5TH ANNUAL AUB BIOMEDICAL RESEARCH DAY

BIOMEDICAL SCIENCES AND ENGINEERING:

SYNERGETIC INNOVATIONS

Saturday, February 21, 2015

Issam Fares Lecture Hall

9:00 am - 2:00 pm

Organizing Committee

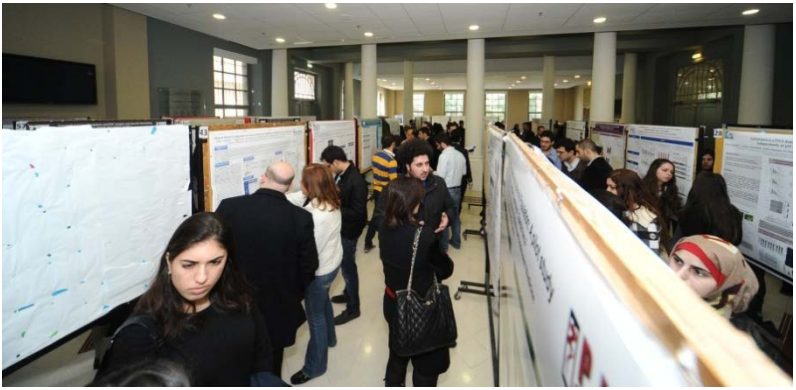
Chairperson

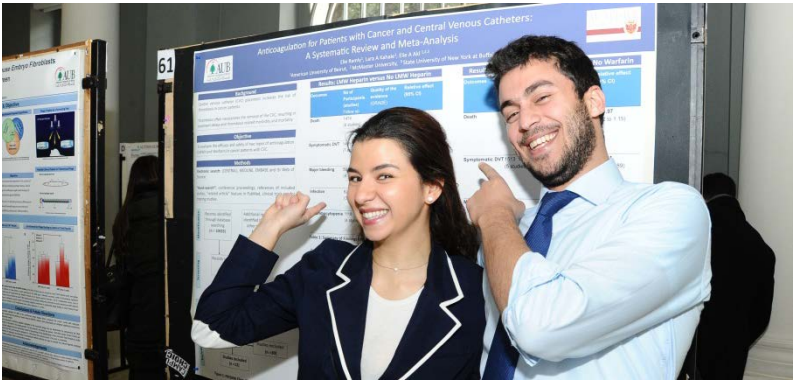
- Ayad Jaffa, Assistant Dean of Graduate Studies & Interdisciplinary Programs, FM, Department of Biochemistry and Molecular Genetics

Members

- Hala Muhtasib, FAS, Department of Biology
- Kamal Bouhadir, FAS, Department of Chemistry
- Marwan Sabban, FM, Department of Anatomy, Cell Biology and Physiological Sciences
- Nahla Hwalla, FAFS, Dean
- Zaher Dawy, FEA, Department of Electrical and Computer Engineering
- Md Anwarul Hasan, FEA, Department of Mechanical Engineering
- Nadine Darwiche, FM, Department of Biochemistry and Molecular Genetics
- Assaad Eid, FM, Department of Anatomy, Cell Biology and Physiological Sciences
- Soha Yazbek, FHS, Medical Laboratory Sciences Program
- Nada Melhem, FHS, Medical Laboratory Sciences Program
- Miran Salameh Jaffa, FHS, Department of Epidemiology and Population Health
- Yumna Maalouf, FM, Medical Dean's Office
- Ali Nabbouh, FM, Graduate Student Affairs


and Aerodigestive Cancers
EMORY
WINSHIP
CANCER




Anticoagulation for Patients with Cancer and Central Venous Catheters:
A Systematic Review and Meta-Analysis
61

Student Awardees of the 2014 AUB Biomedical Research Day

- **Raed Hmadi**, FM: *Molecular Mechanism of Action of the Synthetic Retinoid ST1926 in Imatinib-Sensitive and -Resistant Chronic Myeloid Leukemia*

- **Oula Dagher**, FM: *Bradykinin and Thromboxane Receptors Positively Cooperate on the ERK1/2 Pathway in VSMCs and Co-internalize in Response to Bradykinin*

- **Elie Ramly**, FM: *Anticoagulation for patients with cancer and central venous catheters: a systematic review and meta-analysis*

- **Carine Habchi**, FEA: *A simplified mathematical model for predicting cross contamination in office building air conditioned by displacement ventilation*

2014 Farouk Jabre Award Recipients

- **Dr. Ghanem Oweis**, FEA and **Dr. Asad Zeidan**, FM: *Validation and Optimization of a Novel Aortic Arch Model for Investigation of Shear Stress Role in Atherosclerosis*
- **Dr. Marwan Refaat**, FM and **Dr. Bassem Youssef**, FM: *Noninvasive Linear Accelerator-Based Stereotactic Radiotherapy for Atrioventricular Node Ablation*
- **Dr. Fadi Zaraket**, FEA and **Dr. Ghassan Hamadeh**, FM: *"Automated Symptom and Diagnosis Detection from Clinical Notes*

List of jury members for the 4th Annual Biomedical Research Day

Faculty of Medicine

1. Fuad Ziyadeh
2. Georges Daoud
3. Wassim Abou Kheir
4. Chantal Farra
5. Hiba El-Hajj
6. Marwan Refaat
7. Deborah Mukherjee

Faculty of Arts and Sciences

1. Youssef Mouneimne
2. Noel Ghanem
3. Diagambara Patra
4. Pierre Karam

Faculty of Agricultural and Food Sciences

1. Ammar Olabi
2. Omar Obeid
3. Lara Nasreddin

Faculty of Engineering and Architecture

1. Fadi Zaraket

5TH ANNUAL AUB BIOMEDICAL RESEARCH DAY

BIOMEDICAL SCIENCES AND ENGINEERING: SYNERGETIC INNOVATIONS

Schedule of events

9:00 am - 9:30 am	Welcome note **Dr. Ayad Jaffa**, Assistant Dean for Graduate Studies and Interdisciplinary Programs 2015 Farouk Jabre Award Presentation *Dean Sayegh, Trustee Jabre*
9:30 am - 10:15 am	Key note speaker to be introduced by Dr. Samia Khoury, Associate Dean for Clinical and Translational Research **Dr. Georges El Fakhri**, Director, MGH PET Core; Co-Director, Division of Nuclear Medicine & Molecular Imaging (Research); Director, Center for Advanced Medical Imaging Sciences; Professor of Radiology, Harvard Medical School ***Title:*** *Imaging Molecular Patho-Physiology: Lessons learned in PET-MRI*
10:15 am – 11:00 am	Key note speaker to be introduced by Dr. Anwarul

	Hasan, Assistant Professor of Mechanical Engineering **Dr. Muhammad Zaman**, Associate Chair for Undergraduate Studies and Associate Professor, Biomedical Engineering, Boston University ***Title:*** *Engineering healthy solutions for the bottom billion*
11:00 am – 2:00 pm	Poster viewing followed by lunch, award presentation for the top 5 posters and closing

Objectives

- serve as a platform to bring together the research community of different AUB faculties and to showcase the biomedical research performed at AUB
- provide an intellectual environment for scientific exchange among the various researchers at AUB
- provide a platform for students, postdoctoral fellows and junior investigators to present their scientific findings and to foster collaboration within the AUB family of investigators

Eligibility

- Students
- Trainees
- Residents
- Research Assistants
- Fellows
- Post docs

Keynote Speaker

Dr. Georges El Fakhri, PhD

Dr. Georges El Fakhri is the youngest Professor of Radiology at Harvard Medical School and the founding Director of the Center for Advanced Medical Imaging Sciences (CAMIS) at Massachusetts General Hospital (MGH), Co-Director of the Division of Nuclear Medicine and Molecular Imaging.

He holds a MS in Engineering *magna cum laude* from the Ecole Centrale (France), a MS in Electrical & Computer Engineering from the University of Texas Austin (USA), a MS and PhD in Biomedical Engineering *summa cum laude* as well as a Postgraduate Medical Degree from the University of Paris XI (France).

Dr. El Fakhri is an internationally recognized expert in quantitative medical imaging including SPECT-CT, PET-CT, and PET-MR. He has pioneered novel approaches to compensate for many of the factors affecting quantitative SPECT and PET, develop accurate pharmacokinetic modeling of physiological factors and objectively assess the achieved improvement in diagnostic accuracy, specifically in oncologic, neurologic and cardiac imaging (e.g. flow, perfusion, neurotransmission, metabolism). The methods developed have proven efficient and accurate, and many have been translated to the clinical setting. His research has resulted in more than 130 peer-

reviewed articles, and proceedings, as well as many book chapters.

Keynote Speaker

Muhammad H. Zaman, PhD

Muhammad H. Zaman is Howard Hughes Medical Institute Professor of Biomedical Engineering and International Health at Boston University. He received his PhD from the University of Chicago, where he was Burroughs-Wellcome Interdisciplinary Research Fellow. He then moved to MIT where he worked in the Department of Bioengineering as Herman and Margaret Sokol Foundation Post-Doctoral Fellow in Cancer Research.

Prof. Zaman's current research is focused on two main areas: 1) developing novel tools to understand *in vivo* tumor progression and 2) developing robust technologies for high-value healthcare problems in the developing world. Technologies developed by Prof. Zaman are in various stages of implementation in several countries. In 2013, Scientific American named a technology from Zaman lab, PharmaChk, among the 10 technologies that will change the world.

He has won numerous awards for his research and teaching from IEEE, FEBS, American Society for Engineering Education, USAID, The US National Academy of Sciences, The University of Texas System, Boston University and other national and international

organizations. Most recently, he was named Howard Hughes Professor by the Howard Hughes Medical Institute and was elected as a Fellow of American Institute of Biological and Medical Engineering. His current research is supported by NIH, NSF, USAID, UNECA, CIMIT, Saving Lives at Birth Consortium, and a number of other private foundations.

In addition to his research, Prof. Zaman is actively engaged in bringing quality engineering education in several developing nations. He is currently involved in setting up biomedical engineering departments at universities in Kenya, Zambia, Uganda and Ethiopia. He is co-Director of the UN Africa Biomedical Initiative. He is a regular contributor on issues of STEM education and global health for the Project Syndicate, Huffington Post and writes a weekly column on higher education for leading Pakistan daily, Express Tribune (part of International New York Times).

ABSTRACTS

Abstract # 1

On the detection and prediction of epileptic seizure in noisy environments

Mohammad Hussein Nasrallah (mfn12@mail.aub.edu), Department of Electrical and Computer Engineering, FEA
Prof. Zaher Dawy (zaher.dawy@aub.edu.lb) and Dr. Ahmad El-Hajj (ae37@aub.edu.lb)

Descriptive Statement: This research aims in the long term at developing novel algorithmic techniques for the real-time detection and predication of epileptic seizures based on EEG signals captured regularly from patients while performing their daily activities.

Funding Source: NeuroPro AG (www.neuropro.ch)

Introduction: Epilepsy is a human brain disorder in which cells of the brain's nervous system start malfunctioning. As a result, it may generate the abnormal electrical signals that cause an instant defect of the human brain, leading to a change or complete loss of awareness. Statistics have shown that around 1% of the world's population is affected by this disease. This high figure in addition to the number of annual casualties from sudden unexpected death in epilepsy (SUDEP) have triggered a significant research activity into smart and efficient epilepsy prediction/detection algorithms. Electroencephalography (EEG) is a typical method for acquiring the electrical activity of the brain. It provides a measurement of the electric activity in the brain, translating the chemical currents into voltage recordings. The monitoring of EEG signals is central to the understanding of many brain disorders that may affect a human being. One major use of EEG signal is in the diagnosis of brain diseases, in particular, epilepsy. In this research, we aim at developing efficient seizure detection/prediction algorithms.

Methods: The research relies on multidimensional feature extraction from the recorded EEG signals (time, frequency, rhythmicity, morphology, spatial and temporal correlation, etc.), sophisticated machine learning techniques, and advanced signal processing techniques for data preprocessing and analysis.

Results: Preliminary results demonstrate the merits of the employed multidimensional techniques in the efficient and accurate detection of epileptic seizures. EEG data from professional databases are used in the testing, verification, and analysis.

Conclusion: This ongoing research will provide, upon its completion, a comprehensive framework for the detection and prediction of epileptic seizures, towards safer and better convenience of the patients during regular daily activities due to the continuous monitoring and real-time processing of the brain activity though EEG recordings.

Abstract # 2

Distribution of Area Fraction of Pores in Cortical Bone's Pericortical and Intracortical Regions

I. S. Hage [1], R. F. Hamade [2*]

1: American University of Beirut (AUB), Department of Mechanical Engineering, PhD candidate, ish07@aub.edu.lb.

2*: American University of Beirut (AUB), Department of Mechanical Engineering, Professor, rh13@aub.edu.lb.

Keywords: Cortical porosity, Micro-porosities, Image segmentation, Area fraction, Radius.

Funding sources: Lebanese National Council for Scientific Research (LNCSR/AUB PhD Awards Program). University Research Board (URB) of the American University of Beirut (Doctoral Fellowship).

Abstract: In cortical bone, pores are micro-porosities (i.e., lacunae, clusters of canaliculi, Haversian canals, and resorption cavities). These pores of varying sizes are present with different area fraction, AF %, distributions in pericortical and intracortical regions.

Aims: This pilot study aims to characterize the area fraction (*AF,%*) of said pores as function of distance from the (bovine) bone's geometric center.

Methods: Optical slides at 20X are taken from 2 cortical bone biopsies (named bone 1 and bone 2) and cut at mid-diaphysis femur from 2 different (about 2 year-old) bovine cows. Slides are collected from locations along a straight line that crosses posterior (pericortical) and anterior (intracortical) regions. The area of each of these biopsies is about 2.5mm x 3mm located near the outer cortex of the bone. Computer-automated segmentation using computer vision techniques (functions in MATLAB) are used to locate and identify all pores present in the slides. Values of area fraction (%) are then automatically calculated in primary and secondary osteons for both regions. Plots of AF% are produced vs. distance from bonce center, *R*.

Results: Observations suggest that area fractions (%) of all pores to significantly decrease linearly with *R* (statistically significant $p < 0.01$) in the anterior region where osteonal growth is expected to have continued to develop. However, in the posterior region where osteonal growth appears to have matured, area fraction (%) values seem to have reached a steady state plateau resulting in a fairly flat behavior versus *R*. All observations for biopsies collected from bone 1 are equally applicable for biopsies collected and bone 2.

Conclusion: Variations in area fraction (%) values of all cortical porosities present are plotted against distance from the (bovine) bone's geometric center. Quantitative trends are reported.

Abstract # 3

Semi-Automatic Annotator for Medical NLP Applications

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3 AUB Medical Center Professor

Abstract

Proteins and their relation to diseases became important in recent medical research. In particular, when the main proteins that contribute to a disease are known, one can simply treat the disease by targeting the proteins.

In the field of protein to disease relations, a substantial number of articles that discuss specific diseases like Alzheimer's, Stroke, and backbone injury appeared lately. These articles are stored in online databases like PubMed and SCOPUS. The databases allow using a special API for the retrieval of the data in a predefined format such as EXtensible Markup Language (XML) and comma-separated values (CSV).

Each article presents evidence of the role of one or more proteins in activating a pathway of one or more diseases. To be able to treat a disease, medical researchers are interested in looking at the comprehensive picture In particular, each relevant article must be annotated with the diseases it discusses and the proteins related to the diseases in the article.

It is difficult to complete this task using the existing annotators because: they need a professional software developer to deploy and run them, they could not handle large file sizes such as 100 MB,They cannot parse XML and CSV files, they are single document based, and They do not generate needed statistics.

We propose to build a Semi-Automatic Annotator for Medical NLP Applications (SAMNA), which allows the user to load a large amount of data including abstracts and titles of published articles. We hypothesize that these articles discuss a specific disease. SAMNA shows the articles components with color sensitive annotation, that highlight occurrence of terms of interest in the abstracts and titles, based on a database of terms provided by the specialist, allows the specialist to go between extracted articles one by one, automatically annotates the provided articles, and applies annotation changes across all documents, enables the specialist to add or remove an annotation, enables the specialist to add or remove a label, takes rules from the specialist that specify features of the target annotations, and saves a result file holding the important statistics of annotations occurrences and frequencies for each label.

In this way SAMNA provides a full picture of the label relations to a specific disease, and allow the medical researcher to discover potential treatment targets.

We evaluated SAMNA and used it with stroke disease, where we used SAMNA to construct, annotate and curate a brain ischemia meta-proteome with a corresponding interactome. The curation of the interactome allowed us to analyze the network of proteins and their interactions and to discover a rich-club organization in the interactome. Our findings show that complex network analysis of disease related protein interaction networks may foster a better understanding of

pathogenic mechanisms and provide cost- effective and mechanism-based discovery of candidate therapeutics.

Keywords: natural language processing (NLP), annotator, protein

Abstract # 4

Determining Suture Material Properties Using Computer Vision

Author: Jean Michel Fahmi
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Keywords: suture, design, computer vision, automated grid method

Abstract:

Suture design and implementation is growing more complex day by day to the point where modeling and simulation have become essential. Following previous work on sutures, which should be able to support high value passive and active immobilization forces and guarantee minimum adhesion between the tendon and its surroundings, it was determined that a barbed monofilament suture having a depth of cut of 0.18 mm, an angle of cut of 150^{o}, and major axis to minor axis ratio of 3 was most ideal for the barb itself whereas having a depth of cut of 0.18 mm, an angle of cut of 160^{o}, and major axis to minor axis ratio of 4 was most ideal for the whole suture. Further simulation is required to determine the strength and breaking force for the different material used in sutures. However, most modeling software do not have an enormous database covering all materials in use today, especially those used in suture manufacturing.

To obtain the material properties, tensile and creep tests are performed using a force gauge to measure the force and a high definition camera to measure displacement using the automated grid method. A sample of the material is mounted on the force gauge and the camera is placed 1 meter away. At the beginning of the test, a weight is mounted on the specimen, and while the experiment is running, readings from the force gauge and a frame from the camera are taken simultaneously. After the course of the experiment, the readings and the camera frames enter post processing to get the necessary material properties that the modeling software requires for simulation.

Testing for common material, the results obtained were comparable to values produced from other sources. While this technique is not as precise as others, but it is versatile and can accommodate a wide range of material without much hassle.

Funding source: Lebanese National Council for Scientific Research

Abstract # 5

Efficient and Accurate Algorithm for Cleaved Fragments Prediction (CFPA) in Protein Sequences Dataset based on Consensus and its Variants

Atlal El-Assaada, Zaher Dawya, Georges Nemerb, Hazem Hajja, and Firas Kobeissib
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Department of Biochemistry and Molecular Genetics, American University of Beirut, Riad El Solh, Beirut, Lebanon
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Abstract: Congenital heart defects (CHD) are the most frequent form of major birth defects in newborns and affect close to 1% of newborn babies (8 per 1,000). Signs and symptoms are related to the type and severity of the heart defect. Some children have no signs while others may exhibit shortness of breath, cyanosis, syncope, heart murmur, under-developing of limbs and muscles, poor feeding or growth, and respiratory infections. In this work, we focus on atrial septal defects (ASD) and ventricular septal defects (VSD), which are the most common types of heart defects. Causes of CHD can be either environmental or genetical or a combination of both. Our target is the genetical prospect considering, in particular, the metalloprotease Tll1 gene which has a major role in heart septal development. Upon activation, Tll1 truncates specific extracellular substrate proteins in the heart, and specifically the septum, representing breakdown products (BDPs). These BDPs can leak into the blood, and upon identification, can indicate the absence of heart malfunction, and thus may represent putative markers of such malfunction. This study addresses an up-to-date inter-disciplinary problem from the field of computational biology. It aims at developing an efficient and accurate algorithm, based on the state state-of-the-art sequence matching and alignment algorithms, that can predict the consensus sequence and its variants in a large set of experimentally detected protein sequences, representing substrates of the Tll1 metalloprotease. After detection, it generates all the potential cleaved fragments, representing biomarkers in human serum. The proposed algorithm is efficient in terms of execution time and storage with linear complexity in the number of protein sequences, length of consensus sequence, and length of each protein sequence, respectively. It is flexible as well to handle the different orientations that the consensus and protein sequence can take before cleaving. The developed algorithm is tested against the Mouse Genome and will be validated against the Human Genome, with known BDPs. Ultimately, this knowledge will feed on the long term into the development of a novel tool for researchers to be able to detect/predict cardiovascular septal defects that will guide in the diagnosis and treatment of related diseases.

Keywords: Tll1 gene, Metalloprotease, Congenital Heart Disease (CHD), Heart Septation, Degradomics, Biomarker, Breakdown Product (BDP), Dynamic Programming

Corresponding Author: Atlal El-Assaad

Funding Source: Farouk Jabre Award

Abstract # 6

A New Fast Non Invasive Method to Directly Estimate Ischemia in Human Diseased Coronary Arteries

Iyad Fayssal(*), Fadl Moukalled(a), Samir Alam(b), Robert Habib(b) and Hussain Ismaeel(c)
American University of Beirut
(*)PhD Candidate at FEA, Mechanical Engineering Department
(a)Professor at FEA, Mechanical Engineering Department
(b)Professor at FM, Internal Medicine
(c)Assistant Professor at FM, Internal Medicine
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Abstract

Introduction: Coronary Artery Disease (CAD) is considered among the leading causes of human death worldwide. CAD is classically diagnosed through several invasive techniques such as the coronary catheterization (Cath) method which relies on images of coronary arteries to estimate the geometric % stenosis of a lesion. However, this geometric based method suffers from a certain degree of operator subjectivity in estimating the % blockage of diseased arteries and its physiologic importance, thus misestimating the true functional myocardial perfusion effect of a certain lesion and hence the need for intervention. Such difficulties in characterizing the possibility for myocardial ischemia relying only on 2-D cath images suggested the need to look for additional physiological parameters that will aid in detecting the hemodynamic significance of CAD. Fractional Flow Reserve (FFR), an indicatory index developed by Pijls and De Bruyne, is considered among the physiological parameters used to determine the likelihood that the stenosis impedes oxygen delivery to the heart muscle (ischemia). However, measuring FFR requires invasive intervention through a costly pressure-sensor guide-wire in the diseased artery and administration of a vasodilator (Anticoagulation intravenous (IV) heparin (usually 40 U/kg) and intracoronary (IC) nitroglycerin (100–200 μg bolus) are administered) for inducing hyperemia. To overcome the demands of invasive FFR, comprehensive predictive techniques involving numerical methods have recently gained attention. In specific, the use of Computational Fluid Dynamics (CFD) methodologies combined with patient specific data allows computing in vivo FFR for diagnostic purposes. The ultimate goal of this study is to develop a direct method to assess the level of ischemia rather than relying on traditional FFR and translating it into future clinical practice.

Method: The developed non-invasive method integrates many advantages that were lacking in previous studies which are: (1) the ability to assess whether a stenosis causes ischemia or not through modeling only the diseased artery without including the other connected arterial segments (arterial tree) provided with proper modeling and coupling of boundary conditions at the truncated sections of the domain; (2) directly estimating the actual loss in blood flow rate in a stenosed arterial segment as opposed to the indirect method of measuring/predicting fractional flow reserve; and (3) reducing the solution computational run time via a proposed steady state analysis which compromises between accuracy and complexity.

Results: Preliminary results of the developed method were compared with the traditional FFR method on constructed idealized geometric models of arterial segments. A healthy (hypothetical) artery and three idealized stenosed arterial

segments with plaque profiles of increasing severity were simulated under rest and hyperemic conditions. An excellent agreement was obtained between the ratios $Q_{stenosed}/Q_{healthy}$ ($Q_{stenosed}/Q_{healthy}$ = ischemia level) and $P_{distal}/P_{proximal}$. The computational run time of the new model was reduced by 9 times as compared with a full transient simulation, thus adding a selling feature to the developed method towards translating it into practical clinical applications.

Conclusion : The end product of the developed method will provide a low computational cost assessment tool to estimate stenosis hemodynamic severity by directly predicting the level of ischemia in a diseased coronary artery; thus, replacing the traditional FFR method which requires two pressure values at specified locations (pre-and post-stenotic). The tests done by the method on idealized arterial models have proven viability and were promising towards translating the developed method into clinical, research and educational applications.

Keywords: Ischemia, Coronary arteries, Non-Invasive

Funding Source: FEA

Abstract # 7

Virtual Patient (VP2020)

Khodor Kshoury (kkshoury@gmail.com), Petra Chidiac (pmchid00@gmail.com),
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Advisor: Dr. Mariette Awad, ECE Department (ma162@aub.edu.lb)
Collaborator: Dr. Joumana Antoun, Faculty of Medicine

Keywords: Virtual Patient- Objective Structured Clinical Examination - Artificial Intelligence- Automated Assessment

Descriptive Statement: The aim of this project is to design a virtual patient- VP2020 that interacts with a medical student in order to assess his/her clinical performance during the Objective Structured Clinical Examination (OSCE).

The essential drive of our project is to address the problem faced by many medical schools and acutely by the American University of Beirut Medical Center (AUBMC) when executing the Objective Structured Clinical Examinations (OSCEs) in the medical student training course. This examination is traditionally carried out by employing a standardized patient, typically a hired actor trained to play the role of a patient, for the sake of examining medical students' abilities to engage in clinical interviewing skills with patients. Standardized patients are difficult to find, costly and time-consuming to train, and can prove to be unreliable at times. Our project virtual patient "VP2020" aims to remove these difficulties that arise when attempting to implement OSCE exams by replacing standardized patients with virtual patients. We seek to do so by building a web-based application that delivers a virtual patient, an automated assessment tool, and a Station Authoring tool, all of which contain highly user-friendly interfaces.

