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Abstracts

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Kirill V. Ermakov et al., Biochem Mol biol J 2019, Volume:5
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SINGLE STRANDED DNA FRAGMENTS IN RETINOBLASTOMA PATIENT BLOOD PLASMA: LINK TO ONCOGENESIS AND DIAGNOSTIC VALIDITY

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A significant population of ultrashort (50n – 150n) single-stranded DNA fragments were found in exosome-free blood plasma of retinoblastoma patient (6.84 ng x mL-1), but not in plasma of healthy donors. An original HPLC technique has been employed. 5.0 year old male retinoblastoma (2A) patient and four same age/sex healthy donors were taken for blood plasma cfDNA extraction. A consequent treatment of DNA extract with exonucleases and III, S1 nuclease, and proteinase K was followed then by a cascade ultrafiltration on K75/K25 SPM TechSep membranes (Mirabel, France). /III-nuclease resistant 25K – 75K compounds were analysed by size exclusion/anion exchange HPLC. For this purpose, its key parameters were estimated as the followings: stationary phase – polymethylamidopropylmethacrylamide; column PRP-X600 AE, 4.6 x 150.0mm, 5.0 particles, 1.6 meq/mL (Hamilton Corp., USA); 1,800 p.s.i., 22° – 25°C, 0.8 mL/min elution rate. Both synchronous linear elution LiCl2 (0 – 2.5M) and pH (8.0 – 4.0) gradients were formed on 100mM Tris/acetonitrile (85:15, v/v). Waters/Hamilton compatible Breeze 200SLE Analytical System, W2998 UV-Detector (254nm), W600E gradient former (Waters, Inc., USA). Sample loading: 80 – 100 g DNA in 50 L 100mM Tris-HCl (pH 8.0)/acetonitrile (85:15. v/v). As mentioned above, ssDNA short fragments were found in plasma of retinoblastoma patient. To the contrast, in control donors, a smaller population of ssDNA (2.40 – 2.82 ng x mL-1) was found consisting of essentially larger, 350n – 400n, sequences. A separation efficiency shown by our HPLC technique allows to reveal the size/charge – different populations within an ssDNA pool in cancer plasma which is not always possible in both PCR-based DNA size estimations and a routine agarose gel electrophoretic procedures. The later would mean a possible release of ssDNA directly in the "cancer-booming" DNA defects replacement. HPLC proposed is a simple and reliable tool for further epigenic and diagnostic studies on patients with retinoblastoma.

Biography

Kirill V. Ermakov - Postgraduate Student, Department of Medical Nanobiotechnology Graduated from Pirogov Russian National Research Medical University of the Ministry of Health of the Russian Federation in 2017. Research interests: Spin-selective biochemistry, chemical enzymology, experimental oncology and pharmacology.

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CLINICAL DIAGNOSIS BASED ON TUMOR BIOMARKERS ANALYSIS USING DIGITAL IMAGE PROCESSING AND DIGITAL BIOMARKERS DETECTORS CASE STUDY: EPITHELIAL DYSPLASIA CANCER

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Considerable researches has been devoted to study tumor biomarkers digital images. Information gathered from studying these images are good source for automating clinical diagnosis of tumors. This research investigate the techniques used for analyzing Microscopic images that contain tumor biomarkers in term of diagnosis and also presents an automated method for analyzing epithelial dysplasia microscopic biomarker digital images to provide a clinical diagnosis epithelial dysplasia tumor biomarkers based on both applying the adaptive threshold and the mathematical calculations of processing the tumor biomarkers images. 100 tumor marker digital images for both normal and epithelial dysplasia cases have been experimented. we preprocess the digital images to enhance them, after that we apply the adaptive threshold. We achieved a novel finding for determining the value of the window size for epithelial dysplasia images. We also propose a mathematical method that can be used for processing of epithelial dysplasia images for the clinical diagnosis of these markers to determine if it is normal or contain epithelial dysplasia. The results showed that 94% of epithelial dysplasia tumor biomarkers were correctly diagnosed.

Biography

Hussam N. Fakhouri is a Instructor at the University of Jordan, Amman Jordan he also responsible for the quality assurance in the Facluty. His first published research was in year 2006 about Genome image processing after that he published more than 30 researches in different fields. He studied a PhD in artificial intelligence. He also was an Erasmus scholar to attend many research courses in Denmark, Germany and Russia.

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URINARY MEGALIN AS A POTENTIAL MARKER FOR DIABETIC NEPHROPATHY: CORRELATION WITH VITAMIN D LEVELS

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Background: Urinary albumin excretion has been used as a marker for diabetic nephropathy. Megalin is a 600-kDa protein expressed in renal proximal tubular cells and it is involved in the reabsorption of vitamin D binding protein. Recently, urinary megalin excretion has been evaluated as a potential urinary marker of nephropathy. The aim of this study is to evaluate the correlation between the urinary megalin and serum vitamin D levels.

