

LOW-GRADE CHRONIC INFLAMMATION MEDIATED BY MAST CELLS IN FIBROMYALGIA: ROLE OF IL-37

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Received - January 22, 2018 -Accepted March 12, 2018

It has been observed that acute stress causes the activation of TH1 cells, while TH2 cells regulate and act on chronic inflammation. Fibromyalgia (FM) is a chronic, idiopathic disorder which affects about twelve million people in the United States. FM is characterized by chronic widespread pain, fatigue, aching, joint stiffness, depression, cognitive dysfunction and non-restorative sleep. The mechanism of induction of muscle pain and inflammation is not yet clear. In FM there is an increase in reactivity of central neurons with increased sensitivity localized mainly in the CNS. Mast cells are involved in FM by releasing pro-inflammatory cytokines, chemokines, chemical mediators, and PGD₂. TNF is a cytokine generated by MCs and its level is higher in FM. The inhibition of pro-inflammatory IL-1 family members and TNF by IL-37 in FM could have a therapeutic effect. Here, we report for the first time the relationship between MCs, inflammatory cytokines and the new anti-inflammatory cytokine IL-37 in FM.

0393-974X (2018)

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

EFFECTS OF TUMOR-ASSOCIATED MACROPHAGES ON THE PROLIFERATION AND MIGRATION OF ESOPHAGEAL CANCER-ASSOCIATED LYMPHATIC ENDOTHELIAL CELLS

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Received September 26, 2017 – Accepted February 8, 2018

The aim of this study was to explore whether M2 macrophages can be transformed into M1 macrophages, and to investigate the effect of different types of macrophages on the proliferation, migration and ring-forming ability of esophageal cancer-related lymphatic endothelial cell (LEC). Human monocytic leukemia cell line (THP-1 cell) was induced to differentiate to M1 macrophages (M1 group) and M2 macrophages (M2 group), and co-cultured with esophageal cancer-associated LEC. The individual esophageal cancer co-cultured with LEC was used as control. Different types of macrophages were observed by Cell counting kit-8 (CCK-8). Enzyme-linked immunosorbent assay (ELISA) was used to detect the VEGF-C concentration; the expression of VEGFR-3 protein and its mRNA was detected by Western blot and qRT-PCR, respectively. The positive rate of the M1 group induced by IFN- γ and LPS was significantly higher than that of M2 macrophages (48.57 \pm 5.98% vs 25.83 \pm 1.95%). The expression of VEGF-C in the supernatant of the M2 group was higher than that in the control group, but no significant differences regarding the expression of VEGF-C between M1 and control groups were found. In addition, the expression of VEGFR-3 on both mRNA and protein in esophageal cancer-related LEC of the M2 group was significantly higher than those in the control group; however, the M1 group had a significantly lower VEGFR-3 level on both mRNA and protein than the control group. Human M2 macrophages can be transformed into M1 macrophages, and can promote the proliferation, migration and ring-forming ability of esophageal cancer-associated LEC.

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EFFECT OF CuSO_4 AND NANO COPPER ON SERUM ANTIOXIDANT CAPACITY IN WEANED PIGLETS

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Received December 2, 2017 – Accepted February 13, 2018

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Copper is the most essential trace element in the animal body. Nano-sized copper particles have been widely used in a number of different animal species in modern medicinal practice. The present study was designed to examine the effect of dietary copper sulfate (CuSO_4) and nano copper supplementation on serum antioxidant capacity of weaning piglets. A total of 28 Duroc piglets (21 days, and weighing ~7 kg) were randomly divided into three equal groups. The control group (n=4) was administered with a normal standard diet; however the CuSO_4 (n=12) and nano copper (n=12) groups were treated with 50, 100 and 200 mg/kg/day body weight, respectively. After 28 days, blood serum copper-zinc superoxide dismutase (CuZn-SOD), ceruloplasmin (CP), malondialdehyde (MDA), myeloperoxidase (MPO), total antioxidant capacity (T-AOC), peroxidase (POD), nitric oxide (NO), nitric oxide synthase (NOS), hydrogen peroxide (H_2O_2) and inhibition of hydroxyl radical (CIHR) were analyzed from all groups. The results indicated that nano copper supplementation has significant ($P < 0.05$) effect on the serum antioxidant capability as compared to dietary CuSO_4 group in weaned piglets. Nano-size copper 100 mg/kg/day supplementation was confirmed to improve the immunity level by strengthening the antioxidant capacity of weaning piglets. Dietary supplementation with 100 mg/kg body weight nano copper could be a potential substitute for weaned piglets.