A lot of research has been done on virtual patients. While old virtual patients used simple narrative and multiple choice questions, newer virtual patients utilize advanced AI logic and graphics technologies. All the VPs we found in literature tested the performance of virtual systems in evaluation of communication skills and diagnosis, or teaching, or both. Our project's primary objective is to replace the OSCE by a complete evaluation tool. None of the reviewed VPs successfully accomplish that. And most of them required manual evaluation.

For our VP2020, we have identified the primary subsystems of the project as: the Core AI Engine, the Graphics Engine, the Station Authoring tool, and the assessment module. We started working on the AI Engine and the graphics in parallel, leaving the other modules to be implemented during the Spring semester. We have designed a program using the Stanford NLP tagger and WordNet to form Bag-of-Words for each sentence which will be inputted to the classifier that will categorize the sentence to one of the following classes: Symptomatic (location, duration, descriptive), influential factors, and social or side-talks.

As for the graphics, we looked at tools that will help us generate phonemes and add speech recognition. We will be testing the animation in the spring taking into account that the medical evaluator creating the authoring station should be able to customize it. As previously mentioned, the application will be web-based due to better profile management, security, and better assessment processing and storing. So, we will be setting up the server and building the webpage as well. We will then move on to creating the database which contains the question and answer pairs and use them for testing.

Sponsor: ECE department

Abstract # 8

In-vitro investigation of the effect of applied force on the catheter ablation operation

Hussein Daoud1, Ghanem Oweis2, Marwan Refaat3
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2Associate professor-
Mechanical engineering department, 3Cardiologist and Assistant Professor of Medicine - AUB Faculty of medicine and medical center
Keywords (Cardiac arrhythmia, Atrial fibrillation, Catheter ablation, Steam-pop, Radiofrequency energy)

Introduction: Atrial fibrillation is a common type of cardiac arrhythmia which is caused by a faulty electrical pathway from sections of the heart. Catheter ablation is one of the procedures that are used to treat the atrial fibrillation. During catheter ablation, a series of catheters are put into a blood vessel and they are advanced towards the heart. Radiofrequency energy is then applied through the catheters to ablate the tissues that are in contact with their tips.

In this study we perform in-vitro simulation to the catheter ablation operation in the aim of measuring the effect of the applied force on the temperature profile during the process, the size of the resulting burns, and the chance of steam-pops to appear. These types of data will help the cardiologists to choose the appropriate setup during the actual operations.

Method: Our main concern is to put the sheep heart tissue of our experiment in an environment that is as close as possible to the environment of the heart tissue inside the human body. To do that, the heart peace is immersed in a narrow water tank (25 cm length*3 cm width*8 cm height), a water pump is used to circulate water through the system, and the water is kept at 37 C using heater.

To measure the applied force on the catheter, the water tank is put on the weight scale. When the catheter is just touching the tissue, the scale reads the tank weight. Any additional push on the catheter is recorded as extra weight. In addition, a thermocouple is tapped with the catheter tip to record the temperature profile for the tissue.
Multiple forces (5, 10, 15, 20, 30, & 40 g) are applied on the catheter. Burn's surface diameter, cross section depth, and temperature profile are measured in each case.

Results & conclusion: Higher applied force results in increasing of the temperature-increase rate, the burn size, and the chance of steam-pop to occur. However, we are performing more experiments to provide quantitative results that are statistically valid.

Abstract # 9

Prediction of the aerosol penetration through protective clothing and deposition on the human skin

Nagham Ismail, Nesreen Ghaddar*, Kamel Ghali

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Keywords: Aerosol particles – Protective clothing – Penetration – Deposition – Ventilation model

Descriptive statement: The study addresses the penetration and the deposition of harmful aerosol particles through air-permeable protective clothing used to provide the human safety in toxic environments under wind conditions.

Introduction: Aerosols production is increasing nowadays especially from fossil fuel combustion, biomass burning, automobiles, incinerators, smelters, and power plants. Therefore, there is an increasing concern of occupational exposure of aerosols particles in workplace. This necessitates the use of protective clothing garments to prevent workers from the exposure of harmful aerosols particles. The objective of this study is to develop a sound mathematical model that predicts the aerosol particles penetration through protective clothing and their rate of deposition on the skin.

Methods: A mathematical model is developed to predict the penetration of the aerosol particles through protective clothing and their deposition on the human skin in wind conditions. The penetration of aerosols particles through clothing is influenced by the filtering capacity. The deposition of the penetrated particles on the skin is related to the air that enters the gap between clothing and skin. These two phenomena are integrated with a ventilation model that predicts the air mass flow rate circulating in the gap between clothing and human body.

Results: The simulation results establish the penetration percentage of the aerosol particles, their rate of deposition at the human skin, and their concentration distribution. This model will be used to examine the risks associated with wearing different air permeable protective clothing in order to allow assessment of aerosol protection requirements.

Conclusion: Air permeable protective clothing provides a barrier to harmful aerosol particles and protects the human skin. Although the rate of penetrating is relatively small, additional attention is necessary to find new protective clothing that is light in weight but efficient in protection (nanotechnology).

Funding source: Lebanese National Research Council, Beirut, Lebanon.

Abstract # 10

Tissue Engineering of Heart Valve Leaflets: Experimental Studies and Development of a Model for Computer Simulation of Heart Valves Properties

Dr. Anwarul Hasan, Faculty of Engineering and Architecture, Department of Mechanical Engineering, American University of Beirut.

George Deeb (MS student), Faculty of Engineering and Architecture, Department of Mechanical Engineering, American University of Beirut.

Introduction: Diseased or damaged valve leaflets are amongst the major sources of illness and death globally. The currently preferred treatment of heart valve patients is to mend the damaged valves, but regarding acute cases, most heart valves require replacement since repairing the heart valve is not feasible. The survival of the patient, as well as implantation success rate, depends on the engineered heart valves to instantly ensure adequate mechanical support and function after surgery. Therefore, the engineered heart valves must demonstrate and preserve the biomechanical properties of native heart valves. In the current project, we aim to perform extensive experiments and computational studies on the structure and mechanical properties of heart valves and additionally develop a new computational model for simulation of heart valve leaflets.

Methods: Native bovine heart valve leaflets are excised from bovine hearts collected from local slaughter house in Beirut and cryopreserved as necessary. Tensile mechanical properties are studied using an Instron machine while the shear properties are studied using a parallel plate rheometer. The critical parameters such as, modulus of elasticity, ultimate tensile strength, maximum elongation and the stress-strain relationships as well as the oscillatory shear properties are thoroughly investigated. Furthermore, an FEM based tri-layer composite model is developed using finite element code ABAQUS. The developed model is to be validated against the rheological behavior obtained from rheological measurements.

Results: Results of the rheology experiments on bovine heart valves are available. However, more experimental data is required for larger specimens. The ABAQUS program is to be validated with the experimental results.

Conclusion: The obtained results provide significant insights into the correlations between the nano-microstructure and mechanics of the heart valves and their macroscale behaviors under different healthy and pathological conditions. The proper understanding of the correlations between nano-biomechanics and pathological conditions can pave the ways for generations of new therapeutics for various cardiovascular diseases.

Keywords: Bovine heart, damaged heart valve leaflets, rheology, mechanical properties, and computer-aided simulation.

Abstract # 11

Model-Based Investigation of Blood Gases Exchange in Pulmonary Capillaries Due to Various Modalities of Lung Ventilation Treatments

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Keywords: pulmonary modeling, gas exchange, hemoglobin

Descriptive statement: We present a model-based investigation of oxygen/carbon dioxide transport to/from blood in pulmonary capillaries for various lung ventilation treatment modalities.

Introduction: Lung diseases (IRDS, lung obstructive diseases, apnea, lung interstitial emphysema, etc.) typically lead to malfunctioning of the respiratory system that could cause death. The physical mechanisms behind clinical therapies that rely on various modalities of lung ventilation (CPAP, HFOV) are not yet fully understood. This is, in part, due to the limitations associated with in vivo measurements. The aim of this work is to present a physically based model to investigate the effect of treatment parameters on exchange of gases with the blood.

Methods: This model builds on a previous model to accurately predict exchanges of O_2 and CO_2 with blood in the pulmonary capillaries. The previous model couples lung mechanics, gas transport in the airways and the alveoli, blood oxygenation through diffusion of gases across the alveolar membrane. Unlike lumped models in literature, the model takes into account the dynamics of the spatial distribution of respiratory gases in the airways and within individual alveoli, which leads to more accurate prediction of gases transport. Gas exchange with the blood, which previously did not take into account the binding property of hemoglobin, is enhanced by solving the flow equation in the pulmonary capillaries coupled with the binding capacity of hemoglobin.

Results: Preliminary results show the frequency of the pressure signal perturbation that maximizes oxygenation is strongly dependent on airway resistance and lung tissue compliance. For sufficiently large value of the perturbation amplitude, gas exchange with the blood is insensitive to the frequency within a certain range.

Conclusion: The extended model not only allows investigation of the physical processes underlying various modalities of ventilation treatments, but also enables optimization of treatment parameters in patients suffering from lung diseases and ultimately advances clinical practice.

Funding source: none.

Abstract # 12

Highly elastic hydrogel composed of Acrylated Hyaluronan and Pluoronic for 3D cell culture and cardiovascular tissue engineering

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Keywords: Cardiovascular Tissue Engineering, Elastic Hydrogel, Hyaluronic Acid, Pluronic Acid, Photocrosslinking

Descriptive Statement: Biomaterial is synthesized from Hyaluronan and Pluronic to form a highly elastic and porous three-dimensional network structure suitable for cardiovascular tissue engineering.

Introduction

Background: Each year, 18 million deaths are reported worldwide due to cardiovascular diseases. Available treatment techniques such as myocardial repair via surgery are limited by the insufficiency of transplantable allograft tissue, in addition to the lack of proper implantable biological materials. Synthetic biomaterials currently available, although mechanically stable and biocompatible, induce nonspecific protein adsorption *in vivo*, while natural biomaterials lack the required mechanical properties for *in vivo* applications. Thus, a major challenge in tissue engineering is the ability to develop materials that first, mimic the native extracellular matrix (ECM) and second, possess suitable mechanical properties in comparison with the structural characteristics of native myocardium.

Aims: Composite Hydrogels are emerging to overcome the design constraints for creating 3D scaffolds for tissue engineering. In this work, we develop photocrosslinked HA-Plu hydrogels with optimum mechanical and biological properties and perform cell culture of endothelial and smooth muscle cells. Hyaluronan (HA) is naturally present in the body and enhances cell proliferation and differentiation. The addition of Pluronic (Plu) significantly improves the mechanical properties such as elasticity of the HA hydrogel.

Methods: The hydrogel is synthesized by cross-linking of vinyl group modified HA (AC-HA) and acrylate group end-modified Pluronic F127 (Plu-DA). Different concentrations of AC-HA and Plu-DA are used to determine the HA-Plu hydrogel with the best mechanical and biological properties. Mechanical properties are studied by applying compressive and tensile tests. Cardiac cells are seeded on the surface and encapsulated within the hydrogels and checked for cell adhesion and viability.

Results: Preliminary results reveal a promising biomaterial. HA-Plu hydrogels, when swelled, possess pores bigger than individual cells and allow for cell-cell and cell-matrix interactions. Cytotoxicity of individual components is investigated and show

high cell viability. Mechanical tests are anticipated to reveal soft tissue-like elastic modulus and ultimate tensile strength.

Conclusion: The composite HA-Plu hydrogels retain properties that deem them superior to other natural and synthetic elastic hydrogels. They do not incorporate toxic chemical crosslinking agents, have desirable mechanical characteristics, and their matrices show high porosity.

Funding Source: Dean of Engineering Startup Grant and University Research Board, AUB

Abstract # 13

Recognition of Medical Records Semantics Using Relational Analysis

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Keywords: electronic medical records, semantic recognition, relational analysis, data extraction

Descriptive Statement: An automated framework for the semantic recognition of text in clinical notes.

This work is funded by the FEA Dean Office at AUB and Farouk Jabre Research grant.

Abstract*:* Electronic medical records compose a rich resource for analyzing medical issues and building up related knowledge. Studying the semantics of electronic medical records (EMR) text is considered challenging specifically due to the free text style, numerous medical terms and acronyms, and frequent typos and misspellings in clinical notes. The Middle East region faces further challenges in its EMR analysis because of the diverse background of its physicians which implies a varying recording style and random utilization of non-English words. We present a framework for semantic detection of words in EMR, mainly in the Middle East, based on relational distribution with reference to an English medical text corpus, USMLE exams, as a similar content resource with structured text.

The objective of our current work is to create a local medical terminology thesaurus to facilitate communication between physicians and to build a first component towards advanced EMR analysis applications. Such applications include prediction of epidemic diseases, evidence-based management of public health resources and priorities, and guideline consistency checks in clinical practice, insurance guidelines, or other processes.

We implemented a framework that processes EMR and USMLE text and generates mappings between EMR and USMLE words of similar semantics. The framework is based on words co-occurrence frequencies and relational analysis. It also includes a preprocessing phase that guarantees de-identification of records, noise elimination due to irrelevant words or varied numeric data representations, and a treatment of typos and misspellings. We tested the framework on a collection of clinical notes obtained from local authorized medical institutions. We verified the results with a group of physicians who confirmed a significant percentage of detected relations.

Future work is expected to further improve accuracies in detected relations by employing annotation-related learning and additional processing steps. This includes optimization of the current framework, and application to additional EMR records. Further future work targets the extension of the framework to encompass the implementation of relevant EMR-related practical medical tools.

Finally, the current framework is very promising and up to our knowledge is the first of its kind in Lebanon to explore such new avenues towards resolving the EMR analysis challenges in the Middle East.

Abstract # 14

The effect of gelatin scaffold on 3D cell culture of vascular cells for blood vessel tissue engineering

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Keywords: GelMA hydrogel, tissue engineered blood vessel, 3D cell culture

Abstract

Introduction: Blood vessel grafts are in high demand for patients suffering from cardiovascular diseases. Synthetic polymer grafts have been developed to replaced damaged blood vessels; however, grafts with a diameter less than 6mm are prone to failure due to thrombosis. The challenge of tissue engineering of blood vessels is combining the scaffold, vascular cells and biomolecules that have characteristics of native blood vessels. Hydrogels are a promising scaffold which closely resembles the extracellular matrix of native tissues. Materials used as scaffolds should be biomimetic of native blood vessels and resistant to thrombosis, inflammation, and neointimal proliferation. Thus, it is necessary to understand the cell-material interaction. Different vascular cells carry out specific functions dependent on the substrate properties and over expression of proteins and genes can be observed.

Aim: This project is aimed to study the effect of GelMA hydrogel scaffold properties on 2D and 3D cell culture on rat vascular cells by studying protein and RNA expression.

Methods: GelMA hydrogel will be prepared in 3 different concentrations: 7%, 10% and 15% in 0.5% photoinitiator solution. The GelMA hydrogels will be prepared on TMSPMA coated glass slips and placed in well-plates in DMEM solution. Primary rat vascular cells (smooth muscle and endothelial cells) will be surface-seeded on the GelMA hydrogels separately for 2D cell culture and vascular smooth muscle cells will be embedded in the GelMA hydrogels for 3D cell culture. Protein and RNA expression will be compared between embedded cells and surface-seeded cells on hydrogels versus cells on the well-plates. Cells will be treated with bradykinin for 6 hours and 24 hours.

Results: Preliminary results of global protein expression on 10% GelMA hydrogels resemble their well-plate counterparts with lower expression which is assumed to be due to the less number of cells on the hydrogel to plate.

Abstract # 15

CFD Investigation of the Performance of Localized Air-Conditioning with Upper-Room Ultraviolet Germicidal Irradiation in Reducing Airborne Cross-Infection

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Keywords: Microbiological indoor air quality – Localized air-conditioning – Ultraviolet germicidal irradiation

Descriptive statement: The study addresses the microbiological air quality in spaces with localized airflow and upper room ultraviolet germicidal irradiation (*UR-UVGI*). The distribution of airborne pathogens is determined using computational fluids dynamics (*CFD*) to evaluate the risk of cross-infection within a localized zone with and without *UR-UVGI*.

Introduction: Heating, ventilation, and air conditioning (*HVAC*) industry is moving toward localization for energy efficiency and thermal comfort. The concept of localized air conditioning is to divide a large space into thermally and pollutant independent virtual zones without solid partitions. The aim of this work is to investigate using *CFD* the effectiveness of localized air-conditioning systems combined with *UR-UVG* in reducing the risk of airborne cross-infection within one localized zone.

Methods: A CFD model is developed to predict the dispersion of airborne bacteria in localized spaces with and without the use of *UR-UVGI*. The model is applied to a health care facility containing a source of *Staphylococcus aureus,* a common infectious type of airborne bacteria, and the microbiological air quality is assessed based on the pathogen concentration at the breathing level.

Results: The simulation results reveal that *UR-UVGI* is needed to protect occupants in the localized environmental zone from airborne cross-infection in extreme cases of indoor bacteria generation, and a decrease of 70% in *S. aureus* concentration at the breathing level is achieved when 54 W of *UVC* is delivered to the space.

Conclusion: Although localized air distribution systems can prevent cross-infection between two adjacent environmental zones, they fail to provide such protection for the occupants within a given zone in presence of intense pathogen generation. The use of *UR-UVGI* is then recommended to prevent airborne cross-infection within a localized zone.

Funding source: Lebanese National Research Council, Beirut, Lebanon.

Abstract # 16

Engineering Chondrogenic Niches for Tissue Engineering Applications

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Keywords: Cartilage tissue engineering, biomimetic, GFOGER, alginate sulfate, polyethylene glycol, hydrogel, microenvironment

Mature articular cartilage has very limited ability to self-repair after injury or disease. The cellular microenvironment plays a crucial role in directing proliferation, adhesion, metabolic and catabolic activities of cells. Cells interact with their microenvironment via membrane receptors that recognize changes in the extracellular matrix, oxygen levels, mechanical stimuli and small molecules. The aim of this work was to engineer 3-dimensional (3D) niches which will improve conditions for cartilage tissue engineering applications such as autologous chondrocyte transplantation (ACT). To achieve this goal we designed microenvironments that mimic the proteoglycan or the type II collagen component of cartilage. The proteoglycan mimetic 3D microenvironment was achieved using sulfated alginate hydrogels while collagen was mimicked by incorporating GFOGER peptides in a polyethylene glycol (PEG) hydrogel. We observed that sulfation of alginate induced cell proliferation while maintaining the chondrogenic phenotype of 3D encapsulated chondrocytes. Moreover, interactions of human mesenchymal stem cells with the triple helical collagen mimetic, GPC(GPP)5-GFOGER-(GPP)5GPC-NH2, and the fibronectin adhesion peptide, RGD, were studied in degradable or non-degradable PEG gels. GFOGER-modified degradable gels induced the highest cell proliferation and were the most chondrogenic of the investigated conditions. To conclude, the cell microenvironment can be engineered to induce cell proliferation,

maintain the cartilage phenotype of chondrocytes and promote chondrogenic differentiation of stem cells. The results of this work provide insight into several crucial aspects of the microenvironment and should lead the way to the discovery and application of novel promising materials, for repair and regeneration of cartilage lesions.

Descriptive Statement: In the current work, injectable hydrogels were prepared using synthetic and natural sources to mimic the cartilage extracellular matrix. These biomimetic niches were shown to improve proliferation and chondrogenic capacity of encapsulated cells. The scaffolds above may therefore be used to improve the outcome of autologous chondrocyte transplantation procedures (a common procedure used to treat cartilage lesions).

Funding Source: This work was funded by the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement n° NMP4-SL-2009-229292 (Find&Bind) and the European Regional Development Fund (2013- 2015, postdoctoral fellowship).

Abstract # 17

Synthesis and characterization of novel molecules for new types of hydrogen-bonded organic frameworks (HOFs)

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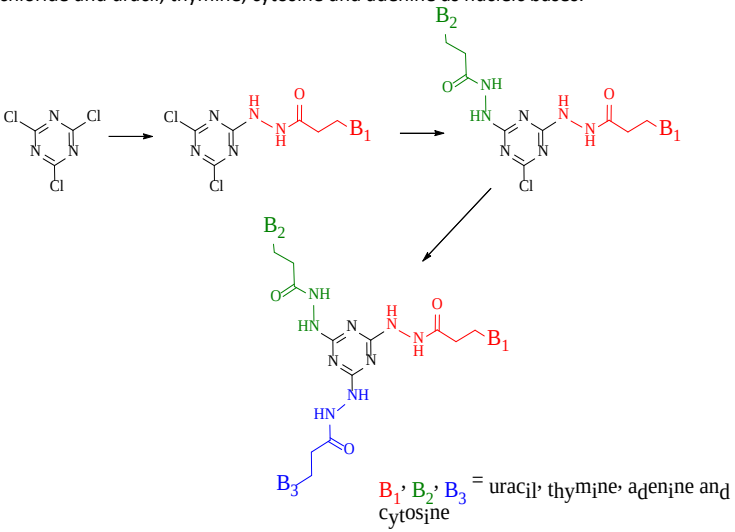
The molecular self-assembly of nucleic bases have generated an increasing interest in the development of novel molecules incorporating pyrimidine and purine groups within. Recently, innovative Hydrogen-Bonded Organic Frameworks (HOFs) have been discovered proposing potential highly crystalline porous materials utilized in gas adsorption and separation applications. We are interested in employing a triazine-based core to integrate H-bond donors and acceptors such as nucleic bases and design novel molecules capable of self-organizing into new types of HOFs. This is achieved via a nucleophilic substitution reaction occurring between cyanuric chloride and uracil, thymine, cytosine and adenine as nucleic bases.



Key Words: Self-assembly, Hydrogen-Bonded Organic Frameworks (HOFs), Triazine-based core, Cyanuric chloride, Uracil, Thymine, Cytosine and Adenine.

Mira Diab-El Harakeh is a Master's student in chemistry.

The authors are grateful to the University Research Board (URB) at AUB and the Lebanese National Council for Scientific Research (LNCSR) for funding this project.

Abstract # 18

Synthesis and analysis of the efficacy and mechanism of action of analogues of the promising anticancer drug Naphthoquinone

Hala Atallah[1], Rana Mezher[1], Jomana El-Aridi[2] and Kamal Bouhadir[1]

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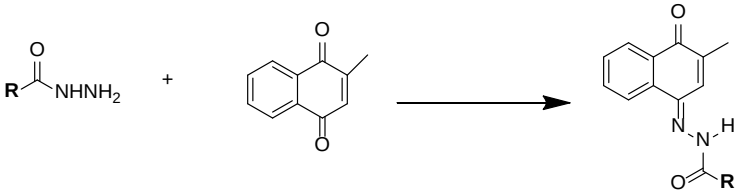
[2] Department of Chemistry, Faculty of Arts and Science, LAU.

Descriptive Statement: Developing anticancerous compounds by coupling hydrazides and menadione .

Background and Aims: Natural occurring quinones, possess anticancerous activities due to the quinone core which is reportedly essential for biological activity. Naphthoquinones, in general, have been reported to possess additional pharmacological properties including antibacterial, antifugal, antiviral, anti-inflammatory, anti-artherosclerotic.(1) Thymoquinones have shown antiproliferation activity against resistant cancer cell lines can be significantly that could be improved by attaching fatty acid-derived alkenyl groups through an acylhydrazone group.(2) In this study, we aim to prepare a series of menadione-hydrazone derivatives as potential anticancer molecules.

Methodology: The synthesis of these hydrazones involves a coupling reaction between menadione (1,4- Naphthoquinone derivative) and several hydrazides (benzoic, octanoic, undecenoic, lauric, stearic, myristic) through an acyl hydrazone group by refluxing overnight with ethanol as a solvent and few drops of TFA (trifluoroacidic acid). The characterization of the resulting compounds was performed by mpt., ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, NOESY and FTIR.



Results: All the collected spectroscopic results confirm the structures of the expected compounds.

Conclusion: We succeeded in preparing and characterizing novel compounds derived from Naphthoquinone. Our future goal is to evaluate the biological activity and prepare a quantitative structure activity database for these derivatives.

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Abstract # 19

Preparation of naphthalene-based nucleosides as potential precursors for stimuli-responsive materials

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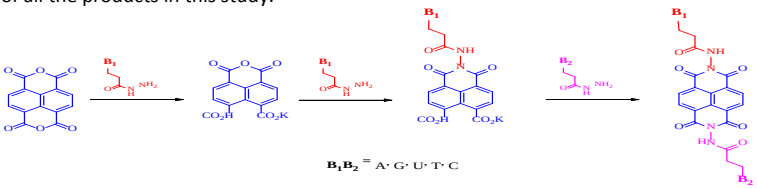
Department of Chemistry, Faculty of Arts and Sciences, American University of Beirut (AUB)

Background and Aims: Molecular recognition probes with the ability to detect specific DNA sequences or mismatches in live cells are attractive in biology and more recently in drug delivery and targeting. We are interested in the design and preparation of small molecules that could potentially self-assemble into hydrogels or rigid porous composites in a controlled and reversible manner, hence, generating stimuli-responsive materials.

Methodology: This study could be realized by incorporating nucleic bases into naphthalene and perylene diimides. We describe herein the chemical synthesis and characterization of some of the naphthalene-based nucleic bases starting with the commercially available naphthalene dianhydride. The purity of the final products was assessed and the structures was elucidated using ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, NOESY, FT-IR, and MS. The collected data confirmed the chemical structures of all the products in this study.[1]



Results: All the collected spectroscopic results confirm the structures of the expected compounds.

Conclusion: We succeeded in preparing and characterizing novel compounds derived from functionalised naphthalenediimides. Our future goal is to evaluate the biological activity of these compounds.

The authors are grateful to the University Research Board (URB) at AUB, the Lebanese National Council for Scientific Research (LNCSR) and the Central Research Science Laboratory (CRSL) at AUB.

Abstract # 20

Synthesis and characterization of menadione-hydrazone derivatives

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2 Departments of Chemistry, Faculty of Arts and Sciences, Lebanese American University

Key Words: Carbanucleosides, nucleic base, quinone, hydrazide-hydrazones.

