzyme-linked immunosorbent assay (ELISA)
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ANORECTAL ATRESIA AS PART OF MICRODUPLICATION SYNDROME 16p13.3

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16p13.3 microduplication syndrome is a rare genetic condition mostly characterized by: typical facial dysmorphisms and variable intellectual disability. Other features include microcephaly, growth retardation, limb anomalies, and defects of the brain, heart, genitalia, palate, and eyes. The aim was to show anorectal atresia as part of microduplication syndrome 16p13.3 which hasn't been found in the literature**.** A 2-year-old girl, born from the second pregnancy, of healthy non-consanguine parents at 20 weeks of gestation, oligohydramnios was suspected. She has been monitored multidisciplinary since birth due to multiple difficulties as part of the malformation syndrome: anal atresia with rectoperinal fistula, open ductus arteriosus - spontaneous closure, atrial septal defect II, congenital laryngomalacia with inspiratory stridor, hypotonia, frenulum linguae breve. Phenotypic dysmorphia includes larger neurocranium compared to viscerocranium, poorly shaped and malpositioned ears, epicanthal folds, wider nasal root, clinodactyly. In the first year of life, anorectal atresia surgery was performed. Laryngomalacia is now less pronounced and less interferes with feeding, established coordination of breathing and swallowing, and no crisis of cyanosis. Chromosomopathy has been ruled out by karyotyping and due to the need for further genetic evaluation, Chromosomal Microarray Analysis was performed - analysis revealed three copies of the 262 kb genome in the region 16p13.3, which includes the RBFOX1 gene. This gene is predominantly expressed in brain neurons and plays a key role in regulating neuronal excitation and influencing the susceptibility to the development of epilepsy. This presentation expanded the range of clinical manifestations for microduplication syndrome 16p13.3 with a unique clinical feature of anorectal atresia. There is no specific treatment for this condition, affected individuals need multidisciplinary monitoring in the direction of improving the quality of life. The therapeutic measures should be on supporting the parents and also suggest genetic counseling for the next planned pregnancies.

Keywords: anorectal atresia, microduplication syndrome 16p13.3, chromosomal microarray analysis, *RBFOX1* gene
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THE IMPORTANCE OF MONITORING TICKS IN THE URBAN REGION CITY OF SARAJEVO, BOSNIA AND HERZEGOVINA

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Changes in soil, vegetation, and climate, as well as the anthropogenic pressure, make living beings adapt, migrate or become extinct. Ticks are very dependent on biotic and abiotic conditions for their local survival and reproduction. Changes in the composition of ticks, as vectors of different pathogens, affect the dynamics of the development of vector-borne diseases in a particular region. Bosnia and Herzegovina does not have a continuous monitoring program on ticks, including the City of Sarajevo, the most crowded urban area. Thus, we aimed to preliminary evaluate tick diversity and abundance in the City of Sarajevo and their impact on pathogen prevalence. Researches were conducted in 2013, 2019, and 2021 in the vegetation period between May and September. Samples from 2013 and 2021 represent ticks collected by dragging method from vegetation in the urban areas, while the 2019 sample consisted of ticks removed from patients in the Emergency Medical Assistance. After morphological identification, several samples could not be identified with certainty as *Ixodes ricinus*, so molecular confirmation was performed targeting the *ITS* region. The *Ixodes ricinus* was the most abundant species in all three periods with a tendency of growth (respectively 51.3%, 98.9%, and 100%), indicating the possibility of a high prevalence of *Borrelia* in this urban area. Additionally, we found *Rhipicephalus sanguineus* (48.7%) in 2013, and *Hyalomma marginatum* (0.53%) and *Dermacentor reticulatus* (0.53%) in 2019. Although from sporadic and discontinuous researches, these results preliminary show a difference in distribution and the diversity of ticks in this timeline. The establishment of continuous monitoring of ticks at the same localities in Sarajevo, accompanied with data on temperature, humidity, potential hosts and main factors influencing the life cycle of ticks, should be a consequential result of this study.