Methods: This was a pre-post study in patients with type 2 diabetes mellitus to examine the effect of 6-month vitamin D on diabetic nephropathy. Results. Urinary megalin was positively associated with SBP (r=0.218, p=0.04) but negatively with GFR (r=-0.16, p=0.023). In addition, when patients were divided according to urinary megalin cutoff point level that qualifies failure, urinary albumin, and TGs were higher in the "high-megalin" group, compared to those with “low-megalin” group. Glycosylated hemoglobin (HbA1c) was statistically and significantly higher in the high-megalin group. A stepwise forward logistic regression which was adjusted for SBP, FPG, and calcium levels showed that there is a significant inverse association between vitamin D levels and megalin levels in urine (OR= 0.281, p-value=0.047). Conclusion. Urinary megalin is a potential marker for diabetic nephropathy is correlated with the extent of vitamin D. Of the 209 patients, 63 patients who had vitamin D deficiency were given supplements of vitamin D. There was a significant improvement in kidney function (*increase* in GFR and *decrease* in ACR), with concomitant decrease in urinary megalin and increase in vitamin D3. The decrease in megalin was more pronounced than ACR, which indicates that megalin is more sensitive than ACR to changes in renal function over a shorter period of time.

Biography

Amal Akour received her Bachelor of Pharmacy from the University of Jordan in 2007 and had completed her Ph.D. in 2012 from Virginia Commonwealth University, Virginia, USA. She is currently working as an Associate Professor of Pharmacotherapy at the School of Pharmacy, University of Jordan, Amman, Jordan. Also, she had been working as Assistant Dean for Hospital affairs and Pharm.D. program in the same institute from September 2015 till September 2017. She is an active member of academic and scientific committees as well as social organizations. She is leading active research group evaluating markers of diabetes progression with the attempt of finding novel progression/therapeutic markers for this epidemic. She has a number of publications in reputed ISI Journals. In addition, she has volunteered to serve her community by providing free health-awareness lectures, supervising free medical days and reviewing publications.

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PERSONALIZED AND REGENERATION MEDICINE REQUIRE A COAGULUM-OMICS MODEL

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Background: In 2017, a program on patient blood management was posted to the National External Quality Assurance Scheme conference for hemostasis and thrombosis [1]. This subsequent coagulum-OMIC framework is a standard for predictive value within personalized and regeneration medicine. An OMIC model is a foresight by the author of this program to achieve OMIC flow [View Fig. 1.]. This model sustains the success of Coagulum-OMICS when supported with the ISO 9000 series. [2] [3] [4].

Study: ISO 9001 and 9004 are powerful tools to identify and define good practice in an OMIC model. ISO 9001 is a process based standard and an ideal standard for OMIC interfaces. The greater challenge in haemostasis and thrombosis is the end to end process involves several parts of healthcare under different clinical management or vendor arrangements. The flexibility of ISO 9004 makes it an ideal tool to access Coagulum-OMICs and sustain the success of personalised and regeneration medicine.

Program Development: A strategy for Research, Family, Organ and Acute Coagulum-OMICs commences biphasic policy objectives for genomics as a primary care with viscoelastic science, coagulum and platelet proteomics [Fig. 2]. Model OMIC development, resources, performance review and innovation, become learned. OMIC teams self-assess the Coagulum-OMICs to identify conformity with the model. Regional committees are supported by a joint working group on quality assurance to manage or improve OMICs

Conclusion: A model for blood coagulum-OMICs is a benchmark to sustain excellence in the future of biological systems. The agility of Coagulum-OMICS to transverse primary and secondary care with genomic [pharma] pre-exams and viscoelastic or proteomic exams makes it a perfect learning initiative, self-assessment tool and governance program. The caveat is a need for expertise to sustain the success of coagulum-OMICs, in situ, with personalised and regeneration medicine.

Biography

James Henry completed his Master of Science (Upper Merit) in 2009 from Middlesex University U.Kin Molecular Pathology. Also he completed his Master of Science (Distinct) in 2014 from University of Greenwich U.K in Patient Blood Management Quality Systems. In 2014 a Patient Blood Management program was overseen by a U.K national governance representative, sponsored by an anesthetic lead and edited by an MHRA inspector who stated "this program is suitable for the NHS". In 2017 that program was posted to the National External Quality Assurance Scheme and then to the British Blood Transfusion Society. In 2018, ISO published the "Quality of an organization — Guidance to achieve sustained success". The author of "Blood Coagulum-OMICs" has developed a model for hemostasis and thrombosis genomic pre-exams and a viscoelastic & proteomic examination to improve predictive value in personalized and regeneration medicine.

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EVALUATION OF MAGNETIC FIELD INFLUENCE ON CHINESE HAMSTER OVARY CELLS

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The influence of magnetic field on mammalian cells and their biotechnological properties has been widely investigated. Several mammalian cells dominate the industry as measured per number of products in the market. Previously, Chinese hamster ovary (CHO) cells have demonstrated increased cell permeability, gene expression, proliferation and micronuclei formation after magnetic field exposure. On the contrary, it has been reported that the cell survival and growth rate, cell cycle distribution and mutation frequency are not influenced. The purpose of this study is to evaluate magnetic field influence on mammalian cell CHO-S proliferation by application of a static magnetic field generated by permanent magnets.

Methodology: **Mammalian cell culture CHO-S was cultivated in cell culture dishes infixed into magnetic yoke under 0.5T magnetic field intensity. Cells were cultivated in a batch regime for 2 and 3 days and in a fed-batch regime for 3 and 4 days by adding the cell culture media after day 2 (2+1 and 2+2 days, respectively). Cell count and viability were determined using trypan blue exclusion method with a hemocytometer**

Results: **Obtained results showed (Fig.1) that viable cell count after 2-4 days of cultivation varied from 13 3 to 48 16 million. Magnetic field has no statistically significant influence on cell proliferation. Factors that influence cell proliferation are cultivation regime and duration. During the fed-batch regime for 4 days, viable cell count increased more than 3 times compared to the batch regime for 2 days.**

Conclusion & Discussion: **The research showed that 0.5T static magnetic field exposure has no statistically significant influence on CHO cell proliferation. However, proliferation increased due to the change of cultivation regime and duration.**

Acknowledgement: **This work has been supported by European Regional Development Fund within the project "Influence of the magnetic field initiated stirring on biotechnological processes" No. 1.1.1.1/16/A/144**

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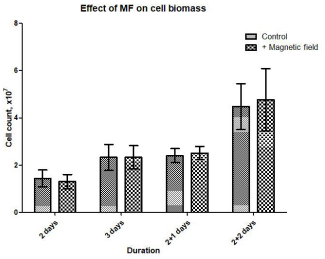

Figure 1: Magnetic field influence on cell proliferation after 2 and 3 days in a batch regime and 3 and 4 days in a fed-batch regime (2+1 and 2+2 days, respectively).

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ELEVATED CIRCULATING MICRORNAS AND VASOACTIVE AMINE METABOLITES IN NEUROCARDIOGENIC SYNCOPE PATIENTS: A PILOT STUDY

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Objective: Syncope is a common clinical problem challenging both cardiologists and general practitioners. In our study, we selected a number of circulating micro-RNAs and vasoactive amine metabolites to be evaluated in attempts to introduce possible biomarkers for syncope. To the best of our knowledge, this is the first study to assess the changes in mi-RNAs in syncope patients.

Materials & Methods: Nineteen patients with history of syncope and nineteen sex and age matched healthy controls participated. A detailed medical history was recorded and cardiovascular examinations followed by head up tilt table testing (HUTT) were performed. Three blood samples were withdrawn, first one at baseline, second during syncopal attack and third one after 30 minutes from the end of the tilt test. The levels of three circulating microRNAs (miR-210, miR-1 and miR-34a) and three vasoactive amine metabolites (endothelin-1, copeptin and serotonin) were quantified.

Results: In Group A, copeptin significantly increased during syncopal attack by 21.7±0.45 pg/mL vs. 4.3±1.209 pg/mL in control subjects (Group B; P=0.002). Similarly, endothelin-1 values significantly rose by an average of 28±1.25 pg/mL in syncope patients vs. 3.35±0.75 pg/mL in healthy controls (P<0.001). Serotonin (5-HT) levels were significantly greater during syncope relative to baseline in HUTT positive patients by 95.89±3.7 pg/mL vs. 9±1.43 pg/ml (p<0.001) in control subjects. In summary, vasoactive amines increased with a 3-5 fold change in group A, but showed 1-2 fold increase in the control group. In group A, miR-210 has increased by a mean of 0.6±0.09 (p<0.001) during syncope (95% CI [0.4-0.79]). While miR-34a values increased by mean of 0.89±0.22 during syncope than baseline value (95% CI [0.42, 1.36]) with significant difference of P=0.001. Likewise, miR-1 levels was elevated by an average of 0.42±0.07 (95% CI [0.26, 0.58]) with a p<0.001 significance. miRNA levels were 3±1 fold higher in the syncope patients (Group A) than in controls.

Conclusion: The selected miRNAs and vasoactive amines have a very promising diagnostic and therapeutic potential as biomarkers in diagnosing syncope.

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ANTI-CANCER EFFECT OF L-GLUTAMINASE ON ACUTE LYMPHOBLASTIC LEUKEMIA (RAJI), BREAST CANCER (MCF7) AND COLORECTAL CANCER (A549) CELL LINES

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Background: There are lots of treatments for cancer but always enzymes are the most efficient. Glutamine can be used to support proliferation in multiple ways. It is a proteinogenic amino acid, and can be act as a nitrogen donor for the synthesis of amino acids as well as nucleotides biosynthesis in cellular processes. The presence of L-glutaminase has been reported in various organisms, including animals, plants, and microorganisms except humans. It's a treatment enzyme for ALL and L-glutaminase also has proved ineffectual for treatment solid tumours such as breast cancer and colorectal. In following research Yarrowia yeast glutaminase is used.

Methods: In this study Raji, MCF7 and A549 cell lines were cultured in RPMI 1640 with 10% FBS and 5% of CO_2 condition. The cytotoxic effects of L-glutaminase on Raji, MCF7 and A549 cells were studied using MTT assay. Then, flow cytometry assay was exploited to measure cell death and apoptosis stage.

Results: MTT assay showed that L-glutaminase significantly inhibited the cell growth. According to the flow cytometry assay result, the L-glutaminase was able to induce apoptosis in Raji, MCF7 and A549 cell lines. The apoptosis of Raji cells was more than other cell lines and A549 was more than MCF-7.

Conclusion: According to our finding, L-glutaminase obtained from Yarrowia, safe yeast could successfully induce apoptosis in Raji, MCF7 and A549 cell lines. Therefore, it could be used as a novel and safe therapeutic candidate for cancer treatment.

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BACTERIOPHAGES AS BIOTECHNOLOGICAL TOOL AGAINST BACTERIAL PLANT PATHOGENS

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Bacterial pathogens are associated with numerous plant diseases and can account for major economic losses to agricultural production. Currently, the plant disease management involving the use of traditional chemicals or antibiotics are losing their efficacy due to the natural development of bacterial resistance to these agents. Bacterial outbreaks are generally problematic to control due to lack of effective bactericides and to resistance development. Most effective plant disease management approaches require an integrated strategy utilizing cultural practices, and biological control agents. In this regard, bacteriophages (also called phages) offer an alternative to conventional management strategies for controlling bacterial plant diseases. Phages are viruses that specifically infect bacteria yet have no direct negative effects on animals or plants. Infection of a bacterium by a virulent phage typically results in rapid viral replication, followed by the lysis of the bacterium and the release of progeny phages. Therefore, phages can be used effectively as part of integrated disease management strategies. A suitable phage candidate for effective biocontrol should have a sufficiently broad host range against a wide variety of strains, which is known as a polyvalent bacteriophage. The relative ease of preparing phage treatments and low cost of production of these agents make them good candidates for widespread in crop protection. Phages have potential for use in integrated disease management strategies, which the phages provided additional reduction in disease and resulted in more efficient foliar disease control than copper-macozeb.

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YEASTS L-ASPARAGINASE INHIBITS CELL GROWTH AND INDUCES APOPTOSIS OF ACUTE LYMPHOBLASTIC LEUKAEMIA (RAJI), BREAST CANCER (MCF-7) AND LUNG CANCER (A549) CELLS IN IN-VITRO SYSTEM

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Background: Cancer is one of the most important problems in the world. Today Enzymes have been intensively studied as a source of antitumor compounds. L-Asparaginase (L-ASNase) is one of the most therapeutic enzyme which used for the cancer therapy. The attendance of L-ASNase has been reported in various organisms, containing animals, plants, and microorganisms (bacteria, fungi, algae, yeast, and actinomycetes) except humans. In this study we used L-ASNase enzyme which isolated from Yarrowia yeast.

Methods: In this study, Raji, MCF7 and A549 cell lines were cultured in RPMI 1640 with 10% FBS and 5% of CO2 condition. Cytotoxic effects of yeasts L-asparaginase was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Then, flow cytometry assay was exploited to measure cell death and apoptosis stage.

Results: According to our findings, yeasts L-asparaginase can inhibit cell growth in a time and dose dependent manner. Flow cytometry assay result showed that yeasts L-asparaginase was able to induce apoptosis in Raji, MCF7 and A549 cell lines. The apoptosis of raji cells is more than other cell lines and A549 is more than MCF-7.

Conclusion: Our results showed that yeasts L-asparaginase could successfully induce apoptosis in Raji, MCF7 and A549 cell lines. Therefore, it could be used as a novel and safe therapeutic candidate for cancer treatment.

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VOXEL BASED ANALYSIS OF PAEDIATRIC BRAIN AND ITS CLINICAL APPLICATION: BIOMARKER TOOL FOR FUTURE DRUG TRIALS

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Voxel-wise analysis is a class of modern methods of image processing in the medical field with increased popularity. It has replaced manual region of interest (ROI) analysis and has provided tools to make statistical inferences at voxel level. Voxel-based morphometry (VBM) is another popular inter individual voxel-based image analysis method that measures differences in local concentrations of brain tissue, through a voxel-wise statistical comparison of multiple tissue density maps. These maps are generated by the normalization of high-resolution structural images (i.e. 3-D T1-W spoiled gradient-echo) and their segmentation to grey matter, white matter and cerebrospinal fluid. Usually VBM is used to compare tissue density maps between two groups and detect regional brain atrophy or hypertrophy. The structural impact of many neuropsychological disorders or birth complications has been studied with VBM in children. Moreover VBM has allowed monitoring of childhood brain development. Combined with advanced normalization techniques it has allowed study of population-related effects. The wide use of standard atlases has facilitated the next level of application, which is voxel based meta-analysis comparing regional effects across studies, providing quantitative summaries with good control of statistical error. Future technological advances are expected to increase the clinical uptake; to improve the impact of quantitative techniques on clinical practice; and to improve our understanding of complex pathological processes. Likely to be an important biomarker in future drug trials to assess treatment effects at the group level.

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AMELIORATION OF CCL4 INDUCED LIVER INJURY IN SWISS ALBINO MICE BY ANTIOXIDANT RICH LEAF EXTRACT OF CROTON BONPLANDIANUS BAILL

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The progress in industrialization has blessed mankind with a technologically superior lifestyle but poor management of industrial waste has in turn poisoned nature. One such chemical is carbon tetra chloride (CCl4), which is a potent environmental toxin emitted from chemical industries and its presence in the atmosphere is increasing at an alarming rate. Presence of CCl4 in human body is reported to cause liver damage through free radical mediated inflammatory processes. Kupffer cells present in the liver are potentially more sensitive to oxidative stress than hepatocytes. Kuffer cells produced tumor necrosis factor-α (TNF-α) in response to reactive oxygen species (ROS), that might further cause inflammation or apoptosis. In this study hepatoprotective capacity of antioxidant rich extract of Croton bonplandianus Baill. (CBL) was evaluated on CCl4 induced acute hepatotoxicity in murine model. Hydro-methanolic extract of C. bonplandianus leaf was used for evaluation of free radical scavenging activity. Liver cells of experimental mice were damaged using CCl4 andsubsequently hepatoprotective potential of the plant extract was evaluated using series of in-vivo and in-vitro studies. In the hepatoprotective study, silymarin was used as a positive control. Antioxidant enzymes, pro-inflammatory markers, liver enzymatic and biochemicalparameters were studied to evaluate hepatoprotective activity of Croton bonplandianus leaf extract. Free radical scavenging activity of CBL extract was also observed in WRL-68 cell line. The phytochemicals identified by GCMS analysis were scrutinized using in-silico molecular docking procedure. The results showed that CBL extract have potent free radical scavenging capacity. The biochemical parameters were over expressed due to CCl4 administration, which were significantly normalized by CBL extract treatment. This finding was also supported by histopathological evidences showing less hepatocellularnecrosis, inflammation and fibrosis in CBL and silymarin treated group, compared to CCl4 group. ROS generated due to H2O2 in WRL-68 cell line were normalize in the highest group (200 µg/ml) when compared with control and negative control (CCl4) group. After molecular docking analysis, it was observed that the compound α-amyrin present in the leaf extract of C. bonplandianus has better potentiality to protect hepatocellular damages than the standard drug Silymarin. The present study provided supportive evidence that CBL extract possessespotent hepatoprotective capacity by ameliorating haloalkane induced liver injury in the murine model. The antioxidant and anti-inflammatory activities also affirm the same. The synergistic effects of the phytochemicals present in CBL are to be credited for all the hepatoprotective activity claimed above.