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THYMOQUINONE PROTECTS AGAINST CEREBRAL SMALL VESSEL DISEASE: ROLE OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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Received January 9, 2018 – Accepted February 5, 2018

Cerebral small vessel disease (CSVD) is a leading cause of progressive decline of cognition and a major risk factor for stroke. Thymoquinone (TQ) is the major biological component of *Nigella sativa* (*N. sativa*) and its extracts. We explored the possible protective effect of TQ against CSVD in stroke-prone spontaneously hypertensive SHRsp rats. Morris water maze and novel object recognition tests were conducted to evaluate memory and cognitive function. mRNA expression of inflammatory factors were determined and oxidative stress was evaluated. We showed that TQ markedly decreased the level of systolic blood pressure in SHRsp rats. TQ reduced the escape latency time and the time spent in the target quadrant in the Morris water maze test in SHRsp rats. TQ also decreased the time spent with the novel object in SHRsp rats in both short- and long-term memory tests. TQ markedly increased the capacity to distinguish between familiar objects and novel objects in the SHRsp rats in the short- and long-term memory tests. The mRNA expression of IL-1 β , IL-6, monocyte chemoattractant protein-1 and cyclooxygenase-2 in the brain of SHRsp rats was remarkably decreased by TQ, indicating the reduction of inflammation. Moreover, TQ increased the activities of superoxide dismutase and catalase, decreased the malondialdehyde level and increased glutathione level in the brain of SHRsp rats, indicating the attenuation of oxidative stress. In summary, we found that TQ could effectively attenuate the blood pressure and the injury of memory and cognition under the condition of CSVD. The anti-inflammatory and antioxidant activities of TQ may be responsible for its protective effect. We demonstrate that TQ is a novel candidate for the treatment of CSVD and its neurological outcome.

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CYSTEINYL LEUKOTRIENES C₄ AND D₄ DOWNREGULATE HUMAN MAST CELL EXPRESSION OF TOLL-LIKE RECEPTORS 1 THROUGH 7

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Received July 17, 2017 – Accepted January 12, 2018

Cysteinyl leukotrienes (CysLT) are potent inflammatory lipid molecules that mediate some of the pathophysiological responses associated with asthma such as bronchoconstriction, vasodilation and increased microvascular permeability. As a result, CysLT receptor antagonists (LRA), such as montelukast, have been used to effectively treat patients with asthma. We have recently shown that mast cells are necessary modulators of innate immune responses to bacterial infection and an important component of this innate immune response may involve the production of CysLT. However, the effect of LRA on innate immune receptors, particularly on allergic effector cells, is unknown. This study determined the effect of CysLT on toll-like receptor (TLR) expression by the human mast cell line LAD2. Real-time PCR analysis determined that LTC₄, LTD₄ and LTE₄ downregulated mRNA expression of several TLR. Specifically in human CD34⁺-derived human mast cells (HuMC), LTC₄ inhibited expression of TLR1, 2, 4, 5, 6 and 7 while LTD₄ inhibited expression of TLR1-7. Montelukast blocked LTC₄-mediated downregulation of all TLR, suggesting that these effects were mediated by activation of the CysLT1 receptor (CysLT1R). Flow cytometry analysis confirmed that LTC₄ downregulated surface expression of TLR2 which was blocked by montelukast. These data show that CysLT can modulate human mast cell expression of TLR and that montelukast may be beneficial for innate immune responses mediated by mast cells.

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STUDY ON PHYSIOLOGICAL AND PSYCHOLOGICAL COMPREHENSIVE NURSING OF ELDERLY TUMOR PATIENTS AFTER SURGERY

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Received December 21, 2017 – Accepted March 12, 2013

The aim of this study is to formulate nursing schemes for elderly tumor patients after surgery according to their clinical characteristics, and give effective guidance for alleviating the patients' psychological anxiety. One hundred elderly tumor patients admitted to the oncology department of the Affiliated Cancer Hospital of Harbin Medical University were included and divided into an intervention group (50) and a control group (50). Nursing intervention was performed on the intervention group, and routine nursing was performed in the control group. One day before surgery, all the patients were asked to fill in a self-rating anxiety scale (SAS) and a self-rating depression scale (SDS), and their blood pressure and heart rate data were measured. After surgery, the patients were asked to fill in a form which investigated their pain degree, recovery situation and satisfaction degree. The heart rate and blood pressure of the patients in the intervention group recovered faster than those of the control group, with lower SAS and SDS scores and shorter recovery time. In conclusion, effective nursing intervention played a crucial role in the postoperative recovery of elderly tumor patients by reducing pain and anxiety degrees, which improved the patients' satisfaction with the nursing.

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LONG NON-CODING RNA SNHG16 CONTRIBUTES TO GLIOMA MALIGNANCY BY COMPETITIVELY BINDING miR-20a-5p WITH E2F1

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Received August 1, 2017 – Accepted December 20, 2017

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Glioma is among the most fatal and highly aggressive primary malignant tumors in the central nervous system. Small nucleolar RNA host gene 16 (SNHG16) is identified to play an oncogenic role in several cancers. However, the exact mechanism of SNHG16 action in the regulation of glioma development remains unknown. LncRNA SNHG16 was increased in glioma tissues and cells compared with normal brain tissues and cells. SNHG16 expression was correlated with the malignancy and poor prognosis of glioma patients. SNHG16 and E2F1 contained a binding site of miR-20a-5p. miR-20a-5p was decreased in glioma tissues and cells compared with normal brain tissues and cells. Downregulation of miR-20a-5p was correlated with the malignancy and poor prognosis of glioma patients. In glioma tissues, the expression of SNHG16 was negatively correlated with miR-20a-5p. Downregulation of SNHG16 increased miR-20a-5p expression. miR-20a-5p mimic reduced the luciferase activity of SNHG16 and E2F1; miR-20a-5p mimic enhanced the inhibition of cell proliferation, invasion, migration, and EMT, and increase of apoptosis induced by SNHG16 knockdown. Anti-miR-20a-5p reversed the effects of shSNHG16. We also found that SNHG16 may act as a ceRNA for miR-20a-5p, enhancing the expression of E2F1. Additionally, knockdown of SNHG16 remarkably reduced the increase of tumor volumes in xenograft mouse models. In tumor tissues, knockdown of SNHG16 increased the expression of miR-20a-5p, reduced EMT and increased apoptosis. In conclusion, SNHG16 promotes glioma tumorigenesis by sponging miR-20a-5p, leading to the enhancement of its endogenous targets E2F1. The data provides a new clue for the role of SNHG16/miR-20a-5p/E2F1 in the development of glioma.

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CLINICAL VALUE OF COMBINED TESTS FOR TUMOR MARKERS FOR GASTRIC CANCER

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Received December 1, 2017 – Accepted January 31, 2018

To study the clinical diagnostic value of treating gastric cancer (GC) with combined tests for tumor markers (CEA, CA199, CA242 and CA724), fifty healthy subjects, 50 patients with GC at different stages and 50 patients with benign GC were randomly selected from our hospital. These subjects were divided into a normal group A, an experimental group B and a control group C. Venous blood was drawn and tested for four serum tumor markers. The SPSS 18.0 analytic system was then used to analyze the data. Tumor markers for GC at different stages, different pathological patterns and tumor incidence are discussed. The difference in expression levels of tumor markers between group C and group A was not statistically significant ($P>0.05$). The differences in expression levels between group B in stage I and stage II and those of groups A and C was statistically significant ($P<0.05$). At the same time, the differences in expression levels of group B in stage III and stage IV and those of groups A and C were also statistically significant ($P<0.01$). For different sizes of tumors, taking 5 cm as a maximum, normal expression and abnormal expression of the four tumor markers was different ($P<0.05$). The tumor incidence of the combined test for the four tumor markers was conspicuously higher than that of single tests. Moreover, the difference between the tumor incidence of the combined test in stages I, II and III and that of single tests in the same stages was of statistical significance ($P<0.05$); however, the difference was not statistically significant in stage IV ($P>0.05$). The combined testing for tumor markers is useful for clinical diagnosis of GC.

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PREVALENCE OF DEGNALA DISEASE IN BOVINE ALONG WITH SCREENING OF TOXIGENIC FUNGI ISOLATED FROM CONTAMINATED RICE STRