

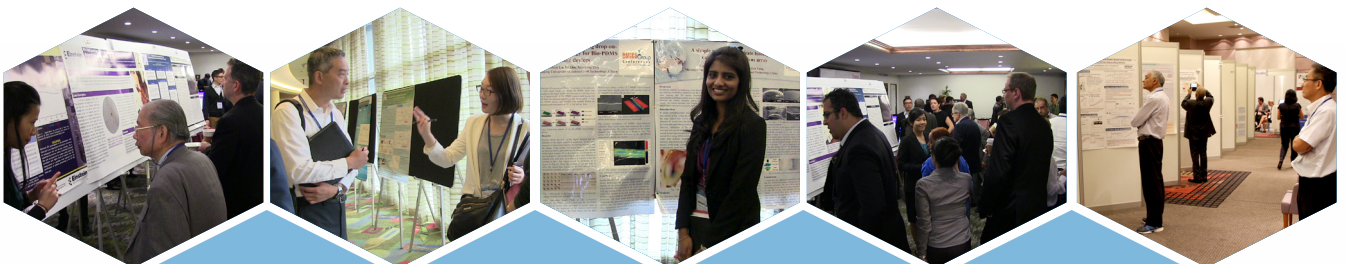
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9th World Digital Pathology & Pathologists Congress

December 05-06, 2016 Madrid, Spain

Posters (Day 2)



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Comparative analysis between growth factors of epiphyseal plate (SOX-9, PTH-rP) and expression of apoptotic modulation factor (BCL-2) in benign and malignant cartilaginous tumors: Correlation with clinical and morphological findings

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Chondrosarcomas (CSs) are a heterogeneous group of tumors with different clinical and morphological manifestations. The distinction between grade I chondrosarcoma (CS1) and enchondroma is difficult. It is necessary to find more precise parameters to assist in diagnosis, histological graduation and prognosis of CSs and therefore its treatment. Some cartilaginous tumors have morphological similarities with epiphyseal plate as: Mesenchymal CS with the immature or rest phase; enchondroma and conventional CS with proliferative chondrocytes and Clear Cell CS with hypertrophic phase cells. Growth and modulation factors interact with chondral cells at different stages of maturation of the plate. SOX-9, PTHrP and BCL-2 act stimulating and/or modulating the growth plate. Probably they have some association with cartilaginous tumors; however their relationship has not been well explored yet. The aim of this study was to evaluate the expression of these molecules with the cartilaginous tumors, correlating with: histological grade; clinical and outcome data. 89 cartilaginous tumors were evaluated: 27 enchondromas, 55 conventional CSs (24CS1; 31CS2+3), 4 Clear Cell CS and 3 Mesenchymal CS. Immunostaining were applied and a score, according to Zhu et al 2013 (modified), was used for analysis. High grade and flat bones cartilaginous tumors and immunolabeling for SOX-9 were associated with poor outcome. PTH-rP over-expression was useful in distinguishing CS 1 from Enchondromas.

Biography

Alexandre do Nascimento is currently pursuing Master's degree in Medical Sciences from Medical School of State University of Campinas (UNICAMP), Brazil, since 2015. He has completed Medical Residency in Anatomic Pathology in 2012 from Clinical Hospital of Federal University of Parana (UFPR), Curitiba, Brazil.

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Immunohistochemical evaluation of Bcl 2, caspase 3, 8 and 9 expressions in canine mammary carcinomas

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The present study investigated immunohistochemical expression of bcl 2, caspase 3, 8 and 9 in the canine mammary carcinomas. We collected 65 paraffin-embedded canine mammary tumor tissue samples and were used as material. These samples were examined histopathologically. After this, routine streptavidin-biotin-peroxidase technique was performed. Data were statistically analyzed using Cronbach's alpha, Kruskal–Wallis and Bonn-Ferroni-Dunn tests. Microscopically, 17 of the cases were diagnosed as tubulopapillary carcinoma, 31 of the cases were diagnosed as complex carcinoma and 17 of the cases were diagnosed as carcinosarcoma. Differences in immunohistochemical expression of bcl-2 was found statistically significant according to tumor types ($P < 0.01$). The other parameters (caspase-3, -8 and -9) were not found statistically significant ($P > 0.05$). In addition, expression of bcl-2 was detected statistically significant in carcinosarcoma cases ($P < 0.01$).

Biography

Ahmet Aydogan has completed his PhD from Adnan Menderes University, Turkey and Post-doctoral studies from Mehmet Akif Ersoy University, Turkey. He is currently an Associate Professor at the Cukurova University, Faculty of Veterinary Medicine, Department of Pathology, Adana, Turkey. He has published more than 30 papers in reputed journals and has been serving as a Reviewer of repute.

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Evaluation of caspases expression in brain lesions of sheep naturally infected with listeriosis

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Background & Aim: Listeriosis is a disease that can cause serious economic losses in sheep. Infection with *Listeria monocytogenes* can lead to many syndromes in animals. The aim of this study was evaluation of caspase expressions in encephalitic listeriosis.