Keywords: ticks, Ixodes, biomonitoring, pathogen
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SIDEROPHORE PRODUCTION BY BACTERIA ISOLATED FROM *ROBINIA PSEUDOACACIA* L. ROOTS AND SERPENTINE SOIL

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Heavy metal contamination of soil poses concerns regarding human health and a balanced ecosystem. Mounting evidence suggests that devastating anthropogenic activities endanger different types of soil across the world and influence plant growth and their survival. Therefore, creating different strategies to alleviate the detrimental impacts of heavy metal burdened soils and understanding the plant-microbe interactions is a promising solution. These interactions are often bolstered with various activities and compounds produced by plant-growth-promoting bacteria (PGPB). As compounds produced by PGPB, siderophores are small molecules responsible for the chelation of dissolved iron and solubilization of iron aggregates. Since siderophores can have a crucial role for plants in situations of low iron availability or nutrient deficiency, the main goal of this study was to isolate soil bacteria and assess their ability to produce siderophores. Since *Robinia pseudoacacia* L. (black locust) exhibits considerable adaptability to nutritiously poor soil, we opted to collect bacterial isolates from serpentine soil and roots of *R. pseudoacacia* (Donja Paklenica, Bosnia and Herzegovina). Bacterial colonies from collected samples were isolated on yeast mannitol agar (YMA) and subsequently tested for the heavy metal tolerance on Tripton Yeast Agar (TYA) supplemented with Cu, Ni, Co in different concentrations. Siderophore production was tested using qualitative (CAS Agar assay) and quantitative (spectrophotometric) methods. The results of spectrophotometric method showed that all 26 selected metal tolerant isolates produced siderophores in a range from 10.96% to 96.66% siderophore units (SU). After seven days of cultivation on CAS Agar, siderophore producing isolates exhibited an orange halo ranging from 3 to 20mm in radius. One root isolate did not produce siderophores on CAS Agar but showed 32.86% SU of production performing the quantitative method. This discrepancy between methods can be explained by the HDTMA activity (component of CAS Agar) which can inhibit or completely stop the growth of gram-positive bacteria. However, this is yet to be confirmed by 16S rRNA sequencing. In comparison to root isolates, isolates collected from the serpentine soil showed intensive siderophore production. The forthcoming study will include screening of other PGP strains and 16S rRNA sequencing for bacterial identification.

Keywords: siderophore production, *Robinia pseudoacacia*, PGP bacteria, heavy metals
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EVALUATION OF *PHYSALIS ALKEKENGI* L. AS THE POTENTIAL ANTIMICROBIAL AGENT

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In this study, antimicrobial activity of ethanolic and methanolic fruit extracts of *Physalis alkekengi* was evaluated against three Gram-positive, three Gram-negative bacterial species, and one fungi: *Enterococcus faecalis* ATCC 29212, Methicillin resistant *Staphylococcus aureus* or MRSA ATCC 33591, *Bacillus subtilis* ATCC 6633, Extended Spectrum Beta-Lactamase producing *Escherichia coli* or ESBL *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 31194, and *Candida albicans* ATCC 1023, through the microbroth dilution method. Assessment of antimicrobial properties included determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of investigated extracts. As antimicrobial reference, dilution series of Amoxicillin and Nystatin were used. Both extracts showed antibacterial and antifungal effects, with no difference in obtained MIC values (256 μg/ml), except in case of *B. subtilis*, where MIC value of methanolic extract was 128 μg/ml. MIC of both extracts for *P. aeruginosa* was 128 μg/ml. Multidrug-resistant strains used in this study were successfully inhibited by *P. alkekengi* fruit extracts, but not with standard antibiotic. In the antifungal assay, MIC value for *C. albicans* found to be 64 μg/ml, which is lower compared to the bacteria. MBC values ranged from 256 to 512 μg/ml, while MFC value for both extracts was 128 μg/ml. Although phytochemical investigations of *P. alkekengi* revealed many different bioactive compounds, the main compounds responsible for antimicrobial properties of *Physalis* species are physalins and neophysalins. In the light of constantly emerging multidrug-resistant pathogens, discovering the new antimicrobial agents of natural origin represents an important issue with both fundamental and clinical implications. According to the available literature, this study represents the first report of antimicrobial activity of *P. alkekengi* fruit extracts.

Keywords: *Physalis alkekengi* L., ethanol extract, methanol extract, minimum inhibitory concentration
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SATUREJA HORVATII SILIC AQUEOUS EXTRACT AS A RADIOPROTECTIVE AGENT IN EXPOSED HUMAN LYMPHOCYTES

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Lamiaceae species are well known for thier beneficial and protective properties. *Satureja horvatii* Silic, an endemic species of a *Lamiaceae* family has shown antigenotoxic and antiapoptotic characteristics in human lymphocyte cultures. Therefore, we aimed to test radioprotective potential of *Satureja horvatii* Silic aqueous extract in the culture of *ex vivo* irradiated human peripheral blood lymphocytes. Isotopic radium was used to irradiate human lymphocyte cultures of a healthy donor. A dose of 100 μg was applied over a 90, and a 9 min period for the high-risk, and low-risk radiation type, respectively. Following irradiation, the cultures were treated with an aqueous extract at a final concentration of 0.1 mg/ml. Negative controls along with the control cultures were set up to monitor the independent effects of radiation and extracts of *Satureja horvatii*. An alkaline comet test was applied, and 100 comets per treatment were analysed. The level of DNA damage was expressed as the percentage of DNA in the comet's tail. Results were compared by one-way ANOVA, followed by Newman-Keuls test with the significance level of $p<0.05$. The results showed a significant increase in DNA damage at the high-risk dose compared to the low-risk dose, but also a significant decrease in DNA damage after aqueous extract treatment indicating radioprotective effect of *Satureje horvatii* Silic aqueous extract. Given various radiation exposures, additional research should address radioprotective effects of widely distributed *Lamiaceae* species. The promotion of sustainable and controlled use of natural resources should be inevitable.