Background and Aims: Naphthoquinones have been reported to possess a variety of pharmacological properties including antibacterial, antifungal, antiviral, anti-inflammatory, anti-artherosclerotic and anticancer activity. Furthermore, derivatives of nucleic bases have also shown anticancer activity such as 5-fluorouracil and anti-HIV activity such as AZT. As a result, we are interested in combining both functional groups in a single molecule via a hydrazone linkage to explore whether the biological activities of the corresponding conjugates are enhanced.

Methodology: In this study, we report the synthesis of a new series of novel menadione-hydrazone conjugates by coupling menadione with three 1-(2-hydrazidoethyl) pyrimidines. The reaction is expected to form on the carbonyl group at position 4 of menadione and not at position 1 due to steric hindrance. The purity of the final products was assessed and the structures were elucidated using 1H-NMR, 13C-NMR, DEPT, COSY, NOESY, FT-IR and MS. The collected data confirmed the chemical structures of all the products in this study.

Results: All the collected spectroscopic results confirm the structures of the expected compounds.

Conclusion: We succeeded in preparing and characterizing novel compounds derived from Naphthoquinone. Our future goal is to evaluate the biological activity and prepare a quantitative structure activity database for these derivatives.

The authors thank the University Research Board (URB) at the American University of Beirut for funding this project.

Abstract # 21

Synthesis, characterization and anticancer activity of a new series of novel 1,4-napththoquinone-2-acyl hydrazides

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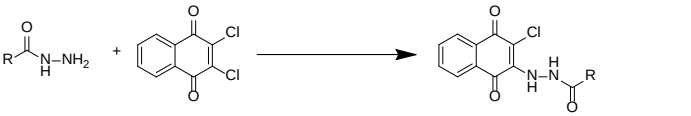
Background and Aims: Naphthoquinones have been reported to possess a variety of pharmacological properties including antibacterial, antifungal, antiviral, anti-inflammatory, anti-artherosclerotic and anticancer effects(1). 1,4-Naphthoquinone contains the quinone core which is linked to the biological activities of these molecules. Our interest in 1,4-naphthoquinone was fortified by literature reports that describe the anticancer and antimicrobial activity of several napththoquinone derivatives. In this project, we aimed to synthesize a new series of novel 1,4-napththoquinone-2-acyl hydrazides as potential adduct with elevated anticancer activities.

Methodology and Results: The synthesis of these hydrazides involves a coupling reaction between 2,3-dichloro-1,4-naphthoquinone and several hydrazides (octanoic, benzoic, undecenoic, lauric, stearic, myristic and pentanoic hydrazide). The characterization of the resulting compounds was performed by melting point, ^{1}H, ^{13}C NMR, FT IR as well as by MS spectrometry.



1,4-naphthoquinone-2-acyl hydrazides

Conclusion: In this work, we have synthesized seven novel compounds derived from 1,4-naphthoquinone. These compounds will be tested for their anti-cancer activity.

KeyWords: 2,3-dichloro-1,4-Naphtoquinone, hydrazides, 1,4-napththoquinone-2-acyl hydrazides, anticancer activity.

Abstract # 22

Choroidal Changes in Amblyopia

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Objective: To detect changes in the choroidal layer at the macular area in amblyopic eyes using enhanced depth spectral domain optical coherence tomography (SD-OCT).

Design: This was a prospective institutional study

Participants: The study included 20 amblyopic patients (12 strabismic and 8 anisometropic) and 20 control subjects; mean ages were 19.4±14.6 years and 16.2±10.2 years respectively.

Methods: A comprehensive eye examination was performed, including best-corrected visual acuity with Snellen charts, slit lamp examination, extraocular motility assessment, cycloplegic retinoscopy and dilated fundoscopy. Choroidal layer imaging was performed using the enhanced depth imaging feature of the Cirrus HD-OCT. Images were exported to analyze the choroidal layer: thicknesses were measured subfoveally and 1500 µm nasal and temporal to the foveal center. Submacular corresponding choroidal areas were also computed and parameters were compared between fellow eyes of amblyopes and between amblyopic eyes and normal controls

Results: No significant differences were detected in the submacular choroidal areas or thicknesses between the amblyopic and fellow eyes (nor between the amblyopic and control eyes). This applied to all amblyopes and to the subgroups of anisometropic and strabismic amblyopia. There was a tendency for the central and temporal choroidal thicknesses to be increased in amblyopic eyes as compared to fellow eyes and normal controls. There were no differences when comparing the fellow normal eyes of amblyopic subjects and the normal controls.

Conclusion: Using enhanced depth SD-OCT imaging, the subfoveal choroidal layer tended to be thicker centrally and temporally in amblyopic eyes when compared to fellow normal eyes and age-matched controls, but this did not reach statistical significance.

Funding source: None

Abstract # 23

THE PREVELANCE OF SLEEP DISORDERS IN A SAMPLE OF HOSPITALIZED PSYCHIATRIC PATIENTS IN LEBANON

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Department of Psychiatry at AUBMC

Key words: sleep disorders, psychiatric patients, co-morbid psychiatric conditions

Funding: Self-funded

Abstract: Sleep is an important part of an individual's health. It is crucial for the restoration of physical aptitudes and mental wellbeing. Unfortunately, studies looking at the prevalence of sleep disorders in psychiatric patients are lacking.
In Lebanon and the Arab region there is a lack of data on the prevalence of sleep disorders in psychiatric patients.
Our hypothesis aims to prove that there exists a high prevalence of multiple sleep disorders in the Lebanese psychiatric patients.

Introduction: A close relationship has been well established among some psychiatric and sleep disorders for some time (Sateia, et al., 2009, Etain, et al., 2012, Peth, et al., 2012, Kalak, et al., 2012). Sleep domains in psychiatric disorders have been restricted to broad descriptive terms such as decreased sleep or increased sleep.
There has been limited research in quantifying the presence of separate sleep disorders among the major psychiatric disorders.

Objectives: Using several validated tools to quantify and describe the prevalence of separate sleep disorders in a psychiatric hospitalized population at AUBMC with an attempt to determine the different types of sleep disorders present in this population.

Methods: Survey-based questionnaires administered to 400 patients. Participants include all adult individuals admitted to the in-patient psychiatric unit
Data collection was done with a face sheet that includes basic demographics and medical information.

Results: Our preliminary results show that out of 70 completed questionnaires only 19 patients are satisfied with their sleep pattern. This coincides with our hypothesis that psychiatric patients have a high prevalence of multiple sleep disorders that are under reported. Data collection and analysis is still ongoing.

Conclusion: Current results highlight that sleep disorders in psychiatric patients are common. Focusing on the various sleep disorders will therefore improve outcomes of psychiatric treatment and reduce relapse rates.
Another goal is to gradually expand sleep research. Our study can be replicated in other departments at AUBMC with populations known to be at high risk for sleep disorders such as oncology, neurology, cardiology, and dialysis patients.

Abstract # 24

Prevalence of Autism in children aged 16-48 months in nurseries in Lebanon: A cross-sectional study.

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Keywords: autism spectrum disorders, prevalence, toddlers, nurseries, Beirut, Mount Lebanon

Descriptive statement: This is a study conducted in nurseries in Beirut and Mount Lebanon to find the percentage of toddlers who potentially have autism spectrum disorders (ASD). All the questionnaires were filled by parents of toddlers going to nurseries. Results of this study show that in fact autism spectrum disorders are increasing in Lebanon. Numbers are comparable to the USA: 1 in 67 children aged 16 to 48 months potentially have ASD as compared to 1 in 68 in the USA. It is the first study of its kind to be conducted in Lebanon.

Funding sources:

- Program Projects in Biomedical Research (2013-2014) (Amount: $80 000)
- OpenMinds fund: $125,360 (private foundation/NGO)

Abstract

Background: Autism Spectrum Disorders (ASD), once considered rare, are now highly increasing in prevalence. According to the Center for Disease Control and Prevention, they affect 1 in 68 children aged 8 years in the USA. The mean prevalence of ASD is 1% according to studies in Asia, Europe and North America. In the Eastern Mediterranean Region, there is a wide variation in reported prevalence, which could be due to the small scale studies conducted (12.5% in Saudi Arabia and 1.4 per 10,000 in Oman). In Lebanon, no estimate for autism prevalence exists, particularly for toddlers. A recent increase in the load of pediatric patients with ASD has been noted at the AUB-MC Special Kids Clinic.

Aims: To examine the prevalence of ASD in toddlers in nurseries in Beirut and Mount Lebanon and to assess some associated factors.

Methods: A cross-sectional study was conducted in 177 nurseries in Mount Lebanon and Beirut governorates (February-September, 2014). A total of 998 children between 16 and 48 months were reached. Parents whose children were eligible

were sent the Modified Checklist for Autism in Toddlers (M-CHAT), a screening instrument for ASD, and a self-administered questionnaire that included factors associated with ASD. Some of the 998 children had 5 or less items missing on their M-CHAT. Data imputation was done to replace missing values by the most frequent answer to each item. Since no follow-up interview for the M-CHAT was conducted to ascertain results, the final prevalence estimate was obtained by multiplying results of the M-CHAT by the positive predictive value of M-CHAT without interview found by a large scale study (0.058).

Results: The prevalence of ASD using M-CHAT was 1 in 67 children.The Male to female ratio was 1.05 (1 in 65 for males and 1 in 67 for females). The Beirut to Mount Lebanon ratio was 1.2 (1 in 57: Beirut and 1 in 68: Mount Lebanon).

Conclusion: Results from this study are comparable to US figures. Many challenges were faced during data collection. Reaching toddlers going to nurseries proved to be a better way to attain large numbers of toddlers than in population-based studies. Future studies using a more representative sample of the Lebanese population and follow-up M-CHAT interviews are needed to determine the true prevalence of ASD in Lebanon. Awareness, early diagnosis and intervention in ASD improve prognosis.

Abstract # 25

Elevated Tricuspid Regurgitant Jet Velocity in Lebanese Patients with Sickle Cell Disease is Associated with Severe Disease and is Clustered in Families

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Keywords: sickle cell disease, tricuspid regurgitant velocity, hydroxyurea, disease severity

Descriptive statement: Elevated tricuspid regurgitant jet velocity on echocardiogram seen in patients with sickle cell is a marker of disease severity.

Introduction: Pulmonary hypertension (PHT), a known complication of sickle cell disease (SCD), is indirectly assessed by measurement of the tricuspid regurgitant jet velocity (TRV). The significance of elevated TRV without PHT and the association between elevated TRV and SCD severity and mortality are still controversial particularly in children and adolescents.

Methods: In this retrospective review of 147 patients with SCD followed at the comprehensive sickle cell clinic at the American University of Beirut Medical Center, elevated TRV was defined as peak TRV of 2.5 m/s or higher. Disease severity was assessed by hemolysis biomarkers, vasoocclusive crises (VOC), acute chest syndrome (ACS) and other SCD complications. Changes in hydroxyurea (HU) doses were examined against TRV variation.

Results: 147 patients were studied, among which 115 patients had homozygous hemoglobin S disease, 8 had sickle β^0-thalassemia, and 24 had sickle β^+-thalassemia. 57 patients (38.8%) had elevated TRV. There were no significant differences between patients with normal and elevated TRV with respect to age, gender, SCD subtype, stroke, avascular necrosis (AVN), ulcers, splenectomy, systolic blood pressure (SBP) and laboratory values including hemoglobin (Hb), mean corpuscular volume (MCV) reticulocyte count, bilirubin, ferritin, lactate dehydrogenase (LDH) and use of HU. VOC and ACS rates were significantly higher in patients with elevated TRV. This association was present despite the possible lowering effect of HU dose escalation on TRV. Familial clustering of elevated TRV was detected in this population.

Conclusion: These findings suggest that elevated TRV is a marker that defines patients with more severe SCD among children and young adults, irrespective of markers of hemolysis and actual presence of PHT. Molecular studies will be needed in the future to further to predict the outcomes and optimize the treatment of patients with elevated TRV.

Abstract # 26

Epidemiologic Surveillance of influenza viruses in Lebanon

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Keywords: Influenza, surveillance, antiviral drug resistance

Funding Sources: The present study was funded by the US-Japan Acute Respiratory Infection Panel (The Ministry of Health, Labor, and Welfare, Japan), JSPS Core-to-Core Program, B. Asia-Africa Science Platforms, the US Department of State Biosecurity Engagement Program (Grant#BEP22033)

General Statement: This ongoing study aims to conduct surveillance of influenza viruses in Lebanon among all age groups, and to examine the efficacy of used antiviral drugs and vaccines.

Abstract

Introduction: Influenza viruses are major causative agents of acute respiratory tract infections. Epidemiologic studies of influenza outbreaks are very limited in the Middle East.

Objective: This ongoing project has conducted surveillance of influenza infections from 2008 till 2014 in Lebanon.

Methods: Nasopharyngeal swabs were collected from patients with influenza-like-illness (ILI) presenting within 48 hours of developing symptoms at the American University of Beirut. Samples were screened by diagnostic kits and further identified by virus isolation and hemagglutination-inhibition test. Genotypic and phenotypic antiviral drug susceptibilities were also assessed.

Results: A total of 666 samples were collected from ILI patients during the seven-year period. Influenza virus was isolated from 189 samples. Among these,

165 were type A and 24 were type B. In general, influenza outbreaks exhibited a seasonal pattern with a peak in winter except for the 2009 pandemic which occurred in summer and fall. Influenza A(H3N2) was the predominant strain in 2008-2009 and 2011-2012 seasons. Seasonal influenza A(H1N1) viruses only circulated in 2008-2009 season after which they were replaced by the 2009 pandemic H1N1 (H1N1pdm09) virus, which was the sole circulating strain in 2009. Influenza B viruses were detected in 2008-2009 and 2010-2011 seasons. All H3N2 viruses were resistant to M2-channel blockers but were susceptible to neuraminidase inhibitors (NAIs). In 2008-2009 season, all seasonal H1N1 influenza isolates were susceptible to M2-channel blockers but resistant to oseltamivir (NAI). H1N1pdm09 viruses were resistant to M2-channel blockers but susceptible to NAIs, except for two isolates that were either resistant or had reduced susceptibility to oseltamivir. Influenza B isolates displayed reduced susceptibility to NAIs compared to influenza A isolates.

Conclusion: Influenza viruses are continuously changing as a result of antigenic shift and drift. Ongoing monitoring of influenza viruses is critical for effective use of vaccines and antiviral drugs.

Abstract # 27

Disease burden and prevalent genotypes of rotavirus causing gastroenteritis in hospitalized children < 5 years of age in Lebanon

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Descriptive statement: In this study, we estimated the disease burden and prevalent genotypes of rotavirus causing gastroenteritis in children less than 5 years of age based on a sentinel hospital-based surveillance program in Lebanon.

Keywords: Surveillance, Rotavirus, genotypes

Funding source: MSD

Abstract:

Background: Rotavirus (RV), a double-stranded segmented RNA virus, is a leading cause of severe diarrhea in young children. Regional epidemiological data are lacking. This study aims to determine the incidence of RV gastroenteritis (GE) in children less than 5 years of age and characterize the prevalent viral genotypes in Lebanon.

Study design: This 3-year prospective hospital-based surveillance study was conducted at AUBMC and 6 other medical centers in Lebanon. Stool samples with demographic and clinical data were collected from children under 5 years of age hospitalized with GE. Samples were tested for RV using rapid kits, and genotypes were determined using hemi-nested RT-PCR followed by sequencing.

Results: A total of 1414 stool samples were collected and tested. Among these, 40% were positive for rotavirus by rapid diagnostic kit. Of the cases, 83% were in children below 2 years of age. Rotavirus infection was confirmed by using PCR in 75% of the rapid-kit positive samples. The most common circulating genotypes were G1P8

(38.9%), G9P8 (24%), G2P4 (17%), and G4P8 (16%). Other genotypes detected at low incidence (<2%) included G12P6, G9P6, G2P8, G9P4, G3P6, G3P9, G1P6 and G2P6. The vaccinated cohort represented 17% of all patients, 86% of which were vaccinated by RV1 (Rotarix®) and the rest by RV5 (RotaTeq®).
Vaccine breakthrough GE was confirmed in 11% of the vaccinated patients. Several genotypes co-circulated during the year with a peak in the winter season. The frequency of genotypes varied slightly by geographic location.
Conclusion: Rotavirus accounts for a significant burden of disease in hospitalized children below 5 years of age in Lebanon, with the majority being under 2 years of age. This study reveals a seasonal pattern of RV infections. Four major genotypes account for the majority of cases in children. Significant breakthrough infections occurred in vaccinated children warranting an analysis of the epitope mutations responsible for these infections.

Abstract # 28

Surveillance of Respiratory Virus Infections among Pediatric Cancer Patients at the Children's Cancer Center of Lebanon

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Key words: Pediatric cancer patients, respiratory infection, co-infection, molecular based screening, epidemiology

Descriptive statement:

Assessing the burden and distribution of respiratory virus infections among pediatric cancer patients remains a key priority for informing patient care. However, little is known about the burden and distribution of respiratory virus infections in Lebanon. We seek to expand and improve the diagnostic capacity for virus detection among pediatric cancer patients in Lebanon in order to reduce unnecessary antibiotic administration, provide evidence to inform infection control practices, and to identify threats from emerging pathogens.

Funding Source: American University of Beirut, Faculty of Medicine Bridge Funding; Children's Infectious Diseases Center (CIDC), St Jude Children's Research Hospital

Abstract:

Background: Respiratory viruses are the most common causes of community and healthcare-associated infections among children. Pediatric cancer patients have a higher risk of morbidity and mortality from respiratory virus infections than other patient populations because of their immunocompromised status. Regional and local data on respiratory virus infections in pediatric cancer patients are scarce.

Aims: To investigate causative viruses of respiratory infections and their burden among pediatric cancer patients and their health care providers at the Children's Cancer Center of Lebanon.

Methods: Nasopharyngeal swabs along with clinical and demographic data were collected from eligible subjects upon obtaining informed consent. Swabs were initially screened with point-of-care rapid kit for dual detection of influenza A/B and RSV. Total nucleic acid was extracted from specimens followed by real time PCR

analysis targeting 16 respiratory viruses to estimate the frequency of infections and their effect on disease outcome.

Results: Thirty pediatric cancer patients have been enrolled with mean age of 4.6 ± 3.7 years. RSV and influenza virus were detected in 27% and 6% of subjects, respectively, by using the rapid kit. Real time PCR analysis confirmed virus infection in 80% of subjects with RSV being the most common virus agent followed by rhinovirus, parainfluenza virus type 3 (PIV3), and human coronavirus OC43 (HCoV-OC43). Co-infections were detected in 55% of patients. RSV was the most prevalent co-infecting virus occurring with rhinovirus, PIV3, and HCoV-OC43. Up to five co-infecting virus pathogens were detected.

Conclusion: The study reveals a high burden of respiratory viral infections in pediatric cancer patients in Lebanon and a high prevalence of co-infections in this patient population. The study also validates the usefulness of real time PCR to improve the diagnostic capacity of virus pathogens in order to advance patient care among pediatric cancer patients in Lebanon and reduce excessive use of antibiotics.

Funding Source: American University of Beirut, Faculty of Medicine Bridge Funding; Children's Infectious Diseases Center (CIDC), St Jude Children's Research Hospital

Abstract # 29

Ocular Manifestation in severe familial hypercholesterolemia patients

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Abstract

Purpose: Studying Ocular Manifestations of patients with severe familial hypercholesterolemia (FHC) in Lebanon.

Methods: 28 patients known to have severe FHC and 24 age matched healthy controls were recruited and underwent full eye exam including fluorescin angiography and ocular computed tomography.

Results: Patients with FHC had significantly higher percentage of early corneal arcus (64% vs 4%) and xanthelasmas (32% vs 0%) compared to control group. These patients had also aprominent yellow rim aroud the optic nerve, not found in the control group (35% vs 0). Peripheral retinal vascular accidents were much more common among patients with FHC compared to controls (21.4% vs 0).

Conclusion: Severe FHC can have many ocular manifestations and complications that may require regular eye exam

Precis: Severe Familial hypercholesterolemia has known ocular manifestations (corneal arcus and xanthelasmas), yet found additionally to carry a higher risk to develop peripheral retinal vascular accidents.

Funding source: None

Abstract # 30

Estimation of Creatinine Clearance for Carboplatin Dosing at Naef K. Bassile Cancer Institute (NKBCI)

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Keywords: Carboplatin, Cockroft-Gault (CG) equation, CKD-EPI equation.

Our study addresses whether the reported way of estimating kidney Creatinine clearance at AUBMC's laboratory (CKD-EPI equation) can be used to calculate Carboplatin chemotherapy dose instead of a more time consuming equation (Cockroft Gault) that's currently routinely being used.

Introduction: Carboplatin chemotherapy is dosed using the Calvert formula that incorporates glomerular filtration rate (GFR) as a key variable. GFR is routinely calculated using the Cockroft-Gault (CG) equation to estimate creatinine clearance. This method may be prone to error, especially in obese patients. Our study aims to compare the dose of carboplatin calculated using CG at Naef K Bassile Cancer Institute (NKBCI) of the American University of Beirut Medical Center (AUBMC) and the dose calculated using CKD-EPI equation which is now provided by AUBMC laboratory with every measurement of serum creatinine as an estimated GFR and the Modification of Diet in Renal Disease (MDRD) equation as an alternative measurement.

Aim: Determine the difference in Carboplatin dose calculated using the Cockroft-Gault equation compared to the dose calculated using CKD-EPI and MDRD equations at NKBCI.

Methods: We conducted a retrospective chart review that included all adult patients who received Carboplatin at AUBMC from January 2012 till January 2014. We obtained the patients list from AUBMC pharmacy records and

collected their clinical data and administered Carboplatin doses. We then re-calculated the carboplatin doses using the different GFR estimation formulas. Paired Sample t-tests were then run using SPSS version 22.0 to check whether a significant difference exists.

Results: Data was available for 227 patients. The mean Carboplatin dose calculated as per CG (579 +/- 260 mg) was significantly different (p-value: <0.00) from that calculated as per CKD-EPI equation (385 +/- 293 mg) and also significantly different (p-value: <0.00) from the dose calculated per MDRD (530 +/- 219 mg).

When stratified by BMI, for non-obese patients, the CG dose (543 +/- 245 mg) was still significantly different (p-value :< 0.00) from the CKD-EPI dose (381 +/- 298 mg). However, there was no statistically significant difference (p-value= 0.30) between the CG dose and the MDRD mean dose (537 +/- 229 mg).

Conclusion: At AUBMC, the reported CKD-EPI GFR estimation cannot be used instead of the routinely used CG equation to calculate Carboplatin dose for chemotherapy.

Our next study will address the accuracy of Carboplatin dosing at our institution compared to the gold standard isotopic methods of measuring GFR and will explore the correlation between sarcopenia and the difference in estimated GFR.

Funding source: None.

Abstract # 31

Urinary Magnesium Creatinine Ratio of Healthy Elementary School Children in Lebanon

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Descriptive Statement: Magnesium Status of Children in Lebanon

Keywords: Urinary Magnesium (Mg) • Urinary Creatinine (Cr) • Urinary magnesium: creatinine ratio • Reference Values • Children • Lebanon

Abstract

Introduction: Urinary magnesium excretion (mg/day or mg/mg creatinine) is known to vary greatly between countries and thus its determination in specific population is essential for the assessment of its status as well as for the diagnosis of abnormalities in its metabolism. A study was conducted to determine urinary magnesium (Mg): creatinine (Cr) ratio of healthy elementary school Lebanese children.

Methods: Using a multi-stage cluster sampling at district, school, and class level, a sample size of 1403 (781 boys and 622 girls) children, from 26 different schools, was selected. Non-fasting morning urine samples and anthropometric data were collected and analyzed.

Results: The mean Mg:Cr ratio was 0.123±0.081 mg/mg and no significant difference was detected between boys and girls (p-value= 0.348). Mg:Cr ratio decreased significantly with age from 0.126 mg/mg at the age of 6 to 0.111 mg/mg at the age of 10. The 5^{th}, 50^{th} and 95^{th} percentiles of Mg:Cr ratio were 0.03, 0.11 and 0.260, respectively. The mean calculated 24-h Mg excretion was 57.2 mg/d (2.07 mg/kg/d) and this implies that children had adequate intake of Mg.

Conclusion: This is the first study that investigates the status of urinary Mg and provides urinary reference values for Mg:Cr ratio which would be important to consider in the diagnosis of abnormalities in Mg metabolism in Lebanese children.

Funding Source: University Research Board

Abstract # 32

Epidemiologic Characteristics, Serotypes, and Antimicrobial Susceptibilities of Invasive *Streptococcus Pneumoniae* Isolates in a Nationwide Surveillance Study in Lebanon

"Lebanese Inter-hospital Pneumococcal Surveillance Program (LIPSP)"

Principal Investigator: Ghassan Dbaibo

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Keywords: Surveillance, *Streptococcus Pneumoniae*, Serotypes

This surveillance study aims at looking at the prevalence and characterisitics of *Streptococcus Pneumoniae*, an important pathogen causing invasive disease with high morbidity and mortality in young children and older adults. It also reviews susceptibilities to different antibiotics and the potential vaccine coverage for better treatment and vaccination strategies.

Abstract:

INTRODUCTION: *Streptococcus pneumoniae* infections are potentially vaccine preventable but yet they still cause severe invasive diseases including pneumonia, bacteremia/sepsis, and meningitis with a relatively high mortality rate. It is a major cause of morbidity and mortality worldwide especially in young children, the elderly patient with chronic medical conditions, and immunocompromised individuals of all ages.

OBJECTIVES: In this study we aim to evaluate the epidemiologic characteristics, serotypes, and antibiotic susceptibilities of *Streptococcus pneumonia* causing invasive pneumococcal disease (IPD) in Lebanon, where such data is lacking.

METHODS: This is a nine-year (Oct 2005- Dec 2014) prospective study involving 84 hospitals from all areas of Lebanon. Active surveillance was performed with personnel from the CIDR calling participating on a weekly or bi-weekly basis inquiring about patients of all age groups with culture-proven IPD. Clinical data was collected using a standardized case report form. Isolates were tested for susceptibility to different antibiotics. The newest CLSI breakpoints for penicillin G and ceftriaxone were used, differentiating between meningeal and non-meningeal isolates. Serotyping was performed using latex agglutination or polymerase chain reaction (PCR) or by using specific anti-sera.

RESULTS: A total of 363 samples were collected during the study period extending from Oct 2005 – Dec 2014, out of which 302 serotypes have been identified so far. Males constituted 58% of samples (n=208). IPD was mostly reported among patients at both extremes of age, 32% of samples were patients above age of 60 and 23% were below 2 years of age. The vast majority of samples were isolated from blood (81.4%, n= 295) followed by CSF (11.3%, n= 41). Of all enrolled patients, 46.6% were admitted with the diagnosis of pneumonia, 25.6% with sepsis, 16.8% with meningitis, and 10% had other diagnoses including mastoiditis, septic arthritis, otitis

media, and hypopyon. Mortality was 11.6% mostly due to sepsis. Children below 5 years comprised 23.7% of total deaths due to IPD. The most commonly isolated serotypes were 19F (36 samples), 14 (25 samples), 3 (22 samples), 1 (18 samples), and 19A (18 samples).

The majority of serotypes isolated were included in the thirteen-valent vaccine (PCV13) (64%, n=193), with 37.7% and 50% vaccine coverage by the seven-valent vaccine (PCV7) and ten-valent vaccine (PCV10), respectively. Only 58% of serotypes were penicillin-sensitive and 24% had intermediate susceptibility to penicillin. Susceptibility was 90.5% to ceftriaxone, 98% to quinolones, and 71% to erythromycin.

CONCLUSION: IPD causes significant morbidity and mortality mainly at extremes of age. The majority of the isolates obtained were vaccine-preventable. This emphasizes the importance of conducting surveillance studies to identify epidemiologic characteristics, antibiotic susceptibility, and serotype prevalence of IPD to raise awareness about vaccination strategies that help decrease the disease burden at the national level.

Funding source: PneumoADIP (partial), Pfizer (partial)

Abstract # 33

Phosphorus Supplementation Abolished Weight Loss of Rats Maintained on Low Protein Diet.

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Key Words: Phosphorus, Low protein diet, food intake, body weight, rats.

Background and Aim: Inadequate dietary protein intake is known to be associated with several adverse health effects including to the development of protein energy malnutrition (PEM). PEM contributes to up to 50% of childhood mortality in developing countries and is usually associated with electrolyte imbalance and low total body phosphate. Recently, maternal protein restriction in rat was reported to favor the development of metabolic syndrome in the offspring during adulthood. Proteins are known to be important dietary sources of phosphorus (P). However, the involvement of P in the outcomes of low protein diets is not clear. The aim of this study was to dissect the impact dietary P manipulation of low protein diet on food intake and weight gain of rats. Egg white protein was used for this purpose since it is known to contain negligible amount of P and have all essential amino acids

Methods: Forty nine male Sprague-Dawley (6 weeks old) rats were randomly allocated to 5 groups and given isocaloric diets. Group 1 (control) with normal protein 20% (from egg white), and 0.3% P, and four groups with low protein diet 10% (from egg white) with different levels of phosphorus: 0.009%, 0.05%, 0.1%, and 0.3%. The rats were fed ad libitum for 9 weeks and their food intake and body weight was monitored.

Food Intake, Weight Gain and Energy Efficiency (EE) of rats over the 9 weeks period.

	Control n=10	P (0.009%) n=9	P (0.05%) n=10	P (0.1%) n=10	P (0.3%) n=10	Anova P-value
Food Intake (g/day)	22.88 [a] ±2.163	19.20 [b] ±2.361	19.70 [b] ±1.635	23.13 [a] ±1.339	23.70 [a] ±2.446	0.000
Weight Gain (g/day)	4.758 [a] ±0.677	1.896 [d] ±0.578	2.651 [c] ±0.407	4.036 [b] ±0.506	4.344 [ab] ±1.261	0.000
EE (g/100Kcal)	5.073 [a] ±0.599	2.382 [d] ±0.875	3.311 [c] ±0.560	4.361 [b] ±0.497	4.408 [ab] ±1.139	0.000

* Results are expressed as Mean± SD. Values that don't share the same superscript are statistically significant

Results and Conclusion: Food intake, weight gain and EE of low protein groups were improved by the addition of P in which that of the 0.3% P group resembles that of the control group. Body changes in the low protein groups are mainly related to decreased P rather than protein intakes. Therefore, the role of P in growth and development requires further investigation, especially under conditions of low protein intake.

Abstract # 34

Prevalence and Correlates of Bullying in a Household Sample of Adolescents Living in Beirut

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Keywords: Bullying, adolescence, mental health

Descriptive Statement for the General Public:
Our abstract is on the prevalence and correlates of bullying from a community sample of adolescents living in Beirut. We have found that 15.4% of our sample had been bullied. Associated factors include being non-Lebanese, male, of younger age, and having symptoms of psychiatric disorders.

Background: Bullying, a distressing experience for many adolescents worldwide. has been linked to the presence and incidence of psychiatric disorders[1,2]. In the Arab world, including Lebanon, population-based data on bullying are scarce. Still, according to the 2011 Global School Based Student Health Survey, 33.6% of middle school students in Lebanon reported ever being bullied[3]. Understanding the types of bullying and their correlates among adolescents thus becomes pivotal to help guide local school-based policies and interventions.
Methods: Data emanates from the 2012 Beirut Epidemiological Investigation of the Psychiatric Status of Youth (BEI-PSY), which used a multi-stage cluster sampling design to recruit 510 adolescents aged 12-18 years from administrative Beirut. Adolescents and their parent/legal guardians filled out an array of questionnaires including the *Development and Well-Being Assessment* (DAWBA) and the *Strengths and Difficulties Questionnaire* (SDQ), while the adolescents completed the *Peer-Relations Questionnaire-12* (PRQ-12) which produces 3 sub-scores: "Bullying" (PRQ-Bully), "Victimized"(PRQ-Victim), and "Prosocial" based on responses to items on a Likert-type scale (ranging 0= "Never" and 3= "Very Often"). The PRQ-Bully and PRQ-Victim subscales had good internal consistency in our sample (α=0.70 and α=0.74, respectively). Adolescents who reported "often" or "very often" as individual-item responses on each of the questions within the PRQ-Bully and PRQ-Victim subscales were considered as having had a bullying experience. Individual correlates of the PRQ-Bully and PRQ-Victim sub-scores were explored using the Mann-Whitney test and Spearman's Correlation, as indicated. Multivariate linear regressions included significant univariate correlates as well as age and gender.
Results: In this sample of adolescents, 15.4% reported having experienced at least one form of bullying, while 21.3% admitted to having bullied others in at least one form. The table attached summarizes the prevalence of the different forms of bullying experienced and perpetuated. On multivariate linear regression models,

higher scores on SDQ-Conduct Problems (self-report) and higher scores on SDQ-Hyperactivity Problems (self-report) were predictors of higher PRQ-Bully scores (B=0.324, p<0.001; B=0.189, p<0.001, respectively). Significant correlates of PRQ-Victim scores were male gender (B=0.082, p=0.046), younger age (B=2.891, p=0.004), being non-Lebanese (B=2.103, p=0.036), higher scores on SDQ-Emotional Difficulties (self-report) (B=5.621, p<0.001), higher scores on SDQ-Conduct Problems (B=4.283, p<0.001), and SDQ-Hyperactivity Problems (B=2.934, p=0.003).

Discussion and conclusions: Despite the prevalence of bullying in our sample being lower than that in other reports from the region, a substantial percentage of adolescents experience bullying at least often. As expected, adolescents with higher conduct and hyperactivity scores on the SDQ were more likely to report bullying. Those reporting being bullied additionally had higher emotional difficulties scores on the SDQ, were more likely to be non-Lebanese, to be males, and to be younger. Socio-economic status (SES) indicators, including attending private versus public schools, did not emerge as predictors of either bullying or victimization. Interventions and strategies to detect bullying addressing schools of different SES backgrounds and at early educational stages are recommended.

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Table 1: Prevalence of the different forms of bullying in a sample of adolescents living in Beirut

	Number	Percentage (%)
"I get called names by others"	34	6.7
"I get picked on by others"	41	8.1
"Others make fun of me"	34	6.7
"I get hit and pushed around by others"	19	3.7
"I am part of a group that goes around teasing others"	57	11.2
"I like to make others scared of me"	48	9.4
"I enjoy upsetting wimps"	18	3.5
"I like to get into a fight with someone I can easily beat"	46	9.1

Funding Source: MPP

Abstract # 35

Neuropsychological functioning in children treated for brain tumors: The AUB-MC experience

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Keywords: Brain tumor survivors, neurocognitive deficits, neuropsychological assessment

Abstract

Introduction: background and aims: Brain tumor survivors may suffer from neuropsychological impairment. Previous studies described deficits in processing speed, memory, attention, visuo-spatial skills and executive function. Multiple risk factors exist including surgery, radiation therapy, chemotherapy, anatomical location of the tumor, antiepileptic drugs and demographic factors. Neuropsychological assessment is recommended by the Children's Oncology Group as part of the long-term management of childhood cancer survivors.

Specific aims: To determine cognitive, behavioral and psychological problems that may arise post therapy. In addition, medical, surgical, social and demographic factors specific to the study population will be taken into consideration.

Methods: Children aged 3 to 17 years with brain tumors followed up at AUB-MC will undergo neuropsychological testing by a trained examiner at diagnosis, 3months after end of treatment and 1 year after end of treatment. At the same points in time, parents will be asked to fill questionnaires related to their child's capabilities, behaviors and background. In addition, information about the anatomical location of the tumor, tumor pathology, surgical resection, chemotherapy regimens, radiation therapy, presence of hydrocephalus and need for shunt, seizures, antiepileptic medications, infections and complications of treatment will be recorded for each patient.

Results and Discussion: Scores and information on cognitive, behavioral and adaptive functioning will be obtained for each patient whether by neuropsychological testing or parental questionnaires. Comparison of the results at baseline, 3 months after end of treatment, and 1 year after treatment will highlight the neurocognitive and behavioral deficits for each patient.

The relationship of the neurocognitive deficits with medical, surgical and social factors will be discussed.

Conclusion: To our knowledge, no previous studies on neurocognitive deficits in brain tumor survivors in the Middle East have been reported so far. This population of children has unique cultural, social and genetic backgrounds.

Funding source: Not determined yet

Abstract # 36

Risk factors of Multiple Sclerosis and associations with anti-EBV antibody titers: a retrospective case-control study in Lebanon

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Keywords: Multiple Sclerosis (MS), Epstein-Barr Virus (EBV), risk factors

Descriptive statement: We studied the risk factors of MS, found in western countries, in a sample from the Lebanese population and searched for an association between the risk factors and the antibody titer against 2 EBV antigens.

Funding source: Philanthropic donation to the AUBMC MS center

Introduction, background and aims: Multiple Sclerosis is a chronic immune-mediated disease characterized by an inflammatory demyelination of the central nervous system. The exact cause of MS is still unknown, but several risk factors have been linked to the disease, and they include: female gender, exposure to Epstein-Bar virus, low vitamin D status, low exposure to ultraviolet (UV) radiation, smoking, childhood and adolescent obesity, and having the HLA-DRB1*15 allele. No previous studies were done on the risk factors of MS in Lebanon, so we aimed at finding out if MS patients in Lebanon share the same risk factors as those in western countries. We also want to look for associations between the titers of EBV antibodies and the different demographic characteristics of our sample.

Methods: The study is a retrospective case-control study on 255 MS patients, receiving care at the Multiple Sclerosis Center of AUB-MC between 2012 and 2014, and 230 controls. Serum samples were tested for anti-EBNA-1 and anti-VCA antibodies via Abbott ARCHITECT System EBNA-1 IgG and VCA IgG chemiluminescent immunoassay. Serum 25(OH)D of controls was measured via a protein binding assay using the DiaSorin RIA (Diasorin, Incstar, Sallugia, Italy). Serum samples of MS patients were tested for

25(OH)D level via Roche 25-OH Vitamin D kit. Case and control demographics were obtained through patient charts.

Results: MS patients were mostly relapsing remitting (78%), with disease duration of 6 ± 7.2 years and a mean EDSS of 2.0±1.7. EBV seropositivity was higher in MS patients (99.5% and 97.3%, respectively) compared to controls (96.3% and 89.4%, respectively) both for anti-VCA and anti-EBNA-1 and the anti-EBNA-1 titers were significantly higher in MS patients (19.6 S/CO) compared to controls (15.0 S/CO). MS patients had a significantly lower vitamin D level (15.8 ng/ml) compared to controls (20.4 ng/ml) and the proportion of MS patients with insufficient and deficient levels was higher (92.7%) compared to controls (83.2%). MS patients smoke more heavily and were more overweight compared to controls. Anti-VCA antibody titers increased with age in controls but were high at all ages in cases. There was no association between demographics and anti-EBV antibody titers. According to a multivariate analysis, risk factors of MS in Lebanon include EBV seropositivity, high anti-VCA and anti-EBNA-1 titers, low vitamin D, and heavy smoking.

Conclusion: Risk factors previously described in western countries appear to correlate with MS in Lebanon. The rate of EBV seropositivity is higher in Lebanon than what is reported in the literature.

Abstract # 37

Assessment of sodium intake using spot urine samples: validation against 24 hour urine collection

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Descriptive statement: Given the established association between salt intake and hypertension, this study aims at assessing whether estimating salt intake from one spot urine sample is a valid approach when compared to 24 hour urine collection.

Keywords: sodium intake; assessment; validity

Abstract: Evidence suggests a direct relationship between sodium intake and hypertension, and salt intake has been shown to correlate with blood pressure in different population groups. Even though 24-hour urine collection is considered the gold standard for the evaluation of dietary sodium intake, spot urine collection is increasingly adopted as a convenient and affordable alternative approach. This study aims at evaluating the validity of spot urine sodium excretion against 24 hour urine collection.

Methods: A convenience sample of 74 adults was recruited to provide a spot urine sample (second morning void) and a 24 hour urine collection. Urinary sodium and creatinine concentrations were assessed. Two published formulas were selected to convert spot urine sodium estimates into estimates of 24 hour sodium excretion (Kawasaki 1993; Tanaka 2002) . Spearman correlation coefficients and Bland Altman tests were calculated to for the relationship between sodium intake as assessed by spot urinary Na excretion (Na_{spot}) and 24 hour urinary excretion (Na_{24h}).

Results: Compared to 24 hour urine collection, the spearman correlation coefficient for the Kawasaki spot urine equation was high and significant (r= 0.570), but the mean difference (1085.53 mg/d) was significantly different from zero. Classification agreement between these 2 methods was of 64 % with the weighted kappa value being of 0.430, indicating moderate agreement between these 2 methods. The Tanaka spot urine equation gave a lower spearman correlation (r=0.556) but a lower mean difference (56.43 mg/d). Classification agreement between Tanaka and 24 hour urine sample was of 54 % with the weighted kappa value being of 0.397, indicating fair agreement between these 2 methods.

Conclusion: Both equations tended to overestimate dietary sodium intake but, compared to 24 hour urine collection, the Kawasaki equation performed better than the Tanaka equation. The results may be limited by the small sample size.

Abstract # 38

Knowledge, beliefs and attitudes of physicians in low and middle-income countries regarding interacting with pharmaceutical companies: a systematic review

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Keywords: Physicians, pharmaceutical company representatives. knowledge, beliefs, attitudes, Low/Middle-income countries.

Descriptive statement: We completed a systematic review about the knowledge, beliefs and attitudes of physicians in low and middle-income countries regarding interacting with pharmaceutical companies.

Abstract

Introduction: Understanding physician's perceptions and attitudes is important for efforts to reduce the impact of their interactions with pharmaceutical industry on clinical practice. It appears that most studies of such perceptions and attitudes have been conducted in high-income countries.

Aims: Our objective was to systematically review the knowledge, beliefs and attitudes of physicians in low and middle-income countries regarding interacting with pharmaceutical companies.

Methods: We included studies addressing any type of interaction between physicians and pharmaceutical companies. The outcomes of interest included knowledge, beliefs and attitudes of practicing physicians. We searched MEDLINE and EMBASE databases. Two reviewers completed in duplicate and independently study selection, data abstraction, and assessment of methodological quality. We narratively summarized the findings stratified by knowledge, beliefs and attitudes.

Results: we included seven papers reporting six eligible studies, each of which had a number of methodological limitations. Three studies found that the top perceived benefits of this interaction were receiving information and rewards. In

three studies, participants perceived that the impact of the interaction on physicians' prescription behavior was minor. In one of the two studies, participants perceived that impact to be lesser when asked about their own behavior. Physicians' attitudes towards information and towards rewards provided by pharmaceutical company representatives (assessed in 4 and 2 studies respectively) varied across those studies. Their attitudes towards developing policies restricting physicians' interactions with pharmaceutical company representatives were positive in two studies. In one study, the majority of participants did not mind the public knowing that physicians were receiving gifts and awards from drug companies.

Conclusion: We identified few studies conducted in low and middle-income countries. While physicians generally perceived the impact of interactions on their behavior as minor, their attitudes toward receiving information and rewards varied across in the interaction and what is more prone to affect physicians' behaviors.

Funding: We did not receive any funding for this study.

Abstract # 39

Factors that impact the academic performance among undergraduate students at the American University of Beirut

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Keywords: Academic performance, factors, undergraduate students, AUB, life-style

Abstract

Background: Academic performance and the quality of education are the key establishers of human and community development. Health and well-being also support effective learning. Thus, acquiring knowledge is crucial for the human living state knowing that health, education and social outcomes are strongly interdependent. Academic performance, measured by Grade Point Average (GPA), is associated with several health related behaviors such as the sleeping pattern, the daily eating habits, exercising, and stress management along with several demographic and socio-economic factors. Although some studies have examined the effect of these indicators on the academic performance, either each factor separately or more than one factor in groups, the associated results found were not all comparable, leading to inconsistent results. Additionally, none was done inside Lebanon or among AUB students.

Objectives: This study determines the prevalence of strong academic achievers among undergraduate students studying at AUB, explores undergraduate students' daily lifestyle factors, and investigates the factors that impact academic performance among university students.

Methods: A cross sectional study was done among a convenient sample of 272 undergraduate students from various faculties at AUB, through a self-administered structured questionnaire.

Results: Descriptive statistics and logistic regressions were performed on the sample. Gender, nationality, faculty, number of meals, alcohol consumption, smoking, and time management were significantly associated with academic performance.

Conclusion: This study revealed that academic performance is affected by several factors. Future studies are needed to validate the study results knowing that no published literature exists on this topic in Lebanon. These results can be used to establish policies, make educational and informational lectures along with workshops, and develop some awareness campaigns to target students' behaviors that can affect their academic performance.

Abstract # 40

Stakeholders' Views and Perspectives on Rehabilitation Services for Individuals Living with Disability: A Survey Study

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Keywords: Stakeholder, Survey, views, perspectives, Rehabilitation, Services, Disability

Descriptive Statement: This is a survey study of stakeholders' **views and perspectives on rehabilitation services for individuals living with disability. We conducted this study to support the World Health Organization in developing** health system guidelines for the implementation of rehabilitation services.

Abstract

Background: Disability is a prevalent issue. It varies between <1% and 30% globally. Rehabilitation services for people living with disability are inadequate. The **World Health Organization** was tasked with developing health system guidelines for the implementation of rehabilitation services.

Aims: The aim of this study is to assess the stakeholders' perceived feasibility and acceptability of a number rehabilitation services and the values they attach to rehabilitation outcomes.

Methods: We disseminated an online self-administered questionnaire through a number of international and regional organizations from the different WHO regions. Eligible individuals included persons with disability, caregivers of persons with disability, health professionals, administrators and policy makers. The answer options consisted of a 9-point Likert scale.

Results: Two fifty three stakeholders participated. The majority of participants were health professional (64%). In terms of outcomes, 'Increasing access' and 'Optimizing utilization' were the top service outcomes rated as critical (i.e., 7, 8 or 9 on the Likert scale) by >70% of respondents. 'Fewer hospital admissions', 'Decreased burden of care' and 'Increasing longevity' were the services rated as least critical (57%, 63% and 58% respectively).

In terms of services, 'Community based rehabilitation' and 'Home based rehabilitation' were found to be both definitely feasible and acceptable (75% and 74% respectively). 'Integrated and decentralized rehabilitation services' was found to be less feasible than acceptable according to stakeholders (61% and 71% respectively). As for 'Task shifting', most stakeholders did not appear to find task shifting as either definitely feasible or definitely acceptable (63% and 64% respectively).

Conclusions: The majority of stakeholder's perceived 'Increasing access' and 'Optimizing utilization' as most critical amongst rehabilitation outcomes. The feasibility of the 'Integrated and decentralized rehabilitation services' was perceived to be less than their acceptability. The majority of stakeholders found 'Task shifting' as neither feasible nor acceptable

Funding: We did not receive any funding for this study.

Abstract # 41

Mild iodine deficiency despite salt iodization in Lebanon.

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Keywords: Urinary Iodine, Children, Lebanon

Introduction: Iodine deficiency affects one third of school-aged children globally, and can impair growth and development. In response to reports of endemic goiter in Lebanon, a national salt iodization program was partially initiated in 1992, and implemented in 1995 by the Ministry of Health. No data exist on iodine status post-implementation of the national iodization program.

Method: A national cross-sectional study of 6-12 year old schoolchildren was conducted between March 2013 and January 2014 using multi-stage cluster sampling. Spot urine samples were collected from 1398 children and urinary iodine levels were measured using a modification of the Sandell-Kolthoff method.

Results: Data are tabulated according to the World Health Organization criteria for assessing iodine nutrition in school aged children. Mean urinary excretion was found to be 80.3(µg/L) indicating the presence of mild deficiency. At the same time, data show that almost 75 % of Lebanese children had insufficient iodine intake.

Table: Iodine status of elementary schoolchildren in Lebanon according to their urinary iodine levels.

Urinary Iodine (µg/L)	Iodine Intake	%	Iodine Status	%
<20	Insufficient	74.75	Severe iodine deficiency	7.51
20-49			Moderate iodine deficiency	27.32
50-99			Mild iodine deficiency	39.91
100-199	Adequate	20.89	Adequate iodine nutrition	20.89
200-299	Above Requirements	2.79	May pose a slight risk of more than adequate iodine intake in these populations	2.79
>300	Excessive	1.57	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)	1.57

Conclusion: Despite the implementation of a national salt iodization program, iodine deficiency remains prevalent in Lebanese schoolchildren

Abstract # 42

Epidemiology and Characteristics of Urinary Tract Infections in Children and Adolescents

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Keywords: ESBL, Urinary tract infection, *Escherichia coli,* Klebsiella, risk factors, children, antibiotic resistance

General statement: This study aimed to determine the epidemiologic characteristics, risk factors for urinary tract infections in children and adolescents admitted to the American University of Beirut- Medical Center and Makassed General Hospital over the last ten years.

Abstract

Background: Urinary tract infections (UTIs) are among the most common infections in the pediatric population. Over the last two decades, antibiotic resistance is increasing significantly as extended spectrum beta lactamase (ESBL) producing organisms are emerging. The aim of this study is to provide a comprehensive view of the epidemiologic characteristics of UTIs in hospitalized children, examine the risk factors of UTIs caused by ESBL producing organisms, and determine the resistance patterns in the isolated organisms over the last 10 years.

Methods: Retrospective chart review was conducted at two Lebanese medical centers. Subjects were identified by looking at ICD-9 discharge codes. Children less than 18 years of age admitted for UTI between January 1st, 2001 and December 31st, 2011 were included. Cases whose urine culture result did not meet our definition for UTI were excluded. Chi-square, Fisher's exact test, and multivariate logistic regression were used to determine risk factors for ESBL. Linear regression analysis was used to determine resistance patterns.

Results: The study included 675 cases with a median age of 16 months and female predominance of 77.7% (525 cases). Of the 584 cases caused *by Escherichia coli (E. coli)* or Klebsiella species, 91 cases (15.5%) were found to be ESBL producing organisms. Vesico-ureteral reflux and previous antibiotics use were found to be independent risk factors for ESBL producing *E. coli* and Klebsiella species (p-value < 0.05). A significant linear increase in resistance to all generations of Cephalosporins (r^2=0.442) and Fluoroquinolones (r^2=0.698) was found.

Conclusion: The recognition of risk factors for infection with ESBL producing organisms, and the observation of increasing overall resistance to antibiotics warrant further studies and new recommendations that guide management of UTIs and antibiotic use in children and adolescents.

Abstract # 43

Assessment of determinants affecting the utilization of dental services: A cross-sectional study on elementary school children in Beirut.

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Key words: Utilization of dental services, oral health, barriers preventing dental visits, awareness of affordable dental care, perception.

Abstract

Background: Studies in Lebanon have shown that oral health problems, such as dental caries, increase with age among children. Despite the availability of dentists to address these problems, their services are not appropriately sought **Aims:** (1) Determine the barriers that prevent parents of elementary school children in Beirut from utilizing the needed dental services despite its abundant presence in Lebanon. (2) Assess their willingness to utilize more these services if insurance coverage becomes available.

Methods: 316 parents of children aged 7-12 years old and attending public and private schools in Beirut constituted the sample of the study. In this cross-sectional study, a financial analysis followed by a self-administered questionnaire distributed to the parents constituted the sources of data collection. The questionnaire included the socio-demographic and economic statuses of the parents, their utilization of dental services for the treatment of their children and their willingness to invest in oral health.

Results: Nearly 73% of the parents had taken their children to the dentist in the past year, but mostly for emergency care. A significant association was found between the school type of the children and the parents' use of dental services. The parents who have their children enrolled in private schools utilize more dental services than those enrolled in public schools. The children oral health status was not found to be significantly associated with the use of services.

Conclusion: Similar to findings in other countries, the financial background was the most restrictive barrier for parents to seek dental services. The lack of correlation between oral health and use of dental services was probably related to the irregularity of and motive for dental visits. The findings underscore the need to implement public health policies such as the establishment of dental insurance coverage.

Funding sources: none

Abstract # 44

Retrospective Study of the Clinical, Serological, and Epidemiological Characteristics of Various Rheumatologic Disorders Seen at AUB-MC

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Keywords: rheumatologic disorders, AUB-MC, SLE, RA, APS, Sjogren's, Scleroderma, vasculitis

Descriptive statement: Rheumatologic diseases are one of the major causes of disability around the world, both in developed and developing countries. Over the past years, as more insight has been learned regarding their pathophysiology of these diseases, significant advances have been made in the management of various rheumatologic conditions. New targeted therapeutic agents have been tried in clinical trials and were found to have significant beneficial effects. However, there is still some debate with regard to whether these results could be extrapolated to our population. The aim of this study is to evaluate retrospectively the clinical, serological, and epidemiological characteristics of patients with different rheumatologic diseases (with an emphasis on Rheumatoid Arthritis, Systemic Lupus Erythromatosus, Anti-Phospholipid Syndrome, ANCA related vasculitis, Sjogrens Syndrome and Systemic Sclerosis) seen at AUB medical center. This registry will serve as a valuable source of information for future clinical trials.

Introduction (background and aims): Rheumatologic diseases are one of the most common causes of disability in the world. They significantly affect patients and their families physically and psychologically. These facts have been recognized by the United Nations and WHO with their endorsement of a Bone and Joint Decade (2000-2010). To date, there is no patient database for the rheumatologic diseases in Lebanon. This study aims to create a patient registry for various rheumatologic diseases. This will enable us to better determine the epidemiological, clinical, and serological characteristics of these diseases in our population.

Methods: Medical records of patients who have received the diagnoses of any of the above mentioned rheumatologic diseases from 1999 till 2009 will be reviewed and included in the registry. Data will be collected and analyzed via SPSS 17.0 using descriptive statistics.

Results: 439 patients fit the criteria of their respective rheumatologic disease and their charts were analyzed for epidemiological, clinical and serological details. 81.5% of patients with rheumatic diseases were females and 18.5% males, the mean age at onset was 39.24 years (+/- 18.76 years). The patient population was predominantly Lebanese. Only 2.1 % of patients were lost to follow-up in all and 4.2% had a family history of rheumatic disease. The most common rheumatic conditions among the 439 patients were rheumatoid arthritis (23%), systemic lupus

erythematosus (22.1%), sjogren's syndrome (15%), scleroderma (8.4%), antiphospholipid syndrome (5%), systemic vasculitis (3.2%), mixed connective tissue disease (2.3%), psoriatic arthritis (1.1%) and dermato/polymyositis (0.9%).

Conclusion: As expected, rheumatic diseases have a wide variety of manifestations and this can be due to multiple factors including geographical distribution and ethnicity. This study shed the light on commonly seen rheumatic diseases at AUBMC, a tertiary referral center, and compared them to the West and other Middle Eastern countries whether clinically or serologically.

Funding source: None

Abstract # 45

Pictoral review of rectovescial and rectouterine pouch hernias and report of two cases

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Funding source: None

Description: The purpose of this work is to present the CT findings and review of the literature concerning two rare cases of surgically confirmed perineal hernias.

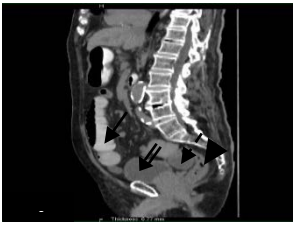
Keywords: Rectovescial pouch, rectouterine pouch, internal hernias, computed tomography.

INTRODUCTION: Internal hernias constitute a rare finding. They are categorized as either acquired or congenital. Predisposing factors for acquired internal hernias include bariatric surgery such as laparoscopic or open Roux-en Y gastric bypass surgery and rarely gastric banding. Amongst the least reported internal hernias are perineal hernias, the rarest of which include rectovesical and rectouterine pouch (pouch of douglas) hernias.

METHODS: An extensive PubMed search for internal hernias was conducted and revealed no accounts of rectovesical hernias and all but three accounts of pouch of douglas hernias making this the first report of a rectovesical hernia in a male.

RESULTS:

Patient 1: A 93 year old male presented to our medical center's emergency department with abdominal pain, vomiting, and constipation. Physical exam was notable for diffuse tenderness over the lower abdomen. CT scan with oral and IV contrast uncovered a small loop of bowel within the rectovesical pouch displacing the rectum laterally with proximally dilated bowel loops and deflated bowel loops distally (Figures 1 and 2). Laparoscopic exploration confirmed a rectovesical hernia.



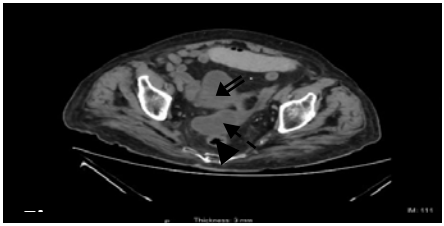
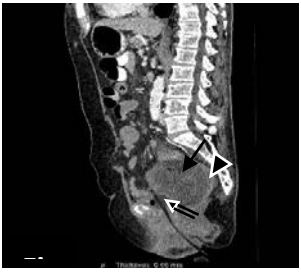


Fig. 1. A sagittal CT scan showing dilated proximal bowels (arrow), rectum (arrow head), small bowel loop (interrupted arrow), and urinary bladder (double arrow) **Fig. 2**. An axial CT scan through the pelvis showing dilated small bowel loop (arrow), rectum (arrow head), and urinary bladder (double arrow).

Patient 2: A 73 year old female with recent history of hysterectomy presented to our emergency department complaining of abdominal pain, food intolerance, vomiting, and obstipation. On physical exam, the patient had diffuse tenderness in the lower abdomen accompanied by involuntary guarding. CT scan with oral and IV contrast revealed a loop of bowel cocooned within the pouch of douglas with dilated bowel loops proximally and distally deflated bowel loops (Figures 3 and 4). Exploratory laparotomy confirmed a pouch of douglas hernia.



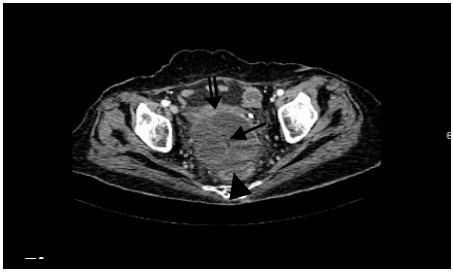


Fig. 3. A sagittal CT scan showing cocooned bowel loops in the cul de sac (arrow), rectum (arrow head), and urinary bladder bearing a foley catheter (double arrow). **Fig. 4.** An axial CT scan through the pelvis showing cocooned bowel loops (arrow), rectum (arrow head), and urinary bladder (double arrow).

CONCLUSION: Both patients described above presented with abdominal pain and a clinical picture of small bowel obstruction by history and physical exam. Loops of bowel found encased within a peritoneal defect of the rectovesical and rectouterine pouches visualized by CT and confirmed surgically, classifies the above cases as two rare reports of perineal hernias.

Abstract # 46

Requirements of the core clinical journals for authors' disclosure of financial and non-financial conflicts of interest: a cross sectional study

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Keywords: financial conflicts of interest, non-financial conflicts of interest, core clinical journals

Descriptive statement: While financial conflict of interest (COI) disclosure is well defined by the majority of the core clinical journals, non-financial COI was only stated in 60% of the journals and therefore should be addressed in a clearer manner.

Funding Source: not funded

Introduction: The Cochrane Handbook describes the funding source the study and the potential COI of the study authors as being "of particular importance" when abstracting data. The aim of this study was to assess the policies of the core clinical journals for authors of trials to disclose their financial and non-financial COI.

Methods: We were interested in both financial and non-financial COI disclosure requirements in 116 core clinical journals indexed under Abridged Index Medicus by the National Library of Medicine. Data abstractors reviewed "instructions for authors" on the journal website and, in order to reflect the actual implementation of the COI disclosure policy, simulated the submission of a manuscript. We used a standard systematic review methodology for the data collection process.

Results: Out of the 116 journals included, 115 had a COI policy. All journals required disclosure of financial relationships pertaining to the authors, 40 (34.8%) and 34 (29.6%) journals required disclosure pertaining to the authors' family members and the authors' institution respectively. 107 journals (93%) required specification of source of payment of which 8 journals required specification of amount of payment irrespective of the amount. 69 (60%) journals required disclosure of at least one form of non-financial COI. Terms used by those 69 journals to refer to non-financial COI included: "non-financial COI" (6.1%), "non-financial affiliations" (4.6%), "other" (70.7%), "academic association" (13.8%),"professional" COI (7.7%) and "intellectual" COI (3.1%). 18 journals (15.7%) claimed an impact of disclosed COI on editorial process, whereas 27 (23.5%) claimed an impact of non-disclosure on the editorial process.

Conclusion: While financial COI disclosure was well defined by the majority of the journals, 60% of the journals required at least one form of non-financial COI to be disclosed. Non-financial COI should be addressed in a clearer manner as part of disclosure policies.

Abstract # 47

Parenteral anticoagulation in ambulatory patients with cancer

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Keywords: Parenteral**,** Anticoagulation, cancer, ambulatory

Descriptive statement: This systematic review evaluates the efficacy and safety of blood thinning agents in ambulatory patients with cancer

Background: Anticoagulation may improve survival in patients with cancer through an antitumor effect in addition to the perceived antithrombotic effect.

Objectives: To evaluate the efficacy and safety of parenteral anticoagulants in ambulatory patients with cancer who, typically, are undergoing chemotherapy, hormonal therapy or radiotherapy, but otherwise have no standard therapeutic or prophylactic indication for anticoagulation

Methods: A comprehensive search included (1) an electronic search (February 2013) of the following databases: Cochrane Central Register of Controlled Trials (CENTRAL) (2013, Issue 1), MEDLINE (1966 to February 2013; accessed via OVID) and EMBASE(1980 to February 2013; accessed via OVID); (2) hand-searching of conference proceedings; (3) checking of references of included studies; (4) use of the 'related citation' feature in PubMed and (5) a search for ongoing studies.

Results: Of 9559 identified citations, 15 RCTs fulfilled the eligibility criteria. These trials enrolled 7622 participants for whom follow-up data were available. In all included RCTs the intervention consisted of heparin (either unfractionated heparin or low molecular weight heparin). Overall, heparin may have a small effect on mortality at 12 months and 24 months (risk ratio (RR) 0.97; 95% confidence interval (CI) 0.92 to 1.01 and RR 0.95; 95% CI 0.90 to 1.00, respectively). Heparin therapy was associated with a statistically and clinically important reduction in venous thromboembolism (RR 0.56; 95% CI 0.42 to 0.74) and a clinically important increase in the risk of minor bleeding (RR 1.32; 95% 1.02 to 1.71). Results failed to show or to exclude a beneficial or detrimental effect of heparin on major bleeding (RR 1.14; 95% CI 0.70 to 1.85) or quality of life. Our confidence in the effect estimates (i.e. quality of evidence) was high for symptomatic venous thromboembolism, moderate for mortality, major bleeding and minor bleeding, and low for quality of life.

Conclusions: Heparin may have a small effect on mortality at 12months and 24months. It is associated with a reduction in venous thromboembolism and a likely increase in minor bleeding. Future research should further investigate the survival benefit of different types of anticoagulants in patients with different types and stages of cancer. The decision for a patient with cancer to start heparin therapy for survival benefit should balance the benefits and downsides, and should integrate the patient's values and preferences**.**

Funding source: National Institute for Health Research Cochrane Review Incentive Scheme

Abstract # 48

Efficacy of Adalimumab Stored in Plastic Vials at Four Degrees Celsius

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Abstract

Purpose: To evaluate the efficacy of anti tumor necrosis factor alpha adalimumab (Humira; Abbott Laboratories, North Chicago, IL, USA) repackaged into plastic polypropylene vials (Eppendorf®; Hamburg, Germany) and stored at 4°C for potential intravitreal use.

Methods: Three samples of adalimumab refrigerated at 4°C for 5 weeks in plastic polypropylene vials (0.1, 1, 10 μg/ml) were used to neutralize the cytotoxic effect of recombinant human tumor necrosis factor alpha (rh-TNF-α) on mouse fibrosarcoma cell line (L929 cells). The RTCA xCELLigence system was used to measure the cytotoxic effect of rh-TNF-α on L929 cells. L929 cell survival was assessed after treatment with the effective dose of rhTNF-α and the different concentrations of adalimumab stored in plastic vials. Rh-TNF-α was also used alone in parallel for each experiment to accurately determine the neutralization effect of the used antibody. Cell survival was measured at the same concentrations of adalimumab stored at 4°C for 5 weeks taken from the original commercial glass vials. The inhibitory response of adalimumab (antiTNF-α) in terms of cell survival was measured at 1-hour intervals for up to 48h at week one and week five using the RTCA xCELLigence system.

Results: The effective dose of rhTNF-α was determined to be 100ng/ml with a 40% mean cell survival at 48 hours. Adalimumab at concentrations of 1 and 10 μg/ml from the repackaged plastic vials stored for 5 weeks at 4°C was able to neutralize the cytotoxic effect of 100ng/ml rhTNF-α. Hundred percent of cells survived compared to 8% survival when treated in parallel with rhTNF- α alone at 48 hours. However, for the very low concentration (0.1 μg/ml), the neutralizing effect of adalimumab decreased by 60 % and 80 % at 48 hours after treatment, at weeks 3 and 5 respectively. On the other hand, all concentrations of adalimumab (0.1, 1 and 10 μg/ml) taken from original glass vials were able to achieve neutralization of the 100ng/ml rhTNF-α with up to 90% cell survival at 48hours after 5 weeks storage at 4°C. This is compared to 30% cell survival when treated in parallel with 100ng/ml rhTNF-α alone.

Conclusions: Adilamumab stored in plastic vials retained its efficacy in neutralizing the effect of rh-TNF-α after five weeks of storage at 4°C at concentration as low as 1 μg/ml.

Keywords: Humira, adalimumab, uveitis, storage, plastic, vials, syringes, TNF-α, anti-TNF-α

Descriptive statement: In this study we measured the efficacy of three different concentrations of anti-TNF-α (Adalimumab) when added to rhTNF-α by determining the mouse fibrosarcoma cells survival. The study was performed using adalimumab that was repackaged and refrigerated in plastic vials at 4°C for 5 weeks. This was also done using adalimumab stored in the original commercial glass vials stored for the same period.

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Funding source: None

Abstract # 49

Efficacy of safety-engineered devices in reducing needle-stick injuries among healthcare workers in intravenous and phlebotomy procedures: a systematic review and meta-analysis

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Funding: This work was funded by the World Health Organization.

Abstract: The acquisition of needle-stick injuries (NSI) in a healthcare setting poses an occupational hazard of transmitting blood-borne pathogens from patients to healthcare workers (HCWs) and vice versa.

Aim: The aim of this study was to systematically review the evidence about the efficacy and safety of using safety-engineered intravenous devices and safety-engineered phlebotomy devices by HCWs.

Methods: We included randomized and non-randomized studies comparing safety-engineered devices to conventional/standard devices that lack safety features for delivering intravenous injections and/or for blood-withdrawal procedures (phlebotomy). The outcomes of interest included NSI rates, and blood-borne infections rates among HCWs and patients. We conducted an extensive literature search strategy using the OVID interface in October 2013. We followed the Cochrane Collaboration's methods for study selection and data abstraction. When possible, we conducted meta-analyses using a random-effects model. We used the GRADE methodology to assess the quality of evidence by outcome.

Results: We identified 22 eligible studies: 12 assessed safety-engineered devices for intravenous procedures, five for phlebotomy procedures, and five for both. Twenty-one of those studies were observational while one was a randomized trial. All studies assessed the reduction in NSIs among HCWs. For safety-engineered intravenous devices, the pooled relative risk for NSI per HCW was 0.28 [0.13, 0.59] (moderate quality evidence). The pooled relative risk for NSI per device used or procedure performed was 0.34 [0.08, 1.49] (low quality evidence). For safety-engineered phlebotomy devices, the pooled relative risk for NSI per HCW was 0.57 [0.38, 0.84] (moderate quality evidence). The pooled relative risk for NSI per device used or procedure performed was 0.53 [0.43, 0.65] (moderate quality evidence). We identified no studies assessing the outcome of blood-borne infections among healthcare workers or patients.

Conclusion: There is moderate-quality evidence that the use of safety-engineered devices in intravenous injections and infusions, phlebotomy procedures reduces NSI rates of HCWs.

Keywords: systematic review, healthcare workers, healthcare setting, needle-stick injuries, safety-engineered devices, Intravenous, Phlebotomy, meta-analysis, blood-borne pathogens.

Abstract # 50

A recent trend towards higher stage prostate cancer at presentation in the Middle East: Are we ready to adopt the USPSTF recommendations for screening?

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Keywords: Prostate, Middle East, screening

Funding source: none

Descriptive statement: The United States Preventive Services Task Force recommends against screening of prostate cancer. Unfortunately, physicians in our part of the world started to advice against screening without insight on the differences in disease epidemiology between different countries and races. In this study our aim was to proof that prostate cancer stage at presentation in our population is different, and we can't adopt such recommendation in Lebanon.

Introduction: A downward stage migration of prostate cancer has been noted in the past 15 years in countries with well-established PSA screening programs. This stage migration spurred the United States Preventive Services Task Force (USPSTF) to issue a blanket recommendation against prostate cancer screening. On the other hand, little is known about changes in prostate cancer stage in developing countries; where PSA screening is not uniformly adopted by health care systems and private hospitals. The aim of this study is to describe the prevalence of pathological stages of PCa a tertiary referral center (AUBMC, Beirut, Lebanon) and the pattern of change in the past 15 years.

Methods: Using the Radical prostatectomy database of AUBMC, changes in tumor stage were examined in 400 men underwent radical prostatectomy from 1998 to 2012.The cohort was split into early PSA (1998-2004), and contemporary PSA (2005-2012) eras based on PSA screening penetrance in our country.

Results: During the early and contemporary PSA eras, 219 and181 men, respectively, underwent radical prostatectomy. The median age in both eras was 62 with mean PSA 9.49. Organ confined disease (T2) was diagnosed in 77% and 67.4% of patients in the early and contemporary PSA eras, respectively (p=0.014). Non-organ confined disease (T3) was present in 22.9% of the patients in the early PSA era vs. 32.6% in the contemporary era (p=0.025); the mean PSA for non-organ confined disease during the early and contemporary eras was 15.92 and 13.38, respectively.

Conclusion: The findings from the AUBMC Database point toward an opposing trend of upward stage migration of prostate cancer. This unexpected opposing trend could be explained, at least in part, by the fact that PSA screening program is not well established in our health care system especially in rural referral areas and a high incidence of high risk disease in the Middle Eastern population. Furthermore, these findings raise a specific concern regarding the universal adoption of the USPSTF recommendation which does not recommend routine PSA screening.

Abstract # 51

TPEN induces DNA damage in human colon cancer cells: Role of Chk1/2 and DNA-PK

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Key words: Metal chelation; redox cycling; copper; reactive oxygen species; anticancer

Descriptive statement: TPEN causes DNA damage in colon cancer cells

Grant support acknowledgment: This work was funded by NPRP grant # 09-047-3-012 from the Qatar National Research Fund (QNRF). The statements made herein are solely the responsibility of the authors.

Abstract

background and aims: The maintenance of optimal metal levels is an essential aspect of cell homoeostasis. However, in many types of cancer these metal levels especially iron, zinc and copper diverge from normal levels. We have recently shown that the transition metal chelator TPEN increases the generation of reactive oxygen species (ROS), which selectively kills colon cancer cells. We have also provided evidence that the redox cycling of copper is responsible for TPEN anticancer effects. In this study, we aimed to further decipher the mechanism of TPEN-induced cell death in colon cancer cells through studying its effect on DNA damage.

Methods: Multiple approaches were employed, including cell viability, comet assay, measurements of production of ROS by DCFH Assay, Western Blot, and Transfection Using Lipofectamine 2000, Immunocytochemistry Detected by Flow Cytometry.

Results: We show that cell death by TPEN is associated with significant DNA damage, an effect that was dependent on ROS generation and on the redox cycling of copper, as evidenced by reversal of DNA damage in the presence of antioxidants (NAC, CAT) or the copper chelator neocuproine (Neo). DNA damage was associated with increased expression of p-H2AX and a significant activation of ATM/ATR signaling molecules, specifically p-ATM, p-ATR and p-Chk1. interestingly, silencing DNApk and Chk reversed DNA damage caused by TPEN, suggesting the involvement of DNApk and ATM/ATR pathways in TPEN-mediated effects.

Conclusion: This study shows for the first time the involvement of DNApk and Chk1/2 in TPEN-induced DNA damage and confirms our previous findings that the redox cycling of copper is the main mechanism by which TPEN induces cell death in human colon cancer cells.

Abstract # 52

Rhabdomyosarcoma derived exosomes exert paracrine signaling.

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Introduction: Rhabdomyosarcoma (RMS) is an aggressive childhood soft tissue tumor, with two distinct subtypes, alveolar (ARMS) and embryonal (ERMS) histologies. The alveolar subtype is characterized by a specific translocation PAX-FOXO1, the protein product of which is thought to contribute to its aggressive and metastatic behavior. Exosomes are small membranous vesicles (30-100 nm in diameter) secreted into body fluids by multiple cell types, including tumor cells. Tumor exosomes contain intact and functional protein, mRNA and miRNA that can alter the cellular environment to favor tumor growth. We hypothesize that the PAX3-FOXO1 fusion protein results in specific effects on exosome cargo and biology, contributing to the invasive and metastatic potential of ARMS cells.

Methods and Results: In the present study, we examined the functional roles of RMS-derived exosomes on tumor cell invasion and motility from a panel of 5 RMS cell lines. We also examined the RNA cargo of RMS-derived exosomes using miRNA array profiling. We found that RMS-derived exosomes exert a positive effect on cellular migration and invasion. We also found potential miRNA expression signatures for both ARMS and ERMS-derived exosomes, identifying pathways that may contribute to paracrine signaling in tumor progression.

Conclusion: Our data suggest a possible direct role of RMS-derived exosomes on the metastatic potential of RMS cells. Current work is focused on further analysis of the identified pathways, and possible biomarkers for RMS to be used in diagnosis and prognosis.

Keywords: Rhabdomyosarcoma, Exosomes, paracrine signaling

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Funding source: Previous CCCL; Current MPP

Abstract # 53

Noxa and ceramide: Crosstalk between Bcl-2 family proteins and p53-dependent ceramide accumulation in mediating intrinsic apoptosis in Molt-4 human T-cell leukemia.

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Keywords: apoptosis; p53; ceramide; noxa; mitochondria.

General Statement: Many cancer treatments rely on initiating programmed cell death, also known as cell suicide. However, most tumors develop mutations in the p53 gene, an essential mediator of programmed cell death. This renders these tumors resistant or poorly responsive to chemotherapeutic agents. Ceramide is a powerful tumor suppressor lipid that can overcome p53 mutation by activating alternative mediators that can induce cell death. A coordinated regulation was found between the trio molecules (i) p53 (ii) the Bcl-2 family member, Noxa and (iii) ceramide in initiating programmed cell death in response to stress response, specifically, to γ-irradiation.

Abstract*:*

Background: The sphingolipid breakdown product, ceramide, has been proposed as a coordinator of many cellular processes, one of which is apoptosis. We have previously shown that γ-irradiation induces p53-dependent apoptosis through ceramide accumulation in the Molt-4 human T-cell leukemia cell line. Noxa, a BH3-only pro-apoptotic protein and a member of the Bcl-2 family, is activated by p53 and regulates downstream pathways that initiate mitochondrial outer membrane permeabilization (MOMP) and subsequently apoptosis.

Aim: Here we identify a novel link between Noxa and ceramide in the apoptotic pathway that were previously thought to work independently.

Methods: Specifically, we knocked down (KD) either p53 or Noxa protein expression in Molt-4 cells and measured ceramide level in response to □-irradiation. Furthermore, lipid and protein analysis of mitochondria isolated from Molt-4 cells were examined.

Results: Our results demonstrate that ceramide accumulation requires the expression of both p53 and Noxa whereby inhibition of either p53 or Noxa totally inhibits ceramide accumulation. Moreover, mitochondria isolated from Molt-4 cells showed a simultaneous ceramide accumulation and cytochrome c release, suggesting that ceramide's role is intricately related to the execution phase of apoptosis at the mitochondrial level.

Conclusion: These findings establish a role for Noxa in regulating ceramide at the mitochondrial level and provide a better understanding of the mechanisms of apoptosis initiated by p53.

This project is fund by Medical Practice Plan (MPP) Grant, AUB.