Methods & Materials: A total of 20 sheep brains were histopathologically and immunohistochemically examined. Histopathologically, microabscesses, perivascular and meningeal cell infiltrations were commonly seen. Immunohistochemically, prominent positive reactions were detected in the caudal brain for caspases (CSPs) -3, -7, and -9.

Results: With regard to cell-specific labeling, necrotic neurons were positive for CSP-3 and -9, Purkinje cells were positive for CSP-3 and -7, CSP-3 was found in neutrophil leukocytes, and CSP-9 was observed in lymphocytes. In addition, only the ependymal cells exhibited immunopositivity for CSP-9.

Conclusion: Collectively, our data from naturally infected sheep indicate that CSP-3, -7 and -9 may play roles in encephalitic listeriosis pathogenesis.

Biography

Mehmet Haligur has completed his PhD from Ankara University, Turkey and Post-doctoral studies from Akdeniz University and Mehmet Akif Ersoy University, Turkey. He is currently an Associate Professor at the Cukurova University, Faculty of Veterinary Medicine, Department of Pathology, Adana, Turkey. He has published more than 40 papers in reputed journals and has been serving as a Reviewer of repute.

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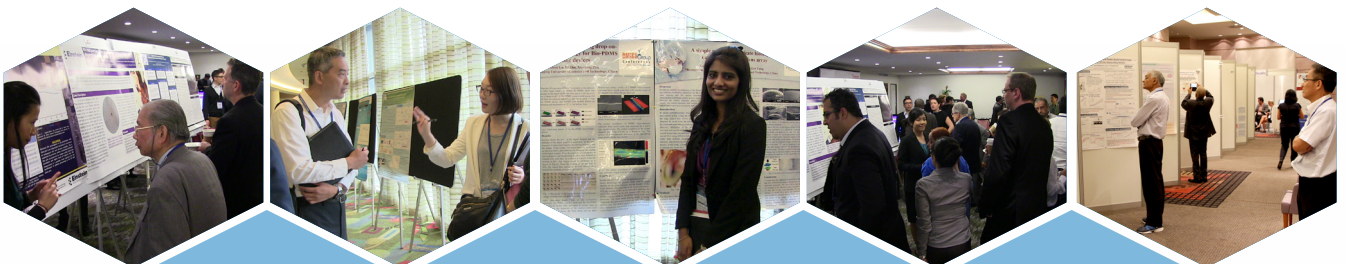
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Demographic and clinical features in combined pulmonary fibrosis with emphysema syndrome patients

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Introduction: Combined pulmonary fibrosis and emphysema (CPFE) has recently been recognized as a new entity. All patients are heavy smokers or ex-smokers. High-resolution computed tomographic (HRCT) scan is 'the gold standard' for the diagnosis. Prognosis is often poor and pulmonary hypertension is common. There is little information on clinical parameters and predictors of mortality.

Aims & Objectives: To identify clinical features and to study some demographic data in Combined Pulmonary Fibrosis and Emphysema Syndrome (CPFE) patients.

Methods: Medical records and HRCT scans from January 2010 through December 2015 were reviewed retrospectively at our hospital. In total 25 patients had interstitial lung diseases (N=25) and from them 12 subjects with CPFE syndrome. Clinical and demographic data were gathered, such as age, gender, smoking history, dyspnoea scale, clubbing, comorbidity, cardiac ultrasound and pulmonary function data. Their values are presented as means±SDs or medians (range), depending on distribution.

Results: In CPFE, predominate smokers or ex smokers and all of them are males. Mean age is 69.3±7.1. Mean UPY (Unit Pack Year) values for smoking status is 40.7±15.6. The mean time from symptoms to diagnosis was 2.08±0.9. The mean partial pressure of oxygen (PaO₂) in CPFE patients was 61.5±9.4 mmHg.

Conclusions: Patients with CPFE syndrome are characterized by dyspnoea often severe, preserved lung volumes, severely impaired gas exchanges. They are severely ill and needs more for oxygen therapy.

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Actinomycosis ovary: A diagnostic dilemma, case report

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Pelvic actinomycosis is a rare presentation of the infection caused by Actinomycetes, presenting as chronic granulomatous inflammation having frequency of 3% and only 2% cases reported in ovary. We present a case of 25 years old female presented in outdoor with complains of abdominal pain and low grade fever for one week. The ultrasonography showed right adnexal mass. The mass was excised and sent for histopathological examination. Histopathological findings were consistent with actinomycosis of the ovary.

Biography

Rabia Butt is Consultant Histopathologist at Chughtais Lab, Lahore, Pakistan. She is an Assistant Professor at Central Park Medical College and Lab Coordinator at Chughtai Lab, Lahore, Pakistan. She has obtained her MBBS degree from University of Health Sciences in 2006 securing one distinction, three silver, five gold medals and first university position.

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Accepted Abstracts



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Strategies for implementation of digital pathology as a primary diagnostic tool

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In the UK, the importance of innovation in pathology is recognized in a recent NHS England report (Digital First: Clinical transformation through pathology innovation). Digital Pathology (DP) is such an innovation with a potential to transform the delivery of diagnostic histopathology services. At the end of 2015, the DP market was valued at US\$327 Million, with a significant increase in market value forecast. However, whilst there have been significant increases in market value along with advances in DP, primary diagnostic use of this technology in the UK is not widespread. Internationally, there are several examples of digital pathology (DP) utilization in Africa; North, South and Central America; Mexico; Asia (Japan, India) and Europe. The main drive for implementation of DP in some countries is the unmet need of the population for quality diagnostic services and appropriately trained staff. This talk will describe the diagnostic histopathology landscape in the UK and discuss strategies that may be adopted to improve the uptake of this technology as a primary diagnostic tool. This will include our own work, in partnership with Philips Digital Pathology Solutions to improve DP training and awareness. Examples of diagnostic DP implementation will be reviewed in order to assess how such examples can inform strategies to improve adoption of DP in the UK.

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An automated method to compute bone marrow density and M:E ratios from H&E slides

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Bone marrow toxicity is an important safety signal in a large variety of pre-clinical drug development programs. Currently, bone marrow cellularity is assessed semi-quantitatively by manual examination of H&E slides by a pathologist. Changes in M:E (Myeloid:Erythroid) cell ratios are challenging to estimate manually from H&E slides. For more accurate assessment, it is necessary to perform a manual differential cell count on bone marrow cytology smears. Both manual procedures are time and resource-intensive. A more efficient quantification process would provide a means to rapidly screen a larger number of slides from studies which exhibited and/or anticipated bone marrow toxicity. An entirely automated image analysis program was designed using Definiens Developer which quantifies changes in overall bone marrow cellularity and approximates M:E ratios and megakaryocyte density in H&E slides. Preliminary validation studies in rats indicate that the automated results correlate well with manual assessment.

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Implementation of digital pathology in the workflow for an integrated health system

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The University of Pittsburgh Medical Center (UPMC) has been at the forefront of implementing and adopting digital pathology. UPMC is an integrated health delivery system, operating more than 20 academic, community and specialty hospitals, more than 500 doctors' offices and outpatient sites, employs nearly 3,600 physicians and offers an array of rehabilitation, retirement and long-term care facilities. Digital pathology has included telepathology as well as primary sign-out. I will discuss the lessons learned from early adoption and recommendations for the optimal utilization of digital pathology will be discussed. We have begun integrating digital pathology in a prospective, in-line fashion within the workflow of our laboratory and this process will be discussed. Some of these lessons include the workflow within the histology/gross laboratory (pre-Imaging variables) as well for the pathologist sign-out work. Additionally, our telepathology efforts will be discussed. We have developed relationships with other sites in the United States as well as several international sites. We have also begun the process of integrating image analysis into the routine workflow for diagnostic pathology. Future areas for development in the field of digital pathology will also be discussed.

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Exposure to excess phenobarbital negatively influences the osteogenesis of chick embryos

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Phenobarbital is an antiepileptic drug that is widely used to treat epilepsy in a clinical setting. However, a long term of phenobarbital administration in pregnant women may produce side effects on embryonic skeletogenesis. In this study, we aim to investigate the mechanism by which phenobarbital treatment induces developmental defects in long bones. We first determined that phenobarbital treatment decreased chondrogenesis and inhibited the proliferation of chondrocytes in chick embryos. Phenobarbital treatment also suppressed mineralization in both *in vivo* and *in vitro* long bone models. Next, we established that phenobarbital treatment delayed blood vessel invasion in a cartilage template, and this finding was supported by the down-regulation of vascular endothelial growth factor in the hypertrophic zone following phenobarbital treatment. Phenobarbital treatment inhibited tube formation and the migration of human umbilical vein endothelial cells. In addition, it impaired angiogenesis in chick yolk sac membrane model and chorioallantoic membrane model. In summary, phenobarbital exposure led to shortened lengths of long bones during embryogenesis, which might result from inhibiting mesenchyme differentiation, chondrocyte proliferation and delaying mineralization by impairing vascular invasion.

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Histopathological and biochemical investigations of protective role of honey in rats with experimental aflatoxicosis

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Aim: The aim of this study was to investigate the antioxidant properties and protective role of honey, considered a part of traditional medicine, against carcinogen chemical aflatoxin (AF) exposure in rats, which were evaluated by histopathological changes in liver and kidney, measuring level of serum marker enzymes, antioxidant defense systems and lipid peroxidation content in liver, erythrocyte, brain, kidney, heart and lungs.

Methods: For this purpose, a total of 18 healthy Sprague-Dawley rats were randomly allocated into three experimental groups: A (Control), B (AF-treated) and C (AF+honey-treated). While rats in group A were fed with a diet without AF, B, and C groups received 25 µg of AF/rat/day, where C group additionally received 1 mL/kg of honey by gavage for 90 days.

Results: At the end of the 90-day experimental period, we found that the honey supplementation decreased the lipid peroxidation and the levels of enzyme associated with liver damage, increased enzymatic and non-enzymatic antioxidants in the AF+honey-treated rats. Hepatoprotective and nephroprotective effects of honey is further substantiated by showing almost normal histological architecture in AF+honey-treated group, compared to degenerative changes in the liver and kidney of AF-treated rats. Additionally, honey supplementation ameliorated antioxidant defense systems and lipid peroxidation content in other tissues of AF+honey-treated rats.

Conclusion: In conclusion, the present study indicates that honey has a hepatoprotective and nephroprotective effect in rats with experimental aflatoxicosis due to its antioxidant activity.

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Serous cystadenoma and fibrothecoma: A rare combination in collision tumor of ovary with pseudo-Meigs syndrome

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Collision tumors are defined as a coexistence of two adjacent but histologically distinct tumors without admixture in the same tissue or organ. They have been reported in various organs but collision tumors involving ovaries are extremely rare. The most common histological combination of collision tumor in the ovary is coexistence of teratoma with mucinous tumors. Very few reports of benign collision tumors involving ovaries have been reported in world literature. In 1937, Meigs described cases of pleural effusion, ascitis and ovarian fibromas and named it Meigs syndrome. In 1954, he limited the syndrome to cases where the removal of tumor cures the diseases. Pseudo-Meigs is a variant, not possessing the original tumor cell types described by Meigs. We report a very unusual combination of fibrothecoma and serous cystadenoma with pseudo-Meigs syndrome. A 63-year old menopausal woman presented with abdominal distention ultrasonography and computed tomography scan revealed large cystic lesion with well-delineated solid area in it. Minimal ascitis was noted. Malignant neoplasm of ovary was suspected. Cytology of ascitic fluid did not showed malignant cells. Carcinoma antigen (CA)-125 was mildly elevated (0.42 IU/ml). Left ovary grossly showed a large uniloculated thin walled cyst with smooth surface and congested vessels. At one end of the cyst, well-demarcated solid homogenous yellowish white mass was seen. Microscopy of multiple sections from solid area revealed benign fibrothecoma. Multiple sections from the cyst wall revealed serous cystadenoma. Final diagnosis of benign ovarian collision tumor of fibrothecoma and serous cystadenoma with pseudo-Meigs syndrome was made.

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Characterizing changes in expression of EMT and metabolic markers during hematogenous dissemination in breast cancer

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Epithelial to Mesenchymal Transition (EMT) and metabolic reprogramming contribute to cancer progression. Here we investigated changes in expression of EMT and metabolic markers during hematogenous dissemination of breast cancer. So, we have performed analysis of EMT (CKs and FOXC1) and metabolic (PGC-1a, COXIV and MCT4) markers in CTCs and tissue samples from naive non-metastatic patients (M0) and metastatic breast cancer patients undergoing therapy (M+). As results, FOXC1 expression was higher in primary tumors than in their correspondent metastases ($p=1.15e^{-4}$). Primary tumors of M+ patients had lower expression of CKs compared to primaries of M0 patients. Both EMT markers were less predominant in CTCs of M+ patients. CTC^{FOXC1+} in M+ patients was associated with HER-2+ primary ($p=0.004$) and T4 tumors ($p=0.036$). Positivity for markers of oxidative metabolism, PGC-1a and COXIV, was significantly higher in CTCs from both M0 and M+ groups when compared to MCT4, an aerobic glycolysis marker. M0 patients presenting CTC^{MCT4+} and CTC^{PGC1a+/COXIV+} had shorter progression-free survival ($p=0.026$). In metastasis, PGC-1a expression was increased while MCT4 was decreased, in comparison to correspondent primary tumors. CTC count and expression of EMT markers changed in CTCs after neoadjuvant therapy while metabolic characteristics were maintained. PGC-1a was the only metabolic marker that presented positivity in CTCs before and after neoadjuvant treatment. This marker also showed increased expression in primary tumor post treatment in one patient. Low or no correlation between CTCs and tumors has been observed for all markers. In this sense, EMT and MET features can be observed in primary tumors and metastasis, respectively and these phenomena can be subjected to alterations by neoadjuvant treatment. The predominance of oxidative metabolism profile in CTCs and metastasis in contrast to aerobic glycolysis in primary tumor suggests that cancer cells reprogram their metabolism from an aerobic glycolysis profile to an oxidative metabolism in order to supply their energetic demand for hematogeneous dissemination. These events can be modulated by neoadjuvant therapy, pointing out metabolic pathways as potential target sites. Furthermore, metastasis is the most common cause of mortality in cancer patients. Therefore, characterization of processes that contribute for the dissemination of tumor cells represents an important approach for impairing colonization of new sites. Here we described changes in expression of EMT and metabolic markers using representative samples of different stages of breast cancer progression: CTCs, primary tumors and metastases. Our data point out CTCs and targeting metabolic pathway as additional therapeutic targets in breast cancer as well as the further investigation of HER2 and FOXC1 connections for understanding and modulation of EMT process in breast cancer.

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Integrated multiregional analysis proposing a new model of colorectal cancer evolution

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Cancer is heterogeneous disease; each tumor in different patients has different cancer genomes. Furthermore, another level of heterogeneity exists: Even a single tumor harbors multiple genetically distinct subclones. This intratumor heterogeneity is presumably one of causes of therapeutic difficulty and its understanding is clinically necessary. In this study, we investigated intratumor heterogeneity in colorectal cancer by analyzing sample obtained from geographically separated regions of 9 colorectal tumors. Our integrated data analyses combined with computational simulation strongly suggest that, after clonally shared alterations were accumulated by aging; numerous subclones were generated by neutral evolution. Importantly, this view can explain the robustness and evolvability of cancer: Therapeutic action inducing an environmental change would convert some of the numerous neutral mutations to driver genes that confer therapeutic resistance. We believe that this study not only provides insights into colorectal cancer pathogenesis but also constitutes a new basis for designing therapeutic strategies.

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Endomicroscopy, new surgical pathology & optical biopsy

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Technological advances in optical microscopy and the diagnostic capacity of a wide variety of optical techniques call for a reappraisal of the role of the pathologist. Today, neither microscopes nor tissue staining and processing are essential for diagnosis. However, in order to set the gold standard for these techniques, more publications on the morphology of in vivo biopsies and non-invasive optical biopsies are needed. Those techniques should be the responsibility of pathologists or alternatively should be a computational pathology left on the hands of Machine Learning techniques and Computer Vision methods that automate image classification to support clinical decisions to thereafter be confirmed by pathologist taken as “gold standards” on any robotic procedure. Following telemedicine similarities, this type of pathology is called: PoCP (Point of Care Pathology) or real time morphological examination at a cellular level. It relies upon software identify procedures showed in the paper such as: Artificial vision, automatic random sub windows and decision trees, content base image retrieval. Together with all novel techniques such as liquid biopsy that should be progressively integrated in a Digital Pathology Lab. We are facing a new sub-specialization that embraces most medical fields, in our case Pathology Informatics. It requires a serious re-definition of medical training to introduce the Body of Knowledge (BoK) of “medical informatics, telemedicine and bioengineering applied to distance or robotic medicine”. We must introduce a transversal and essential topic that assure that the new robotic millennium provide medical support by experienced doctors trained to provide QoC (quality of care) with the forthcoming tools.

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Role of mucosal colonic biopsy in patients with chronic unexplained diarrhea who their colonoscopy is normal

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Objective: There are controversies about the importance of biopsies of normal colonic mucosa in the investigation of patients with chronic diarrhea so the aim of our work to evaluate the significance of mapping biopsy and its yield in patients of apparent endoscopically normal colon who is investigated for chronic diarrhea and to discover the prevalence of hidden diseases.

Methods: Of 300 consecutive patients undergoing colonoscopy by one endoscopist during a five year period, biopsies were taken in 200 cases of unexplained diarrhea of at least 4-6 weeks and their colorectal mucosa appeared macroscopically normal. All biopsies were reviewed by one pathologist.

Results: Of the 200 patients enrolled, 36(18%) cases were classified as no pathological diagnosis and 164(82%) cases showed histopathological changes- 121(73.78%) non specific inflammation, 19(11.58%) ulcerative colitis, 11(6.7%) collagenous colitis, 7(4.26%) lymphocytic colitis, and 6(3.65%) bilharzial colitis.

Conclusion: We conclude that the role of biopsies in chronic diarrhea patients with macroscopically normal colon at endoscopy is high as yielding a histological diagnosis in 26.21% of patients is so benefit to them as they may had modified the treatment after identification of a specific cause as collagenous colitis, lymphocytic colitis, ulcerative colitis and bilharzial colitis. So patients should be subjected to colonoscopy and biopsy to aid specific diagnoses.

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Spatio-temporal analysis of stripe rust on wheat crop in lower Pothohar region

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The present study focused on stripe rust incidences on wheat crop in lower Pothohar region to assess present damaging effects of stripe rust disease on wheat crop through geographic information system tools. As wheat is the main cereal crop that is grown in Jhelum district on both irrigated and non-irrigated lands. This study was done on lower Pothohar region i.e., Jhelum district while comparing the disease incidence data of stripe rust for six years (2010-2015). Climatic data including temperature, precipitation and humidity were analyzed for past six years because these variations are responsible for development of rust diseases in selected regions. Jhelum provides favorable moisture and rainfall to help germinate the rust spores on widespread wheat crop. Geographic information system tools provide valuable information through visual interpretation of attribute data. Standard deviational ellipses showed the extent of variation of disease exhibiting the directional trend for six years in which the ellipse area of stripe rust disease expanded during 2015. Spatial autocorrelation analysis concluded that stripe rust disease is exhibiting dispersed pattern during 2015 with clustered pattern in previous years. Therefore, this study provided the information about spread and shift of stripe rust in Jhelum district through broad spectrum of geospatial analysis that result in real time visualization and predictive analysis. Stripe rust is becoming more prevalent so the wheat varieties resistant against stripe rust needs to be cultivated. Fungicides are also need to be used against development of rust that will ultimately lead to high yield of wheat crop.