Keywords: radiation, comet assay, DNA damage
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GENOTOXIC AND ANTIGENOTOXIC PROPERTIES OF THE *HYPERICUM PERFORATUM L.* EXTRACTS ON *ALLIUM CEPA L*. ROOT TIP CELLS

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For centuries, traditional medicine incorporates the use of plant extracts for the treatment of various diseases. *Hypericum perforatum L.* is among the plants that are widely used in traditional medicine and it is one of the ethnomedical plant species used frequently in Kosovo too. Due to its beneficial effects, *Hypericum perforatum L*. remains popular and it is used also in modern therapies. On the other hand, there is a growing interest in evaluating the toxicological properties of the extracts from this plant. In this study, the genotoxic and antigenotoxic properties of the *Hypericum perforatum L*. extracts were evaluated. The extracts were prepared by infusing plant dry parts in boiling water and keeping in for a total of 10 minutes. The *Allium cepa* bulbs were exposed to concentrations of 0.5, 1, and 5% of plant extracts for 24h. The effects on the frequency of the micronucleated cells as well as on the frequency of chromosomal aberrations in root tip cells were assessed. The mitotic index was also assessed. The protective effects of the plant extracts in the concentrations of 0.5, 1, and 5% against the paraquat-induced damages were evaluated too. The extracts in the concentration of 0.5% showed no effect on the mitotic index, micronucleus frequencies, and chromosomal aberrations. The concentration of 1% had an effect ($p<0.01$) in increasing the frequency of chromosomal aberrations whereas the highest concentration (5%) of the extract caused a decrease ($p<0.001$) in the mitotic index. On the other hand, the concentrations 0.5% and 1% showed a protective effect with regard to chromosomal aberrations induced by paraquat as well as a protective trend against the paraquat-induced decrease in the mitotic index. In addition, all three concentrations of the plant extracts showed protective effects with regard to the micronucleus frequencies caused by paraquat. The obtained data from this study indicate a concentration-dependent genotoxic activity of the *Hypericum perforatum L.* extracts as well as a protective potential of these extracts against paraquat-induced damages in *Allium cepa L.* root tip cells.

Keywords: *Hypericum perforatum* L., genotoxic, Kosovo
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DNA ANALYSIS OF HUMAN SKELETAL REMAINS FROM THE MEDIEVAL NECROPOLES IN BOSNIA AND HERZEGOVINA – A GENETIC STRUCTURE OF OUR ANCESTORS AND PREDICTION OF THEIR MIGRATIONS

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Archaeological sites represent a rich historical and cultural heritage of medieval Bosnia. Among the archaeological sites, the most of them are with stećak tombstone. Stećak is medieval tombstone that appear in the period between the 12th and 16th century AD primarily on the territory of today's Bosnia and Herzegovina (B&H). Even though individual excavations of skeletal remains from archaeological sites have been carried out continuously from the period of medieval Bosnia, there is no data about the genetic structure of ancient B&H populations. The aim of this study was to obtain usable aDNA (ancient DNA) profiles, then compare and potentially find kinship relations, determine haplogroup based on obtained Y-STR profiles and mDNA sequence. Research which includes molecular-genetics characteristics of a population that lived in the medieval Bosnia, would give a new insight into the genetic structure of our ancestors, their potential relationships and migration processes. Molecular genetic characterization of medieval Bosnia populations will be compared with genetic data of recent B&H populations, thus assessing the genetic differentiation between recent and medieval B&H populations.