Abstract # 54

The Anti-Tumor Effect and Mechanism of Action of the Synthetic Retinoid ST1926 on *in vitro* Non-APL AML Models

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Keywords: Acute myeloid leukemia; Retinoids; ST1926; Apoptosis; DNA damage; Nucleophosmin

Background and Aims: Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disorder of hematopoietic progenitor cells which have lost their normal ability to differentiate. Clinical treatment of AML subtypes is highly dependent on the patients' karyotype. In most cases, it is often linked with poor therapeutic outcome due to disease heterogeneity. Retinoids regulate a wide range of biological processes, including development, differentiation, proliferation, and apoptosis, especially in hematopoietic cells. Differentiation therapy using the natural retinoid all-*trans* retinoic acid (ATRA), became the paradigm for the treatment of acute promyelocytic leukemia (APL), an AML subtype. However, in non-APL AML patients, ATRA is only effective on those presenting with *nucleophosmin* (*NPM-1*) mutations but not all the other subtypes. ST1926 is a synthetic retinoid with high specificity, stability and bioavailability showing a significant potential as a therapeutic agent in tumor treatment. Therefore, we aim at investigating the anti-tumor effect of ST1926 on several non-APL AML cells and deciphering its molecular mechanism of action.

Methods and Results: We used representative non-APL AML cell lines harboring different genetic mutations and corresponding to many AML karyotypes. Using MTT and trypan blue exclusion assays, we showed that pharmacologically achievable micromolar concentrations of ST1926 inhibited the proliferation of all tested AML cell lines. ST1926 induced apoptosis as evidenced by the accumulation of treated cells in the preG1 region of the cell cycle, PARP cleavage, and mitochondrial membrane dissipation. Moreover, ST1926 promoted early DNA damage as indicated by an increase in γH2AX expression.

Conclusion: Our results show a promising anti-tumor efficacy of ST1926 in AML treatment. Next, we intend to confirm our results in xenograft mouse models and in primary AML patient cells and to decipher the ST1926 mechanism of action.

Funding: None

Abstract # 55

Targeting colorectal cancer stem cells with the black seed extract Thymoquinone

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Keywords: Colorectal cancer; cancer stem cells; thymoquinone; sphere-formation assay; cancer relapse

Descriptive statement: Thymoquinone targets colon cancer stem cells.

Abstract

Background and aims: Cancer relapse following therapy remains a leading cause of death among humans worldwide. It is believed that cancer stem cells (CSCs), which are a subpopulation of cancer cells that retain the ability of self-renewal and differentiation into different mature cells, are the main factor in cancer relapse. Tumor-derived CSCs form 3D spheres *in vitro* when plated at low densities in low-serum or serum-free defined media in Matrigel. Studies on CSCs, in the context of sphere-forming assays, involve proliferation of spheres, self-renewal and tumor initiation and their behavior upon drug treatment in comparison to adherent cells. Since CSCs are involved in cancer recurrence, they provide a target for drug therapy. Thymoquinone (TQ) extracted from black seed is a promising drug that significantly induces apoptosis, cell cycle arrest, and inhibits tumor growth and tumor cell invasion in 2D; however, studies investigating the effect of TQ on colon CSCs have been lacking. Here we aim to understand the potential effect of TQ on colon CSCs and its mechanisms of action using two HCT116 colon cancer cell lines.

Methods: Sphere formation assay and sphere propagation on Matrigel are mainly performed on HCT116 p53+/+ and p53-/- cells. Spheres are treated with different TQ doses. RT-PCR is performed on spheres to detect expression levels of cancer stem cell markers. Mechanism of drug action, such as invasion and apoptosis are also investigated by wound healing assay, invasion assay and western blot analysis.

Results: Optimal sphere formation in HCT116 cells was observed at 5% serum. HCT116 cells with p53 deletion were more sensitive to TQ treatment than HCT116 p53+/+ cells. Upon treatment with 1 µM and up to 5 µM TQ on HCT116 cells, sphere number and size was decreased in a dose dependent manner. TQ doses affecting sphere formation were 10 fold lower than IC_{50} of TQ on monolayer cultures of HCT116 cell lines. TQ partially inhibited cell migration as evidenced by results of the wound healing assay.

Conclusion: The observed promising effects of TQ and potential inhibition of stemness properties in colon CSCs make it worth translating this natural drug to the clinic as it might potentially inhibit cancer relapse.

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Abstract # 56

Anti-Tumor Efficacy of Arsenic/Interferon in Preclinical Models of Chronic Myeloid Leukemia Resistant to Tyrosine Kinase Inhibitors

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Keywords: Chronic myeloid leukemia, arsenic trioxide, interferon alpha, resistance, T315I

Background and Aims: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by t(9;22) translocation that generates *bcr-abl* fusion gene coding for BCR-ABL oncoprotein with abnormal constitutive tyrosine kinase activity. Tyrosine kinase inhibitors (TKI) have been successfully established for the treatment of CML. Despite high rates of clinical response, CML patients can develop resistance against TKI mainly due to kinase domain mutations. Of special interest is T315I mutation, which accounts for 15–20% of mutations affecting ABL kinase domain. T315I confers resistance to almost all TKI. Ponatinib, the only TKI effective against T315I single but not T315I-inclusive compound mutations, was suspended due to its cardiac side effects and is currently limited to specific cases.

Recently, we demonstrated that arsenic trioxide (ATO) and interferon alpha (IFNα) inhibited proliferation, induced apoptosis, prolonged survival and affected leukemia initiating cells activity in wild-type *bcr-abl* CML models. Here, we investigate the effect of ATO and IFNα on the proliferation of imatinib-resistant CML cell lines and its anti-tumor activity in CML mouse model harboring the T315I mutation.

Methods: Imatinib-resistant K562 and AR230 CML cells were treated with different concentrations of ATO and IFNα. The effect of the treatment on cell proliferation was performed using MTT assay. Synergy analysis was calculated using the compusyn software. Using a retroviral *bcr-abl* T315I transduction murine CML model, we studied the effect of ATO/IFNα on the survival of leukemic mice harboring this famous mutation.

Results: Our preliminary results demonstrated that ATO and IFNα synergized to inhibit the proliferation of imatinib-resistant CML cells. Importantly, this combination significantly prolongs the survival of CML mice carrying the T315I mutation.

Conclusion: Our preliminary data provide clear evidence demonstrating a potential preclinical efficacy of ATO/IFNα in TKI-resistant CML models, specifically in CML mouse models with the T315I mutation, resistant to all available primary and secondary TKI.

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Abstract # 57

Mode of Action of Parthenolide andST1926in Treatment of HHV8-associated Primary Effusion Lymphoma

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Introduction: Primary effusion lymphoma (PEL) is a B-cell neoplasm, caused by the Human Herpes Virus 8 (HHV8) infection and manifested as malignant effusions in body cavities. PEL cells do not present conventional genetic cancer mutations, however the oncogenesis is attributed to HHV-8 latent genes, *LANA-1/2*, *v-cyclin* and *v-FLIP*. PEL is life threatening to immunocompromised and elderly patients since they relapseafter standard chemotherapy treatments, thus the need for new effective and targeted drugs. Among promising drugs, parthenolide (PTL), a natural sesquiterpene lactone and potent NF-κB inhibitor, was reported to have anti-cancer activities against a variety of hematopoietic malignancies and cancer stem cells. ST1926, a novel orally available synthetic retinoid, exhibited a targeted apoptotic and genotoxic effect in numerous human tumor models

The aim of this study was to elucidate the anti-tumor activities and underlying molecular mechanisms, of PTL and ST1926 on PEL *in vitro* and*in vivo*.

Methods: Human PEL (BC1, BC3)and non-PEL (RAJI) cell lines and ascites derived fromPEL-like mouse model were used.The anti-proliferative activities of ST1926 and PTL weredetermined using MTT cell proliferation assay. Flow cytometry was used to detect cell cycle distribution and apoptosis using propidium iodide and Annexin V-FITC kit. The protein expression of apoptosis-regulated genes was examined using Western blot analysis. The drugs effect on latent viral transcripts expression was studied via qRT-PCR.

Results: Our results show that ST1926 and PTLdisplay potent anti-proliferative effects on PEL cell lines and ascites. Each drug separately resulted in increased preG1population and in cell cycle arrest, increased p53 protein levels and PARP cleavage in PEL cells and ascites. In addition, ST1926 downregulated alltested viral latent transcripts in *exvivo* PEL ascites.

Conclusion: The promising anti-cancer and anti-viral effects of PTL and ST1926 drugs could provide a novel basis for clinical application in PEL.

Keywords: Primary Effusion Lymphoma, HHV8, Parthenolide, Retinoid ST1926, Apoptosis

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Abstract # 58

Reciprocal Communication between Niche Stromal Cells and Leukemic Cells: Role of Junctional Complexes and Exosomes in Leukemia.

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Keywords: exosomes, intercellular communication, leukemia, connexins, Tax.

Introduction: A specialized microenvironment in the bone marrow niche is composed of stromal cells including mesenchymal stem cells (MSCs) that might support leukemia progression. In this study, we investigated the reciprocal interaction between MSCs and cell lines from different leukemia types, Chronic Myeloid Leukemia (CML), Acute Myeloid Leukemia (AML) and Adult T-cell Leukemia (ATL), as models of hematological malignancies. We evaluated the role of paracrine interaction between MSCs and leukemic cells through soluble factors, and direct interaction *via* adhesion (N-cadherin), communication (Connexins) and *via* exosomes.

Methods: Indirect co-cultures between MSCs and leukemic cells were assessed in Transwell chambers whereby MSCs were seeded in the bottom well and leukemic cells were in the upper chamber. Direct co-cultures were performed at 1:1 ratio and cells were then sorted and used in co-culture experiments. Leukemic cells-derived exosomes were isolated by ultracentrifugation and co-cultured with MSCs. Following co-cultures for 72 hours, we studied cell proliferation by Trypan Blue Exclusion assay and expression profile of metastatic and stemness markers by Real-time PCR and western blotting.

Results: After indirect co-culture, MSCs induced a moderate increase in AML and ATL cells proliferation. In contrast, MSCs proliferation was only induced by HuT-102 cells. Although indirect co-culture did not cause major changes in leukemic cells expression profile of metastatic markers, direct interaction enhanced the expression of Cx43, SDF-1 and VEGF, especially in the adherent fraction of leukemic cells. Finally, exosomes derived from C81 and HuT-102, HTLV-I positive cells, contained Tax oncoprotein and induced MSCs proliferation. This interaction led to an increase in MSCs cell number, a change in cellular morphology and an increase in the expression of VEGF and stemness markers, Oct-4 and Nanog.

Conclusion: These findings demonstrate that indirect and direct interactions reciprocally affect MSCs and leukemic cells properties. Interaction through leukemia-derived exosomes modulates MSCs properties which might in turn contribute to leukemia progression.

Abstract # 59

Design, Synthesis and Biological Evaluation of Ferutinin and its Analogues on Breast Cancer Cell Lines

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Keywords: Breast cancer, estrogen receptors, *Ferula hermonis*, ferutinin.

Introduction: Ferutinin (FRT), the main active component of *Ferula hermonis*, is a phytochemical with an estrogenic and an anti-proliferative inducing activity on several cell lines including human breast cancer. FRT has been reported as an agonist to estrogen receptor ERα and agonist/antagonist to ERβ. The present study aims to hemi-synthesize FRT analogues in order to investigate their effect along with FRT on *in vitro* growth, cell-cycle progression, apoptosis induction and stem-like properties of breast cancer cell lines.

Methods: FRT analogues were designed using molecular docking then hemi-synthetized by esterification reactions. Cell lines (MCF-7, estrogen dependent and MDA-MB-231, estrogen independent) were treated with FRT and its analogues. Cell proliferation, cell cycle and DNA damage inducing effects of FRT were studied by MTT assay, propidium iodide and DAPI staining, respectively. Sphere formation assays were used to follow the effect of FRT on breast cancer stem cells.

Results: FRT analogues were hemi-synthetized as potential antagonists to ERs. FRT enhanced the proliferation of MCF-7 cells at low concentrations and inhibited the proliferation at higher doses. Conversely, only an anti-proliferative effect of FRT was observed on the MDA-MB-231. FRT induced a pre G_0/G_1 cell cycle arrest in both cell lines. DAPI staining revealed that FRT induces apoptosis in these cells. Cancer stem cell population (CD44 high, CD24 low) was specifically targeted by FRT in MDA-MB-231, whereas it was enriched in MCF-7 cells. Two of FRT analogues remarkably inhibited the growth of the two cell lines by 35-fold on MCF-7 and by 3-fold on MDA-MB-231 when compared to the original molecule.

Conclusion: Our results indicate that the natural compound FRT antagonizes tumor cell growth in both breast cancer cell lines. Two of the investigated FRT analogues showed higher efficiency than the natural molecule and could be suggested as potential candidates for breast cancer therapy.

Abstract # 60

Anti-Cancer Activity of Isoeugenol and Anethole against MDA-MB-231 Breast Cancer Cell Line.

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Keywords: Breast cancer, anethole, isoeugenol, phenylpropenes.

Introduction: Breast cancer is the second most common type of cancer worldwide. Phenylpropanoids, found in daily diet, are widely distributed secondary metabolites in plants with several biological properties. They exhibit potential anti-oxidant, anti-inflammatory and anti-cancer activities. The present study investigates the anti-proliferative activity, cell cycle progression and Epithelial-Mesenchymal transition (EMT) markers of two phenylpropenes (isoeugenol and anethole) against the human breast cancer cell line MDA-MB-231.

Methods: Viable cells were assessed using Trypan blue exclusion assay and cell proliferation was measured using real time cell analysis (RTCA) assay. Cell cycle progression was evaluated by propidium iodide. Transcriptional expression of EMT markers (E-cadherin, N-cadherin and snail) and the pro-angiogenic factor (VEGF) were evaluated using real time PCR. Cell invasion was investigated using RTCA.

Results: Both compounds induced a dose-dependent decrease in cell viability and proliferation after 24, 48 and 72 hours reaching the highest inhibition at 100µM. Both phenylpropenes induced S-phase cell cycle arrest at 50µM after 24h treatment. Conversely, low concentration of isoeugenol (1µM) and anethole (10 and 25µM) caused a cell cycle arrest at the G0/G1 phase. On the other hand, a dose dependent accumulation of cells in the pre-G0/G1 phase was observed with anethole treatment (10-50µM). Upon isoeugenol and anethole treatment, E-cadherin expression was significantly up-regulated while N-cadherin was down-regulated leading to EMT inhibition. These compounds inhibited VEGF expression as well. Nevertheless, they seemed to induce the invasion of MDA-MB-231 cells through the extracellular matrix.

Conclusion: Our data suggest that anethole and isoeugenol have potent anti-proliferative activity against MDA-MB-231 cell line.

Abstract # 61

BioThymoquinone Nanoparticle Formulations Enhance its Anticancer Potential

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Introduction: Thymoquinone (TQ) is a natural product with promising anticancer activity, but its development is hindered by its limited bioavailability. Drug encapsulation has been often used to overcome low drug solubility, bioavailability as well as non specific targeting. For this project, we aimed at synthesizing different TQ nanoparticles (TQ-NPs), characterize them and test their anticancer potential *in vitro* in a panel of breast cells that range from normal to less aggressive cancers and highly aggressive and invasive cancer cell lines.

Methods: TQ nanoparticles (TQ-NPs) were prepared by flash nanoprecipitation using a confined volume impinging jet and controlled flow rates as described by Johnson and Prud'homme in 2003. The size of the TQ-NPs was measured by dynamic light scattering (DLS) and TQ amounts were quantified by High Performance Liquid Chromatography (HPLC). The TQ-NPs stability was monitored over 1 month by DLS. The NPs were further characterized by measuring their entrapment efficiency, loading capacity and zeta potential. Finally, MTT assay was used to assess cell viability *in vitro* after treatment with free TQ, TQ-NPs or blank NPs.

Results: We were successful at preparing four different stable TQ-NPs formulations that had an average diameter size between 45-130 nm. All TQ-NPs had also high entrapment efficiency and loading content that ranged between 72-83% and 70-90%, respectively. When testing TQ-NPs *versus* free TQ and blank NPs by MTT assay against normal MCF-10-A breast cells, MCF-7 breast cancer cells as well as the more aggressive MDA-MB-231 breast cancer cell line, we found that high TQ loading NPs enhance the antitumor activity of the drug when compared to free TQ while being less cytotoxic to the normal cell line. No significant cytotoxicity of the blank NPs was noted.

Conclusion: The results generated from this project describe a new approach for the enhancement of TQ anticancer activity and therefore greatly contribute to the translation of this molecule to the clinic for various applications.

Key words: Thymoquinone, breast cancer, nanoparticles

Abstract # 62

Differential efficacy of p53 restoration in induction and maintenance of senescence in premalignant and malignant cells

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Keywords: p53, oncogene-induced senescence, premalignant tumor

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Abstract

Background and aims: Restoration of p53 in tumors has been suggested as a possible therapeutic approach, based on preclinical *in vivo* and *in vitro* evidence of possible efficacy. However, the relationship between the timing of p53 restoration and its potential for efficacy is still unclear. In the current study, we evaluated the effect of p53 restoration in premalignant and malignant tumors, and assessed the role of p53 in maintenance of tumor suppression.

Results and methods: We used *in vitro* cell culture models, and an *in vivo* model of Cyclin D1-driven pineoblastoma, as well as inducible p53 mouse models. We found that restoration of p53 in murine pre-malignant proliferating pineal tumors resulted in effective cell cycle exit and induction of cellular senescence, while p53 restoration in established malignant pineal tumors did not impact cell proliferation, unless paired with DNA damaging therapies. Interestingly, in premalignant lesions induced to senesce by p53 restoration, inactivation of p53 after senescence resulted in re-entry into the cell cycle, and rapid tumor progression, suggesting that p53 activity is constantly needed to maintain cellular senescence and tumor suppression. These findings were also validated in cell culture models. Evaluation of a panel of human supratentorial primitive neuroectodermal (sPNET) tumors, of which pineoblastoma is a subtype, showed low activity of the p53 pathway, while only one of 6 tumors had p53 deletion or mutation.

Conclusion: Together, this data suggests that restoration of p53 has different effects in premalignant versus pineal tumors, where it may need to be paired with DNA damaging agents, to engage it in tumor suppression. Furthermore, p53 activation may need to be continually sustained for effective tumor suppression in such lesions undergoing senescence. Finally, p53 restoration approaches may be worth exploring in sPNET, where the p53 gene seems to be intact but the pathway inactive in the majority of examined tumors.

Abstract # 63

Regulation of Ceramide by the Novel Synthetic Retinoid ST1926-Induced Cell Death in HTLV-1 Transformed and Malignant T cells

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Background and Aims: Adult T-cell leukemia/lymphoma (ATL) is an aggressive hematological neoplasm characterized by the oligoclonal proliferation of infected T cells after a long latency period of infection with the HTLV-1 virus. The viral oncogene Tax maintains progression and pathogenesis of the disease. The synthetic retinoid ST1926 inhibits growth and induces apoptosis in a wide variety of cancer cells including those that are resistant to all-*trans*-retinoic acid (ATRA). ST1926 showed potent antitumor activities in solid and hematological malignancies with minimal side effects. Therefore, this compound is currently being tested in cancer clinical trials.

Ceramide, a well-known lipid tumor suppressor, induces differentiation, cell cycle arrest, and apoptosis in several types of tumor cells. Depending on different inducers of stress response, ceramide accumulation occurs as a result of sphingomyelin breakdown or by *de novo* synthesis. We investigated the effect of ST1926 on the i) growth and apoptosis of HTLV-1 positive (HuT-102 and MT-2) and negative (Jurkat and Molt-4) malignant T cells, ii) ceramide accumulation and iii) mechanism of ceramide production.

Methodology and Results: Using pharmacologically achievable micromolar concentrations, we have shown that ST1926 inhibits proliferation of HTLV-1 positive and negative malignant T cells using MTT cell proliferation and trypan blue exclusion assays. ST1926 induced massive apoptosis, as evident by PARP cleavage and TUNEL positivity, in all tested malignant cells. ST1926 treatment resulted in Tax oncoprotein degradation in HTLV-1 treated cells, and induced a dose- and time-dependent accumulation of ceramide in HTLV-1 positive and negative malignant T cells, as revealed by DGK assay. Most importantly, ST1926 activated *de novo* ceramide pathway as evidenced by using [^{3}H] labelled palmitic acid.

Conclusion: These results highlight the potential role of ST1926 in increasing ceramide levels, thus lowering the threshold for apoptosis. Future studies will address the regulation of ceramide metabolism by ST1926 in malignant T cells. Finally, *in vivo* studies will decipher the role of ceramide in ST1926 apoptotic response in ATL and T lymphoma.

Keywords: Adult T-cell leukemia, Retinoid ST1926, Ceramide, Apoptosis
Funding Source: CIDR Research funds

Abstract # 64

The synthetic retinoid ST1926 as a novel therapeutic agent in rhabdomyosarcoma

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Keywords: Rhabdomyosarcoma, Retinoid ST1926, Cell cycle, DNA damage

Introduction: Rhabdomyosarcoma (RMS) is the most frequent soft tissue sarcoma in children. Despite multiple attempts at intensifying chemotherapeutic approaches to treatment, minimal improvements in survival have been made for patients with recurrent or advanced stage disease. All-trans retinoic acid is a differentiation agent that has shown some anti-tumor efficacy in RMS cells in vitro, however the effects are of low magnitude. (2E)-3-[3'-(1-adamantyl)-4'-hydroxy[1,1'-biphenyl]-4-yl]-2-propenoic acid (ST1926) is a novel orally bioavailable compound belonging to the class of synthetic atypical retinoids.

Methods and Results: In this study, we used a panel of 5 RMS cell lines in order to investigate the potential therapeutic effects of ST1926 in RMS. By MTT assay, we found that ST1926 reduced RMS cell viability in all tested alveolar (ARMS) and embryonal (ERMS) RMS cell lines, at readily achievable pharmacological micromolar concentrations. ST1926 induced an early DNA damage response, identified by western blot, which led to an S-phase cell cycle arrest (as confirmed by propiduim iodide FACS analysis) and a reduction in CDK1 levels, irrespective of p53 mutational status. Interestingly, in ARMS cell lines, ST1926 treatment resulted in a decrease in levels of PAX3-FOXO1 fusion protein, and this suppression occurred at a translational level. Importantly, ST1926 was effective in reducing the growth of ARMS and ERMS xenografts in nod-SCID mice, and induced a prominent DNA damage response detected by immunohistochemistry.

Conclusion: We conclude that ST1926 has preclinical efficacy against RMS, and should be further evaluated in in clinical trials.

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Abstract # 65

Differential Expression of Kinin and Retinoid Receptors in Human Colorectal Cancer Cell Lines

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Keywords: Colorectal Cancer, Inflammation, Bradykinin Receptors, Retinoids, Retinoid Receptors.

Background: Chronic inflammation is a major characteristic of the development and progression of several tumors, in particular colorectal cancer. The connection between inflammation and tumorigenesis has been well supported from pharmacological, epidemiological and genetic data. However, the molecular mechanism by which inflammation promotes cancer growth constitutes a broad area of ongoing investigation. Hence, multiple key genes involved in oxidative stress and inflammation, such as the kallikrein-kinin system (KKS), should be addressed in this context. Previous studies from our lab showed that bradykinin activates the ERK1/2 pathway which generally promotes cell survival but might in cancer have a pro-apoptotic effect. Retinoids are major regulators of epithelial cell proliferation, apoptosis and differentiation and have shown promise in preclinical colorectal cancer studies. Therefore, we were interested in investigating a potential crosstalk between KKS and the retinoid receptors (RARα, RARγ and RXRα).

Aim: In the present study, we tested for the effects of bradykinin receptor 1 and 2 (B1R and B2R) agonists, all-*trans* retinoic acid (ATRA) and the synthetic retinoid ST1926 on the proliferation and cell death on human colorectal cancer cells with different genetic background. We investigated as well the molecular mechanisms and downstream pathways involved.

Methodology: We used well characterized human colorectal cancer cell lines with different genetic *p53* and *p21* status (HCT116, HCT116 $p53^{-/-}$, HCT116 $p21^{-/-}$, and HT29). Using the MTT assay, we tested for the effect of B1R and B2R agonists and retinoids on cell proliferation. This will be also applied on normal colon epithelial cells to be used as control. Real-time qPCR and Western Blot analysis were performed for characterization and profiling of B1R and B2R and the different retinoid receptors (RARα, RARγ and RXRα) in the tested cell lines.

Results: We showed that the colorectal cancer cell growth was inhibited by B1R and B2R agonists and ST1926 but unaffected by ATRA. We also observed a differential expression of B1R, B2R, and the different retinoid receptors in the tested colorectal cancer cell lines. In the future, we are interested in investigating a potential crosstalk between B1R or B2R and the retinoid receptors, to test whether their activation or inhibition would lead to a decrease in the inflammatory state and/or an increase in the apoptotic rate of cancer cells.

Abstract # 66

Expression of Connexin43 in Breast Cancer Cells (MDA-MB-231): Implications in Cancer Metastasis.

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Keywords: breast cancer, intercellular communication, connexins, VEGF, NSG mice.

Introduction: Connexins regulate cell proliferation, differentiation and function. Several human diseases are linked to mutations or dysfunction of connexin protein, and were shown to be implicated in carcinogenic processes. Several studies have reported channel-dependent and -independent roles of connexins in tumorigenesis with a solid base proving that connexins are tumor suppressive proteins. Our study hypothesize that the over-expression of connexin43 (Cx43) decreases the metastatic potential of a breast cancer cell line MDA-MB-231, while its knock-down by shRNA enhances their invasive properties.

Methods: Upon over-expressing or knocking-down Cx43 in MDA-MB-231, metastatic abilities such as cell proliferation, cell aggregates' morphology in 3D culture system, invasive potential and localization of β-catenin were assessed *in vitro*. *In vivo*, tumor onset and volume, survival rates and cancer cell infiltration to secondary metastatic sites were investigated in immuno-compromised mice injected subdermally with cells over-expressing or knocking-down Cx43.

Results: Results have shown an induction of epithelial phenotype with a suppressed potential to infiltrate lung and liver tissues upon Cx43 over-expression. On the other hand, Cx43 knock-down induced a mesenchymal phenotype with higher expression levels of vascular endothelial growth factor (VEGF), invasive abilities and infiltration to secondary metastatic organ sites.