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ARTIFICIAL INTELLIGENCE IN HEALTHCARE

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Artificial intelligence (AI) in healthcare is the use of software algorithms in the analysis of complex medical data to approximate conclusions without direct human input. All over the world Healthcare is generating tremendous volume of structured & unstructured data through different IT systems & connected devices. Analysis of this data without support of computer algorithms is virtually impossible. With the advances in computer technology it has become possible to process this data and give a well-defined output to the end-user. AI does this through machine learning algorithms, which can recognize patterns in behaviour and create its own logic. Before AI systems can be deployed in healthcare applications, they need to be 'trained' through data that are generated from clinical activities, such as screening, diagnosis, treatment assignment and so on, so that they can learn similar groups of subjects, associations between subject features and outcomes of interest. These clinical data often exist in but not limited to the form of demographics, medical notes, electronic recordings from medical devices, physical examinations and clinical laboratory and images. We look at some specific real world examples in medical world where AI is playing an increasingly important role in bringing the benefits of technology to improve patient care. These include algorithms for analysis of radiology images, robotic surgery, virtual assistants and clinical decision support systems.

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THE KLF6-RELATED SUPER ENHANCER MODULATES CELL PROLIFERATION VIA MIR-1301 IN HUMAN HEPATOMA (HEPG2) CELLS

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Super enhancers, the genome regions including large clusters of transcriptional enhancers, are shown to specifically regulate the genes involved in cell identity and disease, including oncogenes, and to play critical roles in the development and progression of cancer. Recent studies have attempted to elucidate the function of super enhancers by means of microRNAs. Although the functional outcomes of microRNAs have become clearer, the pathways that regulate the expressions of microRNAs remain unclear. In this study, we hypothesized that the inhibition of cell proliferation induced by the disruption of the -related super enhancer may be associated with microRNAs. As a result, it was demonstrated that the over-expression of miR-1301 induced by the disruption of the -related super enhancer leads to a significant inhibition of proliferation in HepG2 cells. Moreover, it was demonstrated that the -related super enhancer propagates the anti-proliferative and pro-apoptotic effects which are mediated, at least in part, by the induction of p^{21} and p^{53} in a p^{53}-dependent manner.

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THE DRYING EFFECT ON THE WATER CONTENT AND ON THE ESSENTIAL OIL CONTENT OF OF LAURUS NOBILIS L

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Medicinal and aromatic plants are promising and are characterized by the biosynthesis of odorous molecules that make up the so-called essential oils (EO), which have long been known for their antiseptic and therapeutic activity in folk medicine.The objective of this study was to evaluate the influence of drying in the shade on the water content and on the content of essential oils extracted from leaves of *Laurus nobilis* L. for better quality control of medicinal and aromatic plants. The water content of Laurus nobilis L. plant material decreases during the drying process. It increased from 100% to 0.006% for the drying in the shade after ten days. The moisture content is practically constant at the end of the drying period. The drying in the shade increases the concentration of essential oils of *Laurus nobilis L*.When the leaves of Eucalyptus Laurus nobilis L. plant are in the shade, the maximum of the essential oil content was obtained on the eighth day, the recorded value was 1.43%±0.01%. Beyond these periods, the content continuously drop in before stabilizing. The optimum drying time is between 6 and 9 days.

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BRCA1/2 TUMOR TESTING AND PARP-INHIBITORS: NEED OR FASHION?

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Somatic BRCA testing (sBRCA) is emerging as a powerful tool to discover and identify more mutations in high serous ovarian cancer patients. Despite its clinical utility, the availability of some technologies, able to enrich somatic mutation from FFPE samples, still requires further adjustments before being completely considered as a validated routine assay. In the last two years, my lab has experienced as the know-how on BRCA germline testing cannot be completely transferred to the somatic NGS pipeline, being the two conditions very different. In light to better underline the main pitfalls and criticisms regarding sBRCA1/2 along with its usefulness in clinical routine setting, the present talk will cover the following issues: Why tumor BRCA1/2 testing is important? What are the pitfalls that can strongly affect sBRCA testing; NGS technology needs to be complemented by other Dx tools; how to solve these issues and accelerate the routine use of *sBRCA1/2* testing; the experience of my lab on 3000 ovarian cancer women screened.

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FACTORS AFFECTING ADHERENCE OF ORAL CHEMOTHERAPY AMONG THE CANCER OUT PATIENTS IN APEKSHA HOSPITAL, SRI LANKA

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Background: Anticancer medications are very expensive and great burden for the Sri Lankan government who provides free healthcare services to the nation. Medication adherence is an important parameter use to measure the effectiveness of therapy. The objective was to identify the correlation factors affecting the effectiveness of oral chemotherapy among the cancer out patients in Apeksha Hospital, Sri Lanka.

Methods: A cross sectional survey was conducted among 202 out patients during period of three months utilizing an interviewer administered questionnaire conceded with five categories. Data was statistically analysed using SPSS version 23. Correlations among the 25 adherence factors were studied to identify the critically affecting adherent factors. Chi square test and R value were calculated to assess factors are statistically significant.

Results: Average medication adherence percentage was 72%. Out of 25, there were 14 critically affecting adherent factors that R within -0.1 to 0.5. Some of them were: interest to obtain more information, discussion about the dosing schedule and side effects with the physician, counsel by pharmacist, living condition. The strongest correlation was showed in understanding therapy with misunderstanding current treatment (r=-0.320).

Conclusion: Majority had satisfactory level in the adherence. But there were considerable proportion of patients who had problems in adherence due to some correctable factors. Therefore, necessary steps should be taken to improve adherence by minimizing deficits in some areas. Conducting counselling sessions and awareness programs might be helpful to enhance adherence and effectiveness of therapy

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CIRCULATING BIO-MARKERS AS A NON-INVASIVE DIAGNOSTIC TEST TO PREDICT LOSS OF PREGNANCY

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The hypothesis of induction of intolerance suggests the role of unique dendritic cell (DC) subsets, NK cells and its ability to prime type 1 regulatory T (Treg) cells in recurrent pregnancy loss (RPL). Thus, we attempted to find if the indices of peripheral Treg/DCs/NK cells and their Th1/2/17 responses can be developed as biomarkers to predict pregnancy loss. Antenatal women with history of RPL (6-20 weeks of singleton pregnancy; n=25), with threatened abortions (TA; 6-20 weeks; n=25), with normal (control) singleton pregnancy (6-12 weeks; n=50), median age of 26±4 yrs were enrolled. Cases with history of chronic diseases/ known anatomical anomalies of womb/genetic or infectious etiology of previous miscarriage were excluded. PBMCs were used to identify dendritic cells as monocytoid and plasmacytoid, co-stimulatory (CD83) and PDL (CD274). Treg cells were also investigated (FACS ARIA III). Cytokines (IL-2/4/6/10/17A, TNF, IFN) were measured using BD™ CBA Human Cytokine Kit. Student t-test/chi-square/Pearson correlation was used to analyse the results and statistically significant if the P value was <0.05. The incidence of pDCs decreases significantly in RPL (1.9±0.56) compared to TA (1.8±0.55; <0.001) and controls (3.09±0.6; <0.001). Treg cells were found to be significantly lowered in RPL patients, 2.02±0.25 (<0.001) and 2.57±1.05% in TA cases, as compared with 3.04±0.69% in normal controls. Expression of activated T-cell was not significantly observed between RPL and control. NK cells were elevated in RPL (7.77±0.74) and TA (4.13±0.58) compared to controls (3.08±0.57). Levels of IL-2, INF , TNFα were found to be higher (<0.001) in TA and RPL cases compared to controls. Th1/Th2 ratio decreases significantly in RPL as compared to normal (<0.001). IL-17A was significantly elevated in RPL (5-fold; 13.2±1.03 ph/ml) and TA (2-fold; 6.9±2.7 pg/ml) as compared to control (2.9±0.6 pg/ml; <0.001), revealing a close association of these conditions with the increased production of Th17 cells. ROC showed IL-2 and IL-17A to be able to distinguish between RPL and TA significantly from control with AUC ranging from 85-98%.

Conclusion & Significance: Significant decreased expression of pDC and Tregs, increase in NK cells and IL-17, exhibit impaired immune regulatory mechanisms in pregnancy which may lead to pregnancy loss. IL17A showed to be one of the most sensitive and accurate marker predicting pregnancy losses. Thus, we propose that analysis of these circulating immune cells in peripheral blood and associated cytokine markers could be used as non-invasive diagnostic test for preganancy loss and miscarriage risk assessment. In future, this test may provide a scientific evidence for improved treatment modalities and immature DCs could be used as an alternative therapy in RPL/TA cases

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THE POTENTIAL INTERACTION BETWEEN ADRIAMYCIN AND HYPERICUM PERFORATUM L

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Several experimental and clinical studies report that *Hypericum perforatum* L. (HP) can be effective in treating various disorders related to the central nervous system, such as depression and substance use disorder. Cancer is known as a disease that not only impairs life and physical integrity, but also affects mental health. This study aimed at investigating the anticancer activity of the HP extract in an *in vitro* model of esophageal squamous cell carcinoma (ESCC) (i.e., KYSE-30 cells) and its interference with Adriamycin (Adr) as a conventional chemotherapeutic drug. To this aim, cancerous and normal cells (5000 cells/mL) were exposed to both anticancer agents in triplicate to be subsequently tested using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. A quantitative real-time polymerase chain reaction (PCR) assay was performed to evaluate the mRNA gene expression of Cyclin D1. Both treatments reduced the cell count in a dose-dependent fashion. The IC_{50} of Adr and HP was ~0.090-0.095 mg/mL and ~0.92-0.94 mg/mL in KYSE-30 cells. The maximum inhibition of KYSE-30 cell was 55.9% and 57.1% by individual Adr and HP, respectively. Combined treatments of KYSE-30 cells with Adr and HP significantly reduced the cell viability and yet elevated the level of cyclin D1 expression as opposed to the individual treatments. The presence of HP was found to decrease Adr concentrations below the therapeutic range. The co-treatment of cancer cells with HP led to poor response to Adr and accordingly multidrug resistance as verified by notable up-regulation of cyclin D1 gene expression. Thus, the simultaneous use of Adr and HP is not recommended in cancer disease.

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DETERMINATION OF COMMON CHROMOSOMAL ABNORMALITIES WITH NIFTY TEST (NON-INVASIVE FETAL TRISOMY TEST) IN TURKEY

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Thanks to scientific developments, detection of many common chromosomal anomalies in the prenatal period has great importance on mother and baby health. Next-generation sequencing technology provides early detection and diagnosis in many medical situations. In this study, Nifty Test (noninvasive fetal trisomy) based on Next Generation Sequencing technology is used to determine fetal chromosomal aneuploidies in the prenatal period. NIFTY (noninvasive fetal trisomy) Test is a kind of the NIPD (noninvasive prenatal diagnosis) tests which determine common trisomy abnormalities (T21, T13, T18) along with detection of 16 different chromosomal abnormalities in fetus chromosomes from cfDNA. The test was performed by using 5-10 ml peripheral blood samples from pregnant women. Wet-lab steps of the test are formed from plasma separation, DNA isolation, library preparation, and sequencing. This part of the test was performed in Turkey while sequencing data results were analyzed and reported by BGI (Shenzhen, China). Between 2013-2018 (until April), Nifty Test was studied on approximately 15.500 pregnant women samples aged 17-57 years in Turkey. 15.250 of the Nifty Test results were negative. High risk was detected in approximately 350 patients and suggested to be directed to invasive prenatal diagnosis, thus confirming the Nifty test results. In addition to detection of fetal aneuploidies by Nifty test, maternal cancer was detected in two of the patients. The studies and research for these two cases continue in communication with BGI and patients' physicians. As a result of this study, valuable statistical data were obtained based on patients profiles and it has been indicated that preferability of NIFTY test has been increasing according to other biochemical-conventional tests and competing NIPD tests in Turkey and near countries because of its high accuracy, sensitivity and specificity ratio.

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IMPORTANCE OF INFLUENZA VACCINATION IN CHILDREN FOR DISEASE CONTROL IN PAKISTAN 2009-2017

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Background: Influenza is a common illness of childhood and the burden of disease is higher among pre-school children with attack rates up to 20%-30%. Limited information about burden of influenza in children is available in Pakistan, therefore the present study was designed to estimate incidence rate of influenza in both outpatient and hospitalize children with underlying risk factors and clinical features.

Methods: During 2009-17, throat and nasopharyngeal swabs collected from children ≤12 years of age were processed for detection of influenza viruses by Real-Time PCR protocol of CDC. SPSS 22.0 was used for statistical analysis presented in this study.

Results: A total of 13,081 influenza-associated outpatients and hospitalized children were enrolled during 2009 to 2017. Influenza virus detection rate was 72% (9418) in the outpatients and 28% (3663) in the hospitalized patients. Of these 54% children (7064) were aged ≥12 years. Influenza viruses were detected in 17% (1216) children, of whom 845 (69%) were positive for Inf A and 371 (31%) as Inf B viruses respectively. The detection of influenza B strain was higher in both groups of children, 29% and 35% respectively following the A/H1N1pdm09 strain. The high frequency of influenza viruses were reported in year 2011 and A/H1N1pdm09 was dominant strain as well. The mean ± SD of children age ≥6 and ≥12 years was 2.4±1.7 and 9.2±2.4 respectively. The gender ratio amongst both groups was equal. Cross sectional analysis showed that fever 1120 (96%), cough 1204 (99%) and sore throat 1021 (84%) was significant factors for influenza infections (p=0.001), however no significant differences were observed with respect to respiratory, liver and metabolic diseases between these groups. Influenza vaccination status was record only in 1% cases. The incidence rate of influenza outpatient and hospitalized was 27/1000 and 13/1000 persons years respectively. The average annual rate of influenza was higher (180 cases/1000) among ≥6 years of age than ≥12 years old children (145 cases/1000).

Conclusions: Higher influenza incidence rate was observed particularly among six year old children, which might contribute to increase in the hospitalization. We believe that vaccination of children will reduce the hospitalization rate and socio-economic burden of influenza in the community if included in national extended program of immunization. Furthermore improvements in existing influenza virus surveillance system are required to estimate the actual burden of influenza in children.

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TRANSCRIPTOME SIGNATURE OF CIRCULATING PBMCS TO UNDERSTAND MECHANISM OF HIGH ALTITUDE ADAPTATION IN NATIVE CATTLE ADAPTED TO TRANS HIMALAYAN REGION OF LADAKH

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Ladakh "land of high passes" is located between the Kunlun mountain range in the north and the main Great Himalayas to the south in state of Jammu and Kashmir. This cold-arid desert located at over 3000 m above mean sea level is characterized by a very harsh climate with extreme temperature (-40 oC in winter and 35 oC in summer): low humidity, precipitation, and reduced oxygen level (nearly 60-70% of the oxygen concentration at sea level). The local cattle from Ladakh region are known as "Ladakhi cattle", a unique germplasm having excellent adaptation potential to high altitude hypobaric stress. In the present study, an effort was made to evaluate the transcriptional signature of circulating PBMCs in local Ladakhi cattle from Ladakh region and Sahiwal cattle from tropical condition using Agilent 44K microarray chip. The top up-regulated genes in Ladakhi cows were *INHBC, ITPRI, HECA, ABI3, GPR171, and HIF-1α* involved in hypoxia and stress response. In Sahiwal cows, the top up-regulated genes *eEF1A1, GRO1, CXCL2, DEFB3 and BOLA-DQA3* were associated with immune function and inflammatory response indicating their strong immune potential to combat the pathogens prevalent in the tropical conditions. The molecular pathways highly impacted were MAPK signalling, ETC, apoptosis, TLR signalling and NF- kB signalling pathway indicating signatures of adaptive evolution of these two cattle types in response to diverse environments. Further, qPCR analysis revealed increased expression of some high altitude related genes viz., *HIF-1, EPAS-1, VEGFA, NOS2, ECE-1 and GLUT-1* in PBMCs of high altitude cattle signifying their importance as an essential component of high altitude adaptation. These genes are supposed to be crucial in maintaining cellular homeostasis in Ladakhi cattle. Based on data generated in the present study, native cattle of Ladakh region was found to be genetically distinct from native cattle adapted to tropical region of India.

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CANCER AND TUMOR IMMUNOLOGY

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Substantiation of the potential relevance to cancer of the list of 115 proteins identified as being targeted solely by tumor selective compounds was performed by two independent perspectives. On the one hand, all 115 proteins were scored on the basis of recently derived oncogene probabilities (Oncoscores) and checked for currently available experimental data on the up- and down-regulation in colon cancer samples. On the other hand, we used all drug target interaction data available from public resources to rank order all drugs based on the number of known targets within the list of 115 proteins and check for whether cancer was the primary indication among the top ranked. The results provide ample support for the use of the DIVISS approach to identifying cancer-relevant targets. A cancer marker or tumor marker is a biomarker found in blood, pee, or body tissues that can be raised by the proximity of at least one sorts of development. There are different tumor markers, each illustrative of a particular alignment. In addition to their use in cancer medicine, biomarkers are often used throughout the cancer drug discovery process. For instance, in the 1960s, researchers discovered the majority of patients with chronic myelogenous leukemia possessed a particular genetic abnormality.

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LOWERING THE LEVEL OF HOMOCYSTEINE BY AGNIKARMA FOLLOWED BY BREATHING EXERCISES

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Background: Strokes nothing but brain attacks which is associated to blocked blood supply to the brain. There are two types of strokes one is short, and another is long with some dangerous effects. Throughout the stroke, the brain does not get sufficient oxygen, effect brain cells to die which is covered by that area of the brain such as memory and muscle control are lost. So many times, seen that increasing the homocysteine levels is one of the cause of the to damage the brain by lining of the arteries. It nothing but the blood clot which blocks blood vessel. A clot inside your blood vessel is nothing but thrombus. A thrombus which may travel in the bloodstream and get stuck in your brain that is associated to stroke.

Materials & Methods: Here, in this study we took the patients with high homocysteine level and patients experience with minor Strokes and TIA (Transient ischemic attack).

Result: We followed the patients with Agnikarma and breathing exercises. Here, Agnikarma works on does depend manner as a pro-oxidant. Breathing exercises provides more oxygen to the brain cell. We experience homocysteine level of blood lower within two months and increase HDL lower LDL of the patients.

Conclusion: Agnikarma improve the immune system of the body which nourish the cell to perform work properly. It also health to lower the homocysteine level.

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A UNIFIED PATHOPHYSIOLOGIC CONSTRUCT OF DIABETES AND ITS COMPLICATIONS, INCLUDING MALIGNANCIES, IN THE CONTEXT OF THE B-CELL CLASSIFICATION OF DIABETES: OPPORTUNITIES FOR DRUG DESIGN

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We have previously presented a proposal for a new, beta-cell centric classification of diabetes based on a consilience of genetic, metabolic, and clinical research that have accrued since the current classification was instituted. It recognizes that the beta-cell is THE core defect in all patients with diabetes. Differences in the genetics, insulin resistance, environment and inflammation/immune characteristics of the damage to the beta-cell in each individual will determine the phenotypic presentation of hyperglycemia and allow for a patient-centric, precision-medicine therapeutic approach , part of which we labeled 'the Egregious Eleven' We now recognize the same pathophysiologic mechanisms that account for damage to the beta-cells that govern the susceptibility of the cells involved in the complications of diabetes to damage by the now well-defined abnormal metabolic environment that typifies beta-cell dysfunction. This abnormal metabolic environment is typified by oxidative stress which alters metabolic pathways a la Brownlee's Hypothesis model, alterations in gene expression, epigenetics, and inflammation. This unified pathophysiologic approach to the complications of diabetes in the context of the B-cell-classification of diabetes allows us to understand the varied risk of developing complications of diabetes with similar levels of glycemic control, how non-glycemic effects of some medications for diabetes result in marked complication risk modification and the value treating co-morbidities of diabetes in effecting complication risk. We also believe that the same pathophysiologic mechanism that account for damage to the beta-cells and govern the susceptibility of the cells involved in the complications of diabetes are likely to explain the association of cancer to diabetes and obesity, and explains why diabetic medications may affect cancer risk and therapy. Opportunities for drug design and development abound.

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