Keywords: Ancient DNA, STR markers, tombstone stećak, medieval Bosnia, kinship, population
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MOLECULAR-GENETIC ANALYSIS OF SKELETAL REMAINS FROM MEDIEVAL ARCHAEOLOGICAL SITES FROM THE TRAVNIK AREA

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Molecular genetic analysis of archaeological skeletal remains is important for gaining insight into the origin and genetic structure of our ancestors. The analysis of ancient DNA (aDNA) is extremely challenging since DNA is frequently found in very low concentrations and is often highly degraded. Regarding mentioned, the main goal of aDNA studies is to successfully isolate DNA from skeletal remains with as little loss of biological material and generate usable electropherograms (DNA profiles) with as many amplified STR loci as possible. Although there is not much information about the people who inhabited the Travnik region during the medieval Bosnia, it is believed that this area was one of the most populated in that period, as evidenced by the numerous archaeological necropolises found in this area. A total of 11 tooth samples from four localities from the Travnik area: Glavica - Han Bila, Fazlići, Alihodže and Klisa were analyzed. Amplification of the isolated aDNA was performed with a commercial multiplex kit *PowerPlex® Fusion System* and *PowerPlex® Y23 System* in samples in which the presence of Y chromosomes was detected. Statistical recalculation of the probability of kinship was determined using *IngebKinship* software. For Y-haplogroup prediction, digital software *Whit Athe's Haplogroup Predictor* and *NEVGEN* were used. Isolation of aDNA and amplification of STR loci from analysed samples was extremely successful, generating full profiles from eight out of 11 samples. The highest degree of kinship was found between the two samples with a kinship probability for relationship brother-brother 99.99996%. Analysis of Y haplotypes showed that all male individuals were related by the male line and that they belonged to the J2a haplogroup.

Keywords: ancient DNA, archeology, skelet remains, STR markers
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THE EFFECT OF STORAGE CONDITIONS OF BLOOD TRACES ON THE DNA ANALYSIS IN FORENSIC CASES

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Analysis of DNA from blood traces and other biological samples from crime scenes is an essential part of work in forensic laboratories, and represents a key step in the criminal investigation that forensic investigators have relied on for decades. Since biological traces found at the crime scene are exposed to numerous factors that can affect the integrity of the trace, the aim of this study was to analyze the impact of certain storage conditions on blood traces left on appropriate surfaces so that they can be used after some time. Blood traces on selected surfaces (glass, metal, fabric and paper) were left in different conditions (light and warm; dark and warm, dark and cold) packed in paper and plastic packaging. After 19 days, dry and wet swabs were sampled and the DNA molecule was isolated using modified Miller protocol. For amplification of isolated DNA PowerPlex® Fusion multiplex system was used. DNA profiles were generated using an ABIPRISM® 310 Genetic Analyzer and GeneMapper®ID 3.2 software. The results of the study indicate significant differences in obtained DNA profiles of individuals whose samples were stored in different conditions. As expected the success of the DNA analysis was highly dependent on type of the surface on which the biological trace was located, and light and temperature exposure. In our study the best DNA profiles were generated from blood traces collected from glass and fabric in all applied storage conditions, except two profiles from glass in light-warm and dark-cold conditions. Our results confirmed the importance of proper sampling and storage conditions of biological traces for successful DNA analysis in forensic cases.

Keywords: forensic traces, DNA profile, criminal investigation
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FREQUENCY ANALYSIS OF 12 SELECTED X-STR MARKERS IN POPULATION OF BOSNIA AND HERZEGOVINA

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X-STR markers are routinely used in forensic practice mainly as a complementary analysis of autosomal STRs analysis in complex cases. However, in certain kinship scenarios, these markers can be the only means to obtain information needed to solve the case. In order to use X-STRs, it is necessary to determine the frequency of those markers in certain population and to establish population databases to be used for comparison in forensic analyses. In this study, a total of 195 individuals from Bosnia and Herzegovina (82 females and 113 males) were included in analysis of 12 X-STR markers. The Investigator® Argus X-12 QS kit was used to obtain allele frequencies for 12 polymorphic STR loci including DXS10103, DXS8378, DXS10101, DXS10134, DXS10074, DXS7132, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB and DXS10148. The main heterogenity parameters for both female and male individuals were calculated in PowerMarker v 3.25 software. There was no statistically significant difference in measured values between males and females in Bosnian population as well as from those observed in neighbouring Balkan populations. DXS10135 locus was found to be the most discriminating locus among all loci included in study in both males and females with highest values of polymorphic information content as well. Expected heterozygosity values among all the studied loci ranged from 65.35% to 92.12%. The least polymorphic locus was DXS8378, with PIC values of 0.581515 in males and 0.63741 in females, respectively. Since only one population study using X-STR markers has been reported previously on the population of Bosnia and Herzegovina, these results expand Bosnian population database with information on eight new X-STR loci. Overall, the studied markers of the Argus 12 X-STR kit proved to be highly polymorphic tool for use in analysis of forensic issues such as missing person identification, incest, immigration disputes, paternity and kinship analysis as well as genealogical studies.

Keywords: X-STR, forensic parameters, Investigator Argus X-12 QS Kit
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