Conclusion: Cx43 over-expression in MDA-MB-231 breast cancer cell line suppresses its metastatic potential *in vivo* and induces an epithelial phenotype *in vitro*. On the other hand, knocking down Cx43 induces a mesenchymal phenotype with aggressive invasive abilities.

Abstract # 67

Biological Evaluation of Ferutinin and its Analogues on Breast and Ovarian Cancer Stem Cells.

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Keywords: Breast cancer, ovarian cancer, *Ferula hermonis*, ferutinin.

Introduction: The incidence of hormone-dependent cancer (breast and ovarian) is increasing worldwide. Resistance to conventional therapies is associated with cancer stem cells (CSCs) capacity of self-renewal and tumorigenesis. Ferutinin (FRT), the main active component of *Ferula hermonis*, is a potent phytoestrogen acting as agonist to ERα and agonist/antagonist to ERβ. FRT and hemi-synthetic analogues exhibit anti-proliferative activity on breast cancer cells. The present study aims to investigate the effect of FRT and its analogues on *in vitro* growth and cell-cycle progression on ovarian cell lines. A comparison study was then conducted on sphere formation capacities of ovarian and breast cancer cell lines.

Methods: Cell proliferation assay (MTT) and Trypan blue exclusion test were performed to evaluate the anti-proliferative activity of FRT and its analogues against ovarian cell lines (OVCAR-3, IGROV-1 and SKOV-3). Cell cycle analysis (propidium iodide staining) was studied by flow cytometry. Sphere formation assays were used to follow the effect of FRT on ovarian and breast cancer stem cells.

Results: FRT exhibited a significant decrease in cell viability on ovarian cancer cell lines. The highest anti-proliferative activity was observed with FRT analogue A1 on OVCAR3 whereas all three derivatives were less potent on SKOV-3 and IGROV-1. Moreover, FRT and its analogues induced G_2-M cell cycle arrest in a dose dependent manner. FRT reduced sphere formation capacity by 53 %, 36% and 100% in OVCAR-3, IGROV-1, SKOV-3 cells, respectively when compared to 40 % in MCF-7 and 54% in MDA-MB-231 cells. FRT targeted CSCs-like population (CD44 high, CD24 low) in MDA-MB-231 but it enriched this population in MCF-7 cells.

Conclusion: Our results indicate that FRT and its potent analogue A1 may be considered as a promising adjuvant compound for treatment of breast and ovarian cancer, whereas more investigation on the mechanism by which this natural compound and its analogue exert their effects are required.

Abstract # 68

Effects of sub-lethal High Intensity Focused Ultrasound (HIFU) exposure on mechanotransduction and cytotoxic response to anti-neoplastic agents in MCF-10A and MDA-MB-231 cells

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Key words: Breast cancer, HIFU, mechanotransduction, chemotherapy

Descriptive statement: Given that HIFU results in pressure/tension waves that can cause cellular deformations, we hypothesize that sub-lethal HIFU could result in mechanotransduction alterations that may modulate cellular response to anti-neoplastic agents.

Introduction: HIFU is a therapeutic modality used to destroy solid tumors. At the focal point, cell death can result from cavitation and/or thermal ablation. However, the effects of sub-lethal HIFU exposure on cell function remain to be elucidated. We aim to examine the alterations in mechanotransduction resulting from the exposure of cells to ultrasonic waves and to determine consequences on cellular response to anti-neoplastic agents.

Methods: A HIFU setup was custom-designed to permit utilization of a 2.158 MHz transducer for *in vitro* exposure of MCF-10A immortalized mammary epithelial cells and MDA-MB-231 invasive breast cancer cells. Real-Time PCR was used to quantify mechanosensitive gene expression. Cellular viability was assessed using trypan blue exclusion assay.

Results: We measured significant enhanced expression of *CAV-1α, PXN*, and *Hic-5;* immediate-early in MCF-10A and delayed in MDA-MB-231 cells. Additionally, we noted an immediate-early transient increase in *TTLL4* expression in both cell lines and in *TWIST1* expression in MDA-MB-231 cells. Notably, sub-lethal HIFU exposure had no significant effect on the expression of *CAV-1*(total pool), *CTSD*, and *HSPA1A* in both cell lines. Moreover, sub-lethal HIFU exposure at 6hr or 30hr prior to the *in vitro* addition of agents sensitized both cell lines to sub-cytotoxic doses of Taxol and Doxorubicin. Furthermore, MDA-MB-231 cells surviving single or dual rounds of HIFU exposure at the focal spot and passaged over 3-to-6 weeks in culture show no significant change in their *in vitro* sensitivity to Taxol or Doxorubicin.

Conclusion: Work is underway to determine if sonoporation plus other mechanisms that are related to mechanotransduction alterations are implicated in the enhanced cellular sensitivity to sub-cytotoxic doses of Taxol and Doxorubicin post sub-lethal HIFU treatment

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Abstract # 69

miRNA Expression Profile Analysis of Lebanese Breast Cancer Tissues

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Keywords: microRNA, microarray, breast cancer, biomarkers, Lebanon.

Descriptive statement: In order to find non-invasive early biomarkers for breast cancer in the Lebanese population, we screened for the expression of microRNA, small noncoding RNA, and their predicted BC initiation targets in normal and cancerous breast tissues.

Abstract: Introduction: Breast cancer (BC) is the most common type of cancer in Lebanese women with a higher percentage of young-aged patients than the West. microRNA (miRNA), a large group of small noncoding RNA, regulate 60% of all protein-coding genes and play a vital role in cancer development. Since the etiology of BC initiation is still not well-studied, we are interested in studying differentially expressed miRNA with potential tumor initiation function. We have recently shown that differential expression of certain miRNA in Lebanese BC tissues could be variable to what is reported in West. Hence, the objective of this study is to investigate the global miRNA profile in Lebanese BC tissues and to identify through in silico tools the relation of dysregulated miRNA to tumor initiation.

Methods: miRNA profiling was performed using Affymetrix GeneChip miRNA 3.0 array after RNA extraction of formalin fixed paraffin embedded 48 tumor and 22

normal adjacent tissues from Lebanese BC patients. Validation of dysregulated miRNA was done using quantitative reverse transcription real time PCR. In silico tools such as Diana tools and ingenuity pathway analysis were used to predict the tumor initiation role of dysregulated miRNA.
Results: A total of 54 miRNA were significantly differentially expressed between tumor and normal adjacent breast tissues. The top differentially expressed miRNA were validated. Using in silico tools, most of dysregulated miRNA were found to be involved in cancer, reproductive system disease, organismal injury and abnormalities and p53 signaling pathway. More analysis of the targets of miRNA and their implication in BC initiation is still in progress.
Conclusion: Lebanese BC patients have a set of dysregulated miRNA expression profile mostly implicated in cancer. Further functional studies will be done on the dysregulated miRNA to comprehend BC onset especially in young patients.

Funding Source: Medical Practice Plan and Kamal A. Shair CRSL

Abstract # 70

Effect of Connexin 43 Loss on Polarity and Initiation of Tumorigenic Pathways in the Phenotypically Normal Breast Epithelium

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Key words: Connexin, Polarity, Mammary epithelium

Descriptive Statement: Connexin 43, a major protein component of gap junctions, through forming the complex assembly with associated partner proteins at the membrane, regulates polarity and direction of cell division in the mammary epithelium.

Position: Ph.D. Student at the Cell and Molecular Biology Program- Biology Department

Funding Source: University Research Board (AUB), Lebanese National Council for Scientific Research-Doctoral fellowship, Lebanese National Council for Scientific Research, Medical Practice Plan (AUB-Medical School), International Breast Cancer and Nutrition Project (Purdue)-Purdue University, IN, USA, UNESCO-L'Oreal International Fellowship for Women in Science

Abstract: Recent evidence suggests a regulatory role for Connexin (Cx) 43, a gap junction (GJ) protein, in apical polarity establishment that is a key property of epithelial tissues and is disrupted early on during tumorigenesis. We have previously demonstrated a Cx43 context-dependent tumor-suppressive role mediated partially by a GJ complex assembly that sequesters Cx43-associated proteins β-, α-catenin and ZO-1 proteins at the cell membrane of breast epithelial tumor cell lines and contributes to lumen formation and maintenance in non-neoplastic breast epithelial cells. However, the mechanism that governs Cx43 role in regulating tumor suppression is not fully elucidated. In this study, HMT-3522 S1 non-neoplastic breast epithelial cells were used to decipher the mechanism through which Cx43 contributes to the homeostasis of the normal mammary epithelium. For this purpose, Cx43 was stably silenced in S1 cells using Cx43-specific shRNA along with a nonspecific (NSS) shRNA as control. S1 cells were cultured in three-dimensional (3D) conditions that permit the formation of physiologically relevant epithelial glandular structures (acini). A significant rise in the proliferation rate of S1 cells was noted in response to Cx43 silencing, as reflected by an increase in the size of S1 acini. Enhanced proliferation was accompanied by improper acinar morphogenesis and

loss of the ability of S1 cells to form monolayered acini. Moreover; preliminary data from invasion assays revealed that the loss of Cx43 gives the capability for S1 cells to invade, through a layer of diluted Matrigel, to a higher extent than the control and T4-2 cells, the tumorigenic counterparts of S1 cells. These changes, in addition to the disruption of apical polarity in acini and mislocalization of both ZO-1, an apical polarity marker, and β-catenin, a protein involved in the control of epithelial to mesenchymal transition (EMT), indicate a shift in the phenotype into one that enables neoplastic development. Furthermore, Cx43 silencing altered the apico-lateral localization in S1 acini of the protein Scrib, a key regulator of apical polarity and a tumor suppressor recently reported to be involved in the control of EMT in murine lens epithelium and in the regulation of murine mammary gland progenitor activity. We propose that Cx43 tumor-suppressive role may be mediated through an impact on pathways involved in EMT.

Abstract # 71

Radiotherapy Induces Injury and Retards Proliferation in Normal Mammary Epithelial Cells within the Treatment Field

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Keywords: Radiotherapy, Mammary epithelium, Inflammation, Proliferation

Descriptive Statement: Radiotherapy retards proliferation and upregulates levels of inflammatory mediators, interleukin-6 (IL-6) and nitric oxide (NO), in normal mammary epithelial cells within the treatment field.

Abstract:

Introduction: Radiotherapy is a well-established cancer treatment strategy that has been used for more than 100 years. However, radiotherapy causes acute or late side effects such as radiogenic second cancers. These effects may result from primary radiation within the treatment field or from secondary radiation outside the treatment field emitted either from the treatment unit or from within the patient. The dose-effect relationship for side effects in radiotherapy settings is poorly understood, especially outside of the treatment field. The purpose of this study was to assess effects of a radiotherapy setting on proliferation of normal epithelial cells and to measure levels of IL-6, nitric oxide (NO) and matrix metalloproteinases (MMPs), inflammatory mediators indicative of cell injury, in four locations: at center of treatment field (i.e. prescription location), at edge of field, outside of field and far outside field.

Methods: An in-vitro model of inflammation was previously established in our laboratory, whereby SCp2 cells, normal mouse mammary epithelial cells, upregulate levels of IL-6, NO and MMPs in response to endotoxin stimulation. In this study, SCp2 cells were exposed to radiation doses and energy spectra characteristic of those received by a cancer patient in a radiotherapy setting, both inside and outside treatment fields.

Results: Our results demonstrate, within 24hrs post-radiation, reduced proliferation in cells located at center or at edge of treatment field, and a concomitant increase in levels of IL-6 and NO, but not MMPs. No significant effect for radiation was noted in cells located outside or far outside of treatment field.

Conclusion: Collectively, results suggest that primary radiation may cause late injury in normal tissues within treatment field. Further studies are warranted to decipher mechanisms associated with normal tissue injury induced by primary radiation and to observe injury outside the treatment field that relates to late effects such as second cancers. This might provide means for targeting specific signaling pathways and counteracting side effects of radiotherapy in order to ensure effectiveness of treatment, while sparing normal tissues.

Funding Source: University Research Board (URB; AUB, Lebanon), Lebanese National Council for Scientific Research (LNCSR; Lebanon), Naef K. Basile Cancer Institute (AUB, Lebanon) and Fogarty International Center (National Institutes of Health; USA)

Abstract # 72

Ceramide Inhibits PKCθ by Regulating its Phosphorylation and Translocation to Lipid Rafts

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Descriptive statement: Excessive activity of protein kinase C theta (PKCθ) is the basis of multiple auto-immune and inflammatory diseases as well as cancer and diabetes. The sphingolipid ceramide regulates the activity of PKCθ during T cell activation raising the possibility that it may have some therapeutic significance in disorders of the immune system where excessive inflammation is the problem.

Abstract

Background and aims: Protein Kinase C (PKC) θ is a novel, calcium-independent member of the PKC family of kinases identified as central player in T cell signaling and proliferation. Upon T cell activation by antigen-presenting cells, PKCθ gets phosphorylated and activated prior to translocation to the immunological synapse where it couples with and activates downstream effectors. Our laboratory has previously shown that PKCθ may be regulated by ceramide, a critical sphingolipid that is known to induce differentiation, growth arrest, and apoptosis. In this study, we intend to elucidate the mechanism of inhibition of PKCθ by ceramide.

Methods: We induced Jurkat cells with PMA or anti-CD3/anti-CD28 antibodies following induction of ceramide by adding exogenous ceramide, bacterial sphingomyelinase, or Fas ligation. The effects of ceramide on the translocation and expression level of total and Thr538-phosphorylated PKCθ were studied in both the lipid raft and non-raft fractions of the plasma membrane. The kinase activity of PKCθ was monitored in response to both exogenous and endogenous accumulation of ceramide as well as in the presence of phosphatase inhibitors.

Results : Ceramide produced by exogenous addition, sphingomyelinase treatment, or Fas ligation prevented Thr538 phosphorylation on PKCθ, a step that is critical for its subsequent activation and translocation to lipids rafts. This inhibition is unlikely to be a nonspecific effect of ceramide on membrane reorganization as it did not disrupt lipid rafts. Other closely related lipids, namely dihydroceramide, palmitate, and sphingosine, did not produce similar effects. Addition of the phosphatase inhibitors okadaic acid and calyculin A reversed the inhibition exerted by ceramide indicating involvement of a ceramide-activated protein phosphatase.

Conclusion: These findings point to a novel mechanism of regulation of the pro-inflammatory PKCθ involved in certain physiological as well as pathological immune responses, and hence ceramide may prove valuable in therapeutic approaches for such disorders involving autoimmunity or excessive inflammation.

Keywords: Ceramide, PKC □, translocation, phosphorylation, lipi

Funding source: None

Abstract # 73

Effect of $1,25(OH)_2D_3$ and $25(OH)D_3$ on Naïve and Total $CD4^+$ T cells' Differentiation and Cytokine Production

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Abstract: The active form of vitamin D $1,25(OH)_2D_3$ was shown to decrease IL-17A expression in $CD4^+$ T cells and exert an anti-inflammatory effect on immune cells. The inactive vitamin D precursor $25(OH)D_3$ has a 1000 fold higher concentration than $1,25(OH)_2D_3$ in serum. We sought to determine the effects of $1,25(OH)_2D_3$ and $25(OH)D_3$ on $CD4^+$ T cells' differentiation and proliferation *in vitro*.

Purified naïve ($CD3^+CD4^+CD45RA^+$) and total $CD4^+$ T cells were activated with beads coated with anti-CD2, anti-CD3 and anti-CD28 in Th17 and Treg polarizing conditions in the presence or absence of $1,25(OH)_2D_3$ or $25(OH)D_3$ at a concentration of 10 nM and 250 nM, respectively. Cells were harvested on day 6 of cell culture, and processed for multiparameter staining and flow cytometry. Cell culture supernatants were reserved for a simultaneous quantitation of multiple cytokines by cytometric bead array for IL-17A, IL-10, IL-6, TNF-α, IL-2, IFN-γ, and IL-4.

Our results show that $1,25(OH)_2D_3$ and $25(OH)D_3$ have a comparable effect on $CD4^+$ T cell responses. Naïve and total $CD4^+$ T cell proliferation was not affected significantly, but in Th17 polarizing cultures, the percentage of $CCR6^+IL\text{-}17A^+CD4^+$ T was significantly decreased in cultures containing vitamin D. Furthermore, the frequency of cells expressing intracytoplasmic IFN-γ and TNF-α was also decreased. Interestingly, in the presence of $1,25(OH)_2D_3$ or $25(OH)D_3$ regulatory T cell (Treg) polarized $CD4^+$ T cells upregulated CD25 and CTLA-4 expression but not Foxp3.

This is the first comparative study of the effect of $1,25(OH)_2D_3$ and $25(OH)D_3$ on naïve and total $CD4^+$ T cells. Our results suggest that $25(OH)D_3$ is active on immune cells and has similar effects as $1,25(OH)_2D_3$ on T cell differentiation and cytokine production. $25(OH)D_3$ is the precursor of $1,25(OH)_2D_3$ and the serum levels of $25(OH)D_3$ have been reported to be low in the Lebanese population. Our findings that $25(OH)D_3$ is active in immune cells suggest that its deficiency may affect immune function even if $1,25(OH)_2D_3$ levels are normal. Whether $25(OH)D_3$ uses the same receptor as $1,25(OH)_2D_3$ still needs to be determined.

Keywords: Vitamin D, Th17, Treg

Descriptive statement: This study aims at addressing the effect of the active and precursor forms of vitamin D on $CD4^+$ T cell differentiation. *In vitro* polarization of T lymphocytes to a Th17 (inflammatory) or Treg (regulatory) T cell phenotype in the presence or absence of vitamin D will help us understand the immunomodulatory effects of vitamin D.

Funding Source: MPP and CNRS

Abstract # 74

HOE140 as a Possible Biased Agonist Modulating the Actions of Bradykinin B2 and Thromboxane Receptors in Vascular Smooth Muscle Cells

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Keywords: Bradykinin, Thromboxane, VSMCs, biased agonist, ERK1/2, βarrestin-2

Abstract

Background: Bradykinin (BK) and thromboxane mediate their actions on vascular smooth muscle cells (VSMCs) through their G-protein coupled receptors, B2R and TP, respectively. We previously demonstrated a synergistic ERK1/2 activation in VSMCs co-stimulated with BK and IBOP (TP stable agonist). Using the TP antagonist, SQ29548, we also provided evidence of possible TP-B2R hetero-dimerization. Equally important, the concept of biased agonism, whereby a ligand preferentially activates one signaling cascade among others downstream of a receptor, has been recently employed in drug discovery to design more selective drugs with fewer side effects.

Aim: We herein aim to investigate the effects of B2R selective antagonist, HOE140, on TP-B2R trafficking and signaling properties, and whether HOE140 could duplicate the findings of SQ29548 and/or possibly be acting as a biased agonist.

Methodology: To monitor fold over basal changes in ERK1/2 phosphorylation, Western Blot analysis was conducted on protein extracts of treated VSMCs. To track the localization of human B2R and TPα, immunofluorescence was performed on HEK293Ts co-transfected with B2R, TPα, and βarrestin-2 (B2R-TPα-βarr2-HEKTs). Previously, we showed that BK, but not IBOP, promotes co-internalization of B2R and TPα in these cells.

Results: Pretreatment of VSMCs with HOE140 attenuated individual BK- or IBOP-induced ERK1/2 activation. However, surprisingly, incubation of VSMCs with HOE140 followed by co-stimulation with BK and IBOP maintained (BK+IBOP)-synergistic ERK1/2 activity rather than reversing it. Besides, both endosomal and membranous co-localization of TPα, B2R, and βarrestin-2 were realized in HOE140-stimulated B2R-TPα-βarr2-HEKTs. However, pretreatment of B2R-TPα-βarr2-HEKTs with HOE140 prior to stimulation with BK or (BK+IBOP) inhibited co-internalization of receptors and βarrestin-2 recruitment. On the contrary, an interesting finding of βarrestin-2-mediated co-internalization of both receptors was realized in B2R-TPα-βarr2- HEKTs stimulated with IBOP alone following HOE140 pretreatment.

Conclusion: These findings present HOE140 as a possibly novel biased ligand and provide additional evidence of possible TP-B2R hetero-dimerization. We are currently investigating the possible mechanism mediating HOE140 actions and its functional implications on vascular physiology.

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Abstract # 75

Kinin signaling in kidney glomerular cells: Role in Diabetic Nephropathy

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Diabetic Nephropathy (DN) is the most common cause of end stage renal disease and the main cause of morbidity and mortality in diabetes. Glomerular injury, a pivotal event initiated by DN, is characterized by mesangial matrix deposition and podocytopathy including podocyte loss. An early marker of DN, microalbuminuria, signifies high risk for progressive renal failure and is strongly correlated with glomerular injury. The risk factors and mechanisms involved in the pathogenesis of DN are still not completely defined. Previous data generated from our laboratory demonstrated new mechanisms and functions of B2 kinin receptors in DN. Diabetic B2R-/- null mice display reduced albumin excretion rate (AER), and reduced glomerular and tubular injury compared with diabetic B2R+/+ mice. In the current study we aimed to understand the cellular mechanisms through which activation of B2 kinin receptors contribute to the initiation and progression of DN. Stimulation of cultured rat podocytes with bradykinin (BK) resulted in a significant increase in reactive oxygen species generation (ROS) and this was associated with a significant increase in NOX1 and NOX4 protein and mRNA levels. BK stimulation also resulted in a signicant increase in the phosphorylation of ERK1/2 and Akt and this effect was inhibited in the presence of NOX 1 and Nox 4 siRNA. Furthermore podocytes stimulated with BK resulted in a significant increase in the protein and mRNA levels of connective tissue growth factor (CTGF) and at the same time a significant decrease in the protein and mRNA levels of nephrin. siRNA targeted against NOX1 and NOX4 significantly inhibited the BK-induced increase in CTGF. Nephrin expression was increased in response to BK in the presence of NOX1 and NOX4 siRNA, thus implicating a role for NOXs in modulating the BK response in podocytes. Moreover, nephrin expression in response to BK was also significantly increased in the presence of siRNA targeted against CTGF. These findings provide new insights into novel aspects of BK signal transduction pathways in pathogenesis of DN and identify novel targets for interventional strategies.

Abstract # 76

Expression and Regulation of Connexins under "Inflammatory" state: Communication between Human Intestinal Epithelial Cells and Immune Cells.

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Keywords: Inflammation, Epithelial cells, Macrophage, Connexin, GJIC, IBD.

Introduction: Inflammatory bowel diseases (IBD) are related to functional impairment of intestinal epithelial cells (IECs) due to infiltration of the sub-mucosa by inflammatory cells. Three essential mechanisms may contribute to the induction of this state: (i) soluble mediators, including exosomes, secreted by inflammatory cells, (ii) direct adhesion and signaling molecules expressed on the surface of immune cells and epithelial cells and (iii) cytoplasmic exchange of specific signals between the inflammatory cells and IECs *via* gap junction (GJ) channels. In this study, we explored the nature of the interaction between human IECs and macrophages (MΦ) in an *in vitro* model of IBD.

Methods: We investigated the role of inflammatory cells in inducing IECs Matrix Metalloproteinases (MMPs) by gelatin zymography as a mechanism that results in breaching the sub-epithelial basement membrane and bringing inflammatory cells in close proximity with IECs. We then identified potential adhesion molecules and connexins (Cxs) involved in intercellular communication and studied the effect of inflammatory mediators on connexins expression at the transcriptional, protein, cellular localization and functional levels.

Results: IECs and MΦ express Cx26, Cx43, and Cx45; however, Cx32 was only expressed in IECs. Connexin 26 and Cx43 expression and MMPs enzymatic activity were significantly up regulated in IECs under inflammatory conditions, which resulted in enhanced functional IECs-MΦ intercellular communication.

Conclusion: We propose that the combination of paracrine and hetero-cellular communication between IECs and MΦs play a pivotal role in the regulation of epithelial cell function by establishing junctional complexes between inflammatory cells and IECs that might stabilize leakiness of intestinal epithelial barrier function.

Abstract # 77

Oxidant Activity of Sumac Fruit Extracts on Human Muscle Stem Cells and Zebrafish Embryos.

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Keywords: Myoblasts, muscle dystrophies, sumac, anti-oxidant activity.

Introduction: Muscle dystrophies are a group of inherited muscle disorders characterized by progressive muscle weakness. Cell therapy performed in skeletal muscle patients with muscular dystrophy is a promising therapy; nevertheless oxidative stress reduces muscle precursor cells (myoblasts) survival. Enhancement of the survival can be achieved by pre-treating cells with anti-oxidant molecules. This study aims to test the anti-oxidant properties of Sumac fruit (*Rhus coriaria L.*) extracts on human myoblasts *in vitro* and on zebrafish embryo *in vivo*.

Methods: The cytotoxic effect of ethanolic crude extract (70%) from sumac and its ethyl acetate (EtOAc) fraction were tested using the Trypan blue exclusion assay. The ability of these extracts to protect cells against induced oxidative stress by hydrogen peroxide (H_2O_2) was studied by cell count, cell cycle, adhesion assays and dihydroethidium (DHE) staining. Real-time PCR was performed to evaluate the transcriptional expression of MyoD and myogenin in human myoblasts (LHCN-M2). *In vivo* experiments using zebrafish model were performed to test the sumac extract effect on the viability of 24, 48, 72 and 96 hours post-fertilized embryos.

Results: These results demonstrate that pre-treatment of LHCN-M2 with sumac extracts increased the viability of cells after inducing oxidative stress. A concentration of 0.3 $\mu g.mL^{-1}$ of EtOAc fraction exhibited the best protective effect against H_2O_2 induced oxidative stress and restored cellular adhesion. Furthermore, we showed that superoxide dismutase mediates the protective effect of ethyl acetate fraction without any modification of MyoD and myogenin expression. *In vivo*, zebrafish embryo pre-treatment with low concentrations of EtOAc fraction protected embryos from H_2O_2 induced death.

Conclusion: Low concentrations of ethyl acetate fraction enhance myoblast survival *in vitro* and increase zebrafish embryo viability *in vivo* after exposure to H_2O_2 induced oxidative stress.

Abstract # 78

Reciprocal Communication between Hematopoietic and Mesenchymal Stem Cells: Role of Junctional Complexes in Hematopoiesis.

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Keywords: Hematopoietic stem cells, mesenchymal stem cells, niche, hematopoiesis, connexins.

Introduction: A specialized microenvironment in the bone "the niche" composed of stromal cells including mesenchymal stem cells (MSCs), support hematopoietic stem cells' (HSCs) self-renewal and differentiation. The role of paracrine interaction through soluble factors in the niche has been extensively explored; however, the direct intercellular communication is yet to be elucidated. This project was developed to study the role of MSCs in modulating HSCs differentiation through direct interaction *via* connexins and N-cadherin.

Methods: Mononuclear cells (MNCs) were isolated from mobilized peripheral blood using ficoll gradient centrifugation. Hematopoietic stem cells were isolated from MNCs by cell sorting using CD34 as specific HSCs marker and then used in co-culture experiments. Indirect co-cultures between MSCs and HSCs were performed using Transwell chambers whereby MSCs were seeded in the bottom well and HSCs were in the upper chamber. Direct co-cultures were assessed at 1:1 ratio and cells were then separated by cell sorting using CD73 as a specific MSCs marker. Following 3 days of co-culture, gene expression of connexins, tight junction and adhesion molecules, migration, mesenchymal and stemness markers was examined by Real-time PCR and western blotting. HSCs differentiation was evaluated using Colony Forming Unit (CFU) assay.

Results: MSCs express different transcripts of connexins including Cx43 and 45, N-cadherin and β-catenin, Oct-4 and Nanog, SNAIL, TWIST, VEGF and SDF-1. A reciprocal interaction exists between MSCs and HSCs whereby the expression of adhesion and communication markers were either induced following direct co-culture or decreased after indirect co-culture. We also demonstrated that MSCs increase the clonogenic potential of HSCs comparing to freshly sorted HSCs.

Conclusion: These preliminary findings demonstrate a potential role for connexins in regulating hematopoiesis and in controlling HSCs fate. Over-expression and down-regulation experiments will be developed to decipher this role.

Abstract # 79

Role of the Retinoblatoma protein, pRb, in the survival of Adult-Born Neurons in the Olfactory Bulb

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Keywords: neurogenesis, Rb, olfactory bulb, conditional knock-out mice, survival, differentiation

Descriptive statement: this study investigates how the tumor suppressor gene, pRb, controls of the survival and function of new-born neurons in the adult brain, specifically the olfactory bulb.

Background and aims*:* Neurogenesis is the developmental process leading to the formation of neurons in the developing brain. This process is ongoing throughout life in the adult mammalian brain, typically in the hippocampus and olfactory bulb (OB). We have recently shown that Rb regulates the rate of proliferation of adult progenitors in the subventricular zone (SVZ) by inducing its deletion using Nestin-CreERT2-YFP/YFP and Rb flox/flox animals. Hence, loss of Rb causes increased progenitor's proliferation with no subsequent effects on the migration of neuroblasts along the rostral migratory stream (RMS) or their differentiation into GABAergic subtypes inside the OB. The aim of this study is to examine and confirm the role of Rb in the survival and terminal differentiation of new born neurons in the adult OB.

Methods*:* 8-week old Rbflox/flox and Rbflox/+ mice were stereotaxically injected in the SVZ with a mixture of CAG-GFP/Cre (Cre virus) and CAG-RFP (control virus) retroviruses, and, sacrificed 28d later. Fast proliferating cells (type C) were randomly transduced with GFP/Cre virus, RFP control virus or both viruses. Exon 19 of the Rb floxed allele(s) was/ere excised by Cre thus inducing Rb's deletion. Brains were sectioned and immunostained with anti-GFP and anti-RFP antibodies to assess neuronal survival, and, NeuN and calretinin (CR), to assess terminal differentiation of new-born neurons. In addition, we used a second Nestin-CreERT2-YFP/YFP line and conducted a birth-dating study by administering a series of BrdU injections following tamoxifen treatment and sacrificing the animals 28d later.

Results*:* we determined the fraction of RFP-GFP double-labeled cells among the RFP-control cells and found the percentage of GFP+;RFP+ cells in the OB identical in both genotypes indicating normal survival of new born neurons in Rb-/- vs Rb+/- animals (n=3Ct and 3 mut). Neuronal maturation was also identical between genotypes as assessed by the percentage of GFP+ cells co-expressing NeuN, and, CR, separately. We did not observe an increase in new-born OB neurons in Rb-/- mice as described earlier which could be due the random/variable nature of viral transduction and/or, alternatively, to the relatively low number of transduced cells compared to the high rate of endogenous recombination seen in Nestin-Cre lines. Finally, after analysis of the second NestinCreERT2 line, we detected a significant increase in GFP+, BrdU+ and GFP+;BrdU+ cells in the mutant OB compared with controls and this was consistent with our previous data.

Conclusion: Rb is not required for the survival and terminal differentiation of adult-born GABAergic neurons in the OB and this is not consistent with its role during development when its loss severely affected neuronal differentiation and migration in the telencephalon. Our data indicate that the Rb pathway may be manipulated in order to expand the pool of adult progenitors for regenerative purposes in the future.

Abstract # 80

Knock-out of the bradyzoite marker *p18* in *Toxoplasma gondii*: insights towards a functional characterization during neurotoxoplasmosis

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Keywords: *Toxoplasma*, Bradyzoite, *p18*

Descriptive Statement: The dynamics of disease progression and development in case of Toxoplasmosis is an interplay between acute and chronic infection phases. The mechanisms of interconversion between these phases are poorly understood and the molecular players are yet to be identified. We investigated the involvement of a chronic-phase-specific surface marker *p18*.
Funding: Seed fund, Medical Practice Plan and Cèdre.
Background and Aims: *Toxoplasma gondii* is an apicomplexan protozoan parasite that infects all warm blooded animals including humans. *T. gondii* causes a severely morbid or fatal disease in fetus and immunocompromised patients. During its life cycle, *T. gondii* exhibits three morphologically infectious stages: tachyzoites, bradyzoites, and sporozoites. Tachyzoites are rapidly multiplying and responsible for the acute toxoplasmosis leading to tissue damage. Bradyzoites are slow-growing and responsible for the chronic neurotoxoplasmosis that often reactivates in immunocompromized patients. Lastly, sporozoites are the infective forms found in oocysts. The back and forth switch between tachyzoite and bradyzoite stages is a key modulator of the progression of toxoplasmosis between acute and chronic phases. However, this switch remains very poorly understood.
We have investigated the role of the bradyzoite marker p18 in this frame for which the gene sequence is annotated on www.toxoDB.org.
Methods: We have used the selection vector (P2854) containing the selectable marker cassette hypoxanthine-xanthine-guanine-phosphoribosyl-transferase (HXGPRT) and cloned the 5' and 3' flanking regions of *p18*. This vector was introduced by electroporation to the Pru *Δku80* strain which favors its integration by double crossing over and homologous recombination. We have successfully generated and cloned the Pru *Δku80Δp18* knock-out parasites and investigated their phenotype *in vivo*.
Results: Deleting *p18* led to the formation of more bradyzoite cysts in the brains of mice. However, these bradyzoites reactivated much later than the wild type strain. This result drove us to investigate the phenotype of the Pru *Δku80Δp18* knock out during the acute phase of infection. We could clearly see a better survival rate of mice infected with the knock-out strain as compared to the wild type strain, furthermore, we have seen less parasites in all tested organs except in the brain where the amount of tachyzoites from both strains was similar.
Conclusion: These results strongly suggest a role of *p18* in the reactivation process and require further investigation on the immune profile of the knock-out parasites.

Abstract # 81

Screening for the Prevalence of EGFR and ALK Mutations in LungAdenocarcinoma Patients in the Levant Area, a Prospective Analysis.

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Co-Investigators: Maya Khalil, Alain Mina, Hind Rafei, Najla Fakhreddin, Rami Mahfouz, Fadi Farhat, Shadi Hamouri, Haifa Dbouk, Ziad Salem, Haidar Rabee, Ghazi Zaatari.

Keywords: Lung Adenocarcinoma, EGFR mutation, EML4-ALK translocation, Levant Area.

Our study is a multicenter prospective study of formalin fixed paraffin embedded (FFPE) tissues from patients diagnosed with lung adenocarcinoma to assess the prevalence of EGFR and ALK mutations in the Levant Area.

Submitting Co-Investigator: Alain Mina, MD, Post-Doctoral Research Fellow.

Funding source: Pfizer Pharmaceutical.

Introduction: background and aims: Recent evidence suggests that a significant percentage of lung adenocarcinomas have a driver mutation that is responsible for the development of the tumor and promotion of its growth and spread. Significant variation in the prevalence of these mutations has been documented with race and smoking status being two major factors (more common in Asians and/or non-smokers). To date, no data has prospectively assessed the prevalence of these mutations in a Middle Eastern population and that will be the aim of our study.

Methods: Patients with lung adenocarcinoma were prospectively enrolled as part of a multicenter, multinational study. Tumors were tested for various EGFR mutations in exons 18-21 by PCR and for EML4-ALK translocation by FISH Break-Apart test. Study was open in 10 sites in Lebanon, Jordan and Iraq and was restricted to patients of Middle Eastern origin.

Results: 180 patients have been recruited so far. 120 (67%) males and 60 females (33%) with a mean age of 62.6 years. EGFR testing was done in 166 patients and has been found to be Wild type in 142 (85.6%) patients, mutated in 24 (14.4%). Tissue has been insufficient in 12 (6.7%) of the samples and 2 (1.1%) of the results are still pending. As for the EML4-ALK translocation, it has been tested in 115 patients. Results were negative in 112 (97.4%) patients and positive in 3 (2.6%). 21 attempts at ALK testing failed (11.7%) while the remainder of results are either pending (6 (3.4%)), unattainable due to insufficient tissue (12(6.7%)) or undone because patients were either EGFR or KRAS mutation positive (26(14.4%)).

Conclusion: Because of overwhelming evidence in favor of the survival benefits of targeted therapies, assessing the prevalence of clinically significant mutations has become a must. Although the frequencies of such mutations (KRAS, EGFR, ALK…) have been well established in Western populations, our study will be the first to establish their frequencies in the Levant area. Preliminary results have shown an EGFR mutation rate of 14.4% and an ALK translocation rate of 2.6% in our patient population as compared to a 15-20% and 5% in western literature respectively.

Abstract # 82

A Lebanese population-based study of copy number variations in autism

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Keywords: Autism; Copy number variations; RYR2; TBCE

Funding source: The work is supported by a generous grant from OpenMinds and from AUBMC (Program Projects in Biomedical research).

Abstract

Introduction: Autism is a neurodevelopmental disorder characterized by ritualistic-repetitive behaviors, impaired non-verbal communication and language development. Prevalence estimates of autism have increased over the last 20 years. Although autism is considered to be genetic in 20-25% of cases, its extreme heterogeneity has defied genetic classification. Recent studies show an increased implication of copy number variations (CNVs) in the pathogenesis of the disease.

Methods: Affymetrix Cytogenetics Whole-Genome 2.7 M and CytoScan™ HD Arrays were used to detect CNVs within 41 Lebanese autistic children. Cohorts of 33 normal participants and 35 non-autistic developmentally delayed and/or intellectually disabled patients were used as controls.

Results: We identified 16 *de novo* CNVs and 57 inherited CNVs, of which 16p11.2 duplications and 2p16.3 deletions have been previously reported as pathogenic. We also identified two likely pathogenic duplications at 1q42.3 and 1q43 encompassing RYR2 and TBCE, respectively, as ASD candidate genes. A further duplication of unknown significance at 10q11.22 has been proposed as a modulator for the phenotypic expression of the disease.

Conclusion: Our results identify new genetic targets in autism spectrum disorder (ASD). Uncovering a set of genes responsible for autism in the Lebanese population will facilitate diagnostic work-up and genetic counseling for ASD in Lebanon. Discovery of novel ASD genes may also lead to untapped and novel therapeutic targets.

Abstract # 83

Association between *Neuronal Ceroid Lipofuscinosis CLN3* gene expression and clinical characteristics of breast cancer patients

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Keywords: *CLN3*, breast cancer, HER2, ceramide, sphingolipid signaling

Abstract

Introduction: Breast cancer (BC) is one of the most common cancers in women worldwide. Elucidation of underlying biology and molecular pathways is necessary for improving therapeutic options, hence, improving clinical outcomes. Neuronal Ceroid Lipofuscinosis 3 protein (CLN3p) is anti-apoptotic, and defects in the *CLN3* gene cause accelerated apoptosis of neurons in juvenile Batten disease and upregulation of ceramide. Dysregulated apoptotic pathways are often implicated in the development of the oncogenic phenotype. Predictably, CLN3p was upregulated in a number of human and murine breast cancer cell lines.

Method and Results: Here, we determine *CLN3* expression in non-tumor vs. tumor samples from fresh and formalin-fixed/paraffin-embedded (FFPE) breast *tissue and* analyze the association between *CLN3* overexpression and different clinicopathological characteristics of breast cancer patients. Additionally, gene expression of 28 enzymes involved in sphingolipid metabolism was determined. *CLN3* mRNA is overexpressed in tumor vs. non-tumor breast tissue from FFPE and fresh samples, as well as in MCF7 BC compared to MCF10A normal cells. Of the clinicopathological characteristics of tumor grade, age, menopause status, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), only absence of HER2 expression correlated with *CLN3* overexpression. Sphingolipid genes for ceramide synthases 2 and 6 (*CerS2*; *CerS6*), delta(4)-desaturase sphingolipid 2 (*DEGS2*) and acidic sphingomyelinase (*SMPD1*) displayed higher expression levels in breast cancer vs. control tissue, whereas, ceramide galactosyltransferase (*UGT8*) was underexpressed in breast cancer samples.

Conclusion: *CLN3* may be a novel molecular target for cancer drug discovery, and this may be achieved via modulation of ceramide pathways.

Abstract # 84

Performance of chromosomal microarray for patients with intellectual disabilities/developmental delay, dysmorphism, and/or multiple congenital anomalies in a Lebanese cohort

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Keywords: Chromosomal microarray analysis, duplication/deletion, mosaicism, developmental delay/intellectual disabilities

Introduction: Chromosomal microarray analysis (CMA) has become the standard-of-care by the American College of Medical Genetics for individuals with developmental delays (DD), intellectual disabilities (ID), birth defects, dysmorphic features and autism spectrum disorders (ASD). CMA not only allows the identification of smaller chromosome abnormalities (deletions and duplications) than those detected with conventional chromosome analysis but it also allows the investigation of their integral genes and the detection of loss-of-heterozygosity (LOH) regions. Here we present our experience in implementing this technology in 97 cases referred to the AUBMC Special Kids Clinic.

Methods: CMA were assessed for 97 unrelated children referred for DD/ID, dysmorphic features and/or multiple congenital anomalies (MCA) using the Affymetrix® Cytogenetics 2.7M and CytoScan HD arrays.

Results: Out of 97 cases studied, 15 had abnormal CMA results harboring a total of 16 chromosomal abnormalities. Two of these abnormalities were mosaic aneuploidy: One case of mosaic trisomy for the entire chromosome 9 and one case of segmental mosaic duplication for chromosome 12p13.33q12. In addition, we identified a copy-neutral mosaic LOH on chromosome 11q in a third case of mosaicism. One of the patients had a rare genotype consisting of two chromosomal aberrations: a deletion on chromosome 5p15.33p1531 and a duplication on chromosome 12p13.33p13.2. Of the remaining 11 abnormal CMA results, 5 were duplications (on chromosomes 1, 9, 16, and 17), and 6 deletions (on chromosomes 1, 13, 17, 18, 22 and X).

Conclusion: With a detection rate of chromosomal abnormalities around 15.4%, which is 4-5 times that of traditional chromosome analysis, this study further supports CMA use as a first line diagnostic tool for Lebanese individuals with DD/ID, ASD, and MCA. Elucidating the etiology of these patients' developmental delays will result in appropriate patient management and care and will help assessing recurrence risk in their families.

Abstract # 85

Tbx5: The missing culprit gene in Thalidomide toxicity

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Abstract

Holt-Oram syndrome (HOS) is a rare autosomal dominant disease associated with upper limb malformation, congenital heart defects (CHD) and/or conduction abnormalities caused by a haploinsufficiency of T-box transcription factor 5 (Tbx5). Congenital heart disease (CHD) is a leading cause of death, with an incidence of approximately 6–8 in 1,000 live births. Only 13 % of all CHD cases are thought to be inherited and the rest are sporadic in nature. Some CHD are caused by environmental factors and teratogens like thalidomide. Thalidomide a sedative drug that was used by pregnant women for morning sickness caused severe malformations similar to HOS in the newborns of those women and thus it was removed from the market. Previous studies showed that Tbx5 transcription was reduced as a response to thalidomide detected by semi-quantitative RT-PCRs on RNA extracted from wing buds of chicken embryos.

We aimed to investigate the effect of thalidomide on Tbx5, suggesting an interaction between them that participated in the cause of the malformations. We used the electric mobility shift assay (EMSA) to show that thalidomide decreases the binding affinity between Tbx5 protein and consensus sequence of T-box. Thalidomide didn't affect the cellular localization of tbx-5 as indicated by Immuno-fluorescence. To assess the effect of thalidomide on transcriptional regulation of Tbx5 on Vascular endothelial growth factor (VEGF) promoter we used Luciferase assay. Suppressed expression activity of VEGF promotor was observed in the presence of thalidomide. Thalidomide could also suppress the interaction of Tbx5 with Gata4 presented by VEGF promoter expression, while it couldn't affect this interaction on the protein level as shown by co-immunoprecipitation assay.

Since the exact mechanism of action of thalidomide is not known yet, our results promise for a novel explanation of how this drug causes CHD and limb deformities by acting on tbx5.

Keywords: Congenital heart disease (CHD), T-box transcription factor 5, Thalidomide, Vascular endothelial growth factor (VEGF)

Funding source: NA

Abstract # 86

Identification of several mutations in *ATP2C1* in Lebanese families: Insight into the pathogenesis of Hailey-Hailey disease

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Background and aims: Hailey-Hailey disease is an inherited blistering dermatosis characterized by recurrent erosions and erythematous plaques that manifest in intertriginous areas. Genetically, HHD is an autosomal dominant disease, resulting from heterozygous mutations in *ATP2C1*, which encodes a Ca^{2+}/Mn^{2+}ATPase. In this study, we aimed at identifying and analyzing mutations in five patients from unrelated families diagnosed with HHD and study the underlying molecular pathogenesis.

Methods: We performed DNA sequencing for the coding sequence and exon-intron boundaries of *ATP2C1*. Heat shock experiments were done on several cell types. This was followed by real-time and western blotting for ATP2C1, caspase 3, and PARP proteins to examine any possible role of apoptosis in HHD. TUNEL staining was done to confirm the western blotting results. We then performed heat shock experiments on neonatal rat primary cardiomyocytes.

Results: Four mutations were detected, three of which were novel. The manifestation of HHD requires both genetic and environmental factors; therefore we performed heat shock experiments on different fibroblasts and HaCaT cells, mimicking the environmental factor seen in HHD. It was found that stress stimuli; temperature stress, leads to an increase in the mRNA and protein levels of ATP2C1 in heat-shocked cells as compared to non-heat shocked ones. However, the increase in ATP2C1 and heat shock protein hsp90 is significantly lower in HH fibroblasts in comparison to normal fibroblasts and HaCaT cells. NO role for apoptosis in the pathogenesis of HHD was found. Heat shock done on rat cardiomyocytes, led to a significant variation in ATP2C1 mRNA and protein levels.

Conclusion: This is the first genetic report of HHD from Lebanon in which we identified three novel mutations in *ATP2C1* and shed light on the molecular mechanisms and pathogenesis of HHD by linking stress signals to the phenotypes. This link was also found in cultured cardiomyocytes suggesting thus a yet uncharacterized cardiac phenotype in HHD patients masked by its in-expressivity in normal health conditions.

Keywords: ATP2C1, heat shock, cardiomyocytes, Hsp90

Funding source: This work was supported by a fund from the MPP and URB of the American University of Beirut to M.K

Abstract # 87

Effect of Type 1 Diabetes on the DNA Methylation, Gene Expression Status of Target Genes Involved in Vascular Complications

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Keywords: Type-1 Diabetes, Vascular Complications, Bisulfite DNA conversion, DNA Methylation, and High resolution Melt analysis.

Background: Epigenetic alterations are known to be implicated in microvascular and macrovascular diabetic complications. Metabolic Memory is the phenomenon in which diabetic individuals who are exposed to high blood glucose, exhibit diabetic complications even after achieving normoglycemia, which is partially resulted from mitotically inheritable changes in DNA methylation patterns on promoter region of key genes involved in inflammation, oxidative stress, cellular fibrosis, renin-angiotensin, apoptosis, migration, and proliferation.

Aim: Correlate the methylation level of the promoter region and gene expression of genes that contribute to micro- and macrovascular complications in type 1 Diabetes, and to check the Global Post-Translational Modifications of the total proteome.

Methods: 30 Male Sprague Dawley rats divided into Controls, Diabetics and Insulin-treated Diabetics, were used. Type-1 Diabetes was induced by a single injection of Streptozotocin (65 mg/Kg) at day 0 of experiment while control groups were injected with a subsequent Saline injection. Two daily subcutaneous insulin injections were supplemented to the treated diabetic rats group, after 2 weeks of diabetes induction, to normalize the blood glucose levels. All rats were monitored up to 6 weeks, and were sacrificed afterwards. Protein, RNA, and DNA were extracted from the rats Aortas (denuded), and kidney cortex. On the other hand, primary rat aortic smooth muscle cells (RASMCs), divided into Controls, High Glucose-treated (20 mM) and Osmotic pressure controls (Mannitol 20 mM) were used. All cells were incubated for 4 weeks. qRT-PCR was utilized to assess the gene expression status for different gene. Bisulfite specific PCR followed by Methylation-Sensitive High-Resolution Melt (HRM) was employed to assess the relation between DNA methylation and gene expression of different genes. LC-MS/MS analysis was utilized to access the global proteomic profile modification due to Hyperglycemia and their Post-Transitional modification.

Results: qPCR results showed High Glucose treatment of RASMC for 4 weeks induces gene expression of Bradykinin receptor B1 (B1R), and B2 (B2R), Connective Tissue Growth Factor (CTGF), Fibronectin (Fn1), and NADPH Oxidase 1 (Nox1). However, it reduced Nox 4 gene expression. On the other hand, the Methylation analysis showed differential hypomethylation of the promoter regions of B1R, B2R, TNFα, TGFβ1 and 2, and IL6 between the *in vitro* and *in vivo* samples. Mannitol did not have the same effect as glucose and hence the observed changes are not due to osmotic pressure.

Abstract # 88

Ceramide accumulation during adenoviral infection: Role of *E4orf4*

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Keywords: Cancer, Adenovirus, E4orf4, Ceramide, Cell death.

Cancer is a leading cause of death worldwide. Current therapies are of limited value because most agents depend on the tumor suppressor p53 for their cell killing. *E4orf4*, one of the early adenoviral genes, seems to overcome this obstacle as it can induce cell death in a wide range of cancer cells independent of p53 status. This raises the possibility of using *E4orf4* in cancer gene therapy in order to overcome resistance to treatment and prevent cancer recurrence. Understanding the mechanisms of its action is critical for its development as a therapeutic agent.

Background: Cancer remains a leading cause of mortality globally. Adenoviruses are promising as therapeutic agents in cancer treatment since they are relatively safe, capable of infecting both dividing and non-dividing target cells, and can be produced in high titers. *E4orf4*, one of the adenoviral genes, represents a major potential tool in cancer therapy. It is able to trigger p53 and caspase-independent apoptosis selectively in cancer cells. The aim of this study is to investigate the mechanism of action of *E4orf4* by examining its regulation of ceramide accumulation, one of the major pathways of p53-independent apoptosis.

Methods: Since expression of *E4orf4* in the resting or dividing cell induces apoptosis, a T-Rex tetracycline-inducible system was constructed in two human cell lines (A549 and MCF7 cells) to characterize its mechanism of action under controlled conditions. Flag-*E4orf4*-GFP gene was inserted in an inducible expression vector pcDNA4/T0 and verified by sequencing. pcDNA4/T0/*E4orf4* and the regulatory plasmid pcDNA6/TR were co-transfected in A549 and MCF7 cells. The clones A549-C5 and MCF7-C8 were established. Real-time PCR and western blot analysis were used to confirm the expression of *E4orf4* in these clones. Analysis of viability by Trypan Blue, cell cycle distribution, and ceramide levels were measured.

Results: Trypan blue analysis revealed no significant changes in cell viability in MCF7-C8 and A549-C5 clones after 6 and 12 hours of induction with respect to their controls. There were cell-type specific changes in cell cycle distribution. In A549-C5 cells, an increase in S and G2/M phases after 6 and 12 hours of induction was found. In MCF7-C8 cells, an increase in sub-G0 phase after 6 hours of induction was observed. Moreover, a 2-fold increase in ceramide levels was observed in MCF7-C8 and A549-C5 clones after 6 hours of induction as compared to their controls.

Conclusion: These findings indicate that there are cell type-specific responses to E4orf4 expression: cell cycle arrest or apoptosis. This correlates with the increase in ceramide levels, a known inducer of both cell cycle arrest and apoptosis. This raises the possibility that some of the actions of *E4orf4* are mediated by ceramide. Better understanding of the mechanisms of *E4orf4* action will help in developing it as a therapeutic reagent for treatment of cancers.

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