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Primary adrenal hemangiopericytoma: The first reported case

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Hemangiopericytomas are rare tumors originating from pericytes in the wall of capillaries. It is a type of soft tissue sarcoma which commonly involves the lower extremities, pelvic retroperitoneum and head and neck. The age of initial diagnosis of HPC is 40.3 years-old (range 16-86) usually presenting as a painless mass. A 32-year old female presented with a 5 year history of slowly-growing right flank mass. She denied any history of hematuria, dizziness, headaches or hypertensive episodes. Physical examination findings showed a bulging right flank and an approximately 15 x 15 cm, palpable, non-tender mass on bimanual examination. CT-scan with IV contrast was requested and revealed a large suprarenal mass, right. She underwent adrenalectomy with en-bloc nephrectomy, right with uneventful post-operative course. Histopathology of the specimen was read as hemangiopericytoma. Further testing by Fluorescence *In-Situ* Hybridization confirmed the diagnosis. Metastatic work-up was done and was negative. Hence, this is the first reported case of primary adrenal hemangiopericytoma. Surgical removal is the mainstay of treatment of hemangiopericytomas. Radiotherapy and chemotherapy have no role in the management of the disease. The lesson learnt from this case is that behind every mask there is a face and behind that, a story. What seemed to be a simple and ordinary as an adrenal mass turned out to be as special and unique as a hemangiopericytoma.

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Histopathology pattern study of breast disease in Aden, Yemen by use digital pathology

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Pattern of breast diseases in Yemen is inadequately studied; we studied the histopathological breast diseases in order to find out the histopathological pattern in patients suffering from breast diseases in Modern Histology Lab, Aden, Yemen. We performed a retrospective and prospective study conducted at Private Modern Histology Lab, Aden, Yemen during the period from January 2005 to December 2008. The data were collected from the referral sheets. A total of 286 biopsies cases of breast diseases 275 (96.2%) were female and 11 (3.8%) were man. In female Benign breast tumor was the most common lesion found is comprising 32.4% in age group between 20-29 years, followed by fibrocystic changes 28.4%, inflammatory lesion 15.3% and accessory breast 4.3%, while malignant cases 19.6% with an incidence pike between 50-59 years (53.8%). Invasive ductal carcinoma 39 (72.1%) was the more common breast carcinoma founded, the tumor size between 2 to 5 cm (56.4%). 35.2% of female had metastasis in axillary nodes, in conclusion the female gender affected by breast diseases more than men with predominance of benign conditions over malignant lesions in both sex. To allocate a special budget for future researches in breast cancer including campaign against breast cancer and raising the awareness of the public about digital pathology breast cancer through mass media, the quality of the histopathological laboratories should be improved by introducing modern techniques for better assessment of surgical pathology specimen. It is important for pathologists, radiologists, to be aware of or try to detect or search for DCIS or LCIS in benign breast tumor.

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Angiomorphometric characteristics of the breast cancer in the peripheral and internal tumoral areas

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The objective of this study is to correlate the morphometric microvascular characteristics of the breast cancer in the peripheral and internal areas of the tumor with the prognostic factors. Histologic sections from 80 cases of infiltrative breast cancer were immunostained with CD34 and evaluated with the image analysis IMAGE J program following the method of Giatromanolaki A., et al. The microvascular count is significantly higher in the tumor margin with gradual decrease toward the internal areas. The microvascular count in both tumoral areas peripheral and internal associated with the tumor size, histological grade and vascular invasion. The microvascular count in the peripheral area show association with the expression of cerbB-2, ki67, while in the internal area associated with the expression of cerbB-2, ki67 and p53. In the peripheral area the decrease of vascular perimeter and compactness and the increase of the factor of shape associated with higher histologic grade. Whereas the decrease of compactness and increase in the factor of shape associated with the positive expression of cerbB-2 and negative expression of estrogen receptor. Regarding the microvasculature in the internal tumoral area the increase of the area, perimeter and ferret (all represent increase vascular size) associated with the expression of ki67, whereas the area associated with the vascular invasion, indicating that in the peripheral tumoral area microvasculature with smaller perimeter and more regular and circular shape associated with poor prognostic factors while in the internal tumoral area microvasculature with larger caliber associated with tumors more proliferative and invasive.

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Protective effects of *Urtica dioica* seed extract in aflatoxicosis: Histopathological and biochemical findings

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The ameliorative potential and antioxidant capacity of an extract of *Urtica dioica* seeds (UDS) was investigated using histopathological changes in liver and kidney of broiler, measuring serum marker enzymes, antioxidant defense systems and lipid peroxidation (malondialdehyde (MDA)) content in various tissues of broilers exposed to aflatoxin (AF). A total of 32 broilers were divided randomly into 4 groups: Control, UDS extract-treated, AF-treated and AF+UDS extract-treated. Broilers in control and UDS extract-treated groups were fed on a diet without AF. The AF-treated group and AF+UDS extract-treated groups were treated with an estimated 1 mg total AF/kg feed. The AF+UDS extract groups received in addition 30 ml UDS extract/kg diet for 21 days. The AF-treated group has significantly decreased body weight gain when compared to the other groups. Biochemical analysis showed a small increase in the concentrations of serum aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transpeptidase and lactate dehydrogenase in the AF-treated group compared to that of the control group, whereas concentrations of these enzymes were decreased in the AF+UDS group compared to that of the AF-treated group. Administration of supplementary UDS extract helped restore the AF-induced increase in MDA and reduced the antioxidant system towards normality, particularly in the liver, brain, kidney and heart. Hepatorenal protection by UDS extracts was further supported by the almost normal histology in AF+UDS extract-treated group as compared to the degenerative changes in the AF-treated broilers. It was concluded that UDS extract has a protective hepatorenal effect in broilers affected by aflatoxicosis, probably acting by promoting the antioxidative defense systems.

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The investigation of effect of *Nigella sativa* on the prevention of aflatoxin induced liver lesions in rats

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Aflatoxicosis is a mycotoxicosis developing acute or chronic conditions caused by aflatoxins in domestic animal and humans. Aflatoxicosis is a widespread problem especially in the underdeveloped and the developing countries. Aflatoxins are potential threat to humans and animal and cause severe economic losses in animal industries. The chronic toxications especially suppress the immune system which facilitates the occurring of many diseases. Liver is main organ affected by aflatoxicosis and are histopathologically observed necrosis, fibrosis and hepatocarcinogenesis. It is not well known the effective protection in aflatoxicosis. However, it is reported that some vitamins, proteins and inorganic substances have a protective effect. In lastly performed studies, it was indicated that *Nigella sativa* (NS) had many pharmacologic effects as such antioxidant, immunomodulatory and anticancer. However, there is scanty study about their protective effects on aflatoxicosis. This study was planned to investigate the effect of NS on the prevention of aflatoxin-induced liver lesions in rats in term of biochemical, histopathological and immunohistochemical methods. This purpose, a total of 30 rats was allotted into one of three experimental groups: A (Control), B (AFB1-treated) and C (AFB1+NS-treated) each containing 10 animals. The rats were sacrificed at 90th day of the experiment. Blood samples for the biochemical analysis and tissue samples from livers for histopathological examination were taken. On the basis of biochemical and histopathological findings, it is concluded that treated plant extract decrease the lipid peroxidation and liver enzymes, increase the antioxidant defense system activity and prevent the liver damage in the AFB1-treated rats. The study indicates that hepatoprotective effects are obtained from the group C (AFB1+NS -treated).

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Role of mucosal colonic biopsy in patients with chronic unexplained diarrhea who their colonoscopy is normal

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Objective: There are controversies about the importance of biopsies of normal colonic mucosa in the investigation of patients with chronic diarrhea so the aim of our work to evaluate the significance of mapping biopsy and its yield in patients of apparent endoscopically normal colon who is investigated for chronic diarrhea and to discover the prevalence of hidden diseases.

Methods: Of 300 consecutive patients undergoing colonoscopy by one endoscopist during a five year period, biopsies were taken in 200 cases of unexplained diarrhea of at least 4-6 weeks and their colorectal mucosa appeared macroscopically normal. All biopsies were reviewed by one pathologist.

Results: Of the 200 patients enrolled, 36(18%) cases were classified as no pathological diagnosis and 164(82%) cases showed histopathological changes- 121(73.78%) non specific inflammation, 19(11.58%) ulcerative colitis, 11(6.7%) collagenous colitis, 7(4.26%) lymphocytic colitis, and 6(3.65%) bilharzial colitis.

Conclusion: We conclude that the role of biopsies in chronic diarrhea patients with macroscopically normal colon at endoscopy is high as yielding a histological diagnosis in 26.21% of patients is so benefit to them as they may had modified the treatment after identification of a specific cause as collagenous colitis, lymphocytic colitis, ulcerative colitis and bilharzial colitis. So patients should be subjected to colonoscopy and biopsy to aid specific diagnoses.

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Association of MDR1 gene polymorphism (G2677T) with Imatinib response in Egyptian chronic myeloid leukemia patients

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Background: Despite the excellent efficacy results of imatinib treatment in CML patients, resistance to imatinib has emerged as a significant problem. Genetic variations in genes involved in drug transportation might influence the pharmacokinetic and metabolism of imatinib. The genotype of a patient is increasingly recognized in influencing the response to the treatment. **Aim:** To investigate the genotype frequencies of single nucleotide polymorphisms (SNPs) G2677T in CML patients undergoing imatinib treatment to determine whether different genotype pattern of these SNPs have any influence in mediating response to imatinib. **Methods:** A total of 96 CML and 90 control samples were analyzed for the human multidrug resistance gene 1 (MDR1) gene polymorphism (G2677T) using polymerase chain reaction-restriction fragment length polymorphism technique. **Results:** Genotype distribution revealed a significant lower frequency of TT genotype in CML patients and non-significant difference in the GG, GT genotype frequencies between patients and controls ($P=0.004$, 0.138 , 0.210 , respectively). GG genotype was significantly higher in chronic phase ($P=0.046$), while GT genotype was significantly higher in Blastic crisis phase ($P=0.002$). There was a significant difference in genotype frequency of G2677T among patients showing response and resistance to imatinib in chronic phase ($P=0.02$). TT genotype was associated with complete hematological response ($P=0.01$), complete cytogenetic response ($P<0.001$), and better molecular response with a significant association ($P<0.001$). GT genotype was associated with partial hematological response ($P=0.01$) and minor cytogenetic response ($P<0.001$). Optimal and suboptimal responses were observed for patients with TT genotype ($P=0.003$). Failure of drug response was associated with GT genotype ($P=0.02$); however, GG had no association with drug response. Multivariate analysis considered GT genotype as independent risk factor for resistance ($P=0.037$), while TT genotype as protective factor against resistance to imatinib ($P=0.008$). **Conclusion:** Determination of MDR1 polymorphisms (G2677T) might be useful in response prediction to therapy with imatinib in patients with CML.

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Meningioma 1 (MN1) expression: Refined risk stratification in acute myeloid leukemia with normal cytogenetics (CN-AML)

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Background: Prognostic stratification of cytogenetic normal acute myeloid leukemia (CN-AML) is an area of active research. **Aim:** The aim of this study was to determine the prognostic importance of the meningioma 1 (MN1) gene expression levels in CN-AML. **Methods:** One hundred patients with CN-AML were diagnosed and MN1 expressions were analyzed using quantitative real-time polymerase chain reaction. **Results:** High expressions were detected in 48 (48%) patients (expression range: $2.35-31.99$, mean: 13.9 ± 8.49) in comparison with 52 (52%) patients with low expression (expression range: $0.02-2.3$, mean: 0.68 ± 0.77). The course of the disease in patients with high MN1 expression was unfavorable. Patients with high MN1 expression was associated with significant low complete remission rate (62.5 vs. 8.4% , high vs. low MN1, $P=0.001$) and high mortality rate (75% vs. 46.1 , $P=0.03$). AML patients with high MN1 expression tended to be refractory (37.5 vs. 19.2% , $P=0.00$) and relapse risk (54.1 vs. 23% , $P=0.02$). Multivariable analysis confirmed high MN1 expression as an independent risk factor for disease-free survival and overall survival. **Conclusion: MN1 over expression independently predicts bad clinical outcome in CN-AML patients.**

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Genomic analysis of intra-tumor heterogeneity unveil cancer evolution

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The ultimate goal of our study is to improve the clinical outcomes for patients with malignancies, particularly of the gastrointestinal (GI) tract; however, such tumors are often refractory to treatment. One of the main causes of this intractability is the genomic heterogeneity of cancer, which complicates the development of genetically based therapeutics. We have proposed two approaches to clarifying when or how malignant cells acquire genomic heterogeneity. One addresses inter-tumor heterogeneity and the other addresses intra-tumor heterogeneity. In order to assess genomic heterogeneity in samples from human cancers, we have applied next generation sequencing and super-computational analysis with simulation. We believe that understanding the development of genomic heterogeneity in cancer cells can help elucidate the evolution of malignancy and may suggest interventions to eliminate the progression of malignancy and ultimately improve prognosis for affected patients. Our work to date has focused primarily on characterizing inter-tumor genomic heterogeneity in cases of esophageal cancer, which is the most intractable malignancy among GI cancers. Based on mutational signatures in 144 cases of Japanese esophageal squamous cell carcinoma (ESCC), the diverse patients could be clustered into three risk-factor subtypes: (1) Environmental factors, i.e., drinking and smoking, (2) polymorphisms in the aldehyde dehydrogenase 2 (*ALDH2*) gene and (3) Polymorphisms in the cytochrome P450 2A6 (*CYP2A6*) gene. To address the analysis of genomic variability within a primary tumor, we employed a novel approach, analyzing multiple regions within a tumor to identify genomic heterogeneities and to determine as much as possible about the order in which they arose. Computational analysis with simulation allowed us to deduce the evolution of a tumor's heterogeneity. In this study, we dissected multiple samples from mutually exclusive tumor regions of nine cases of colorectal cancers and interrogated them with exome sequencing, gene copy number analysis, DNA methylation arrays and microarray-based gene expression studies. In each case, we were able to identify "founder" mutations, which were detected in all regions sampled and progresser mutations that were found in some regions but not all. We found that founder mutations were associated with aging. At the gene copy number level, focal amplifications were observed to occur more frequently in founder mutations, while focal deletions were more common in progresser mutations. Epigenetic annotation indicated that CpG-island hyper methylation was an age-related, early event in tumor development and that global hypomethylation was a feature of tumor progression. This multidimensional survey, coupled with computational simulation, revealed that most intra-tumor genomic heterogeneity is likely to be generated by "neutral evolution" not by "Darwin's Theory of Evolution". In other words, most mutations observed in a tumor arise incidentally and are neutral in terms of tumor progression. Such neutral mutations are called "passenger mutations", to distinguish them from the "driver mutations" that actively promote tumor cell proliferation. This may suggest that the refractoriness to treatment observed in some tumors may be caused when a therapeutic treatment has the unintended effect of converting one or more passenger mutations to driver mutations, thereby conferring therapeutic resistance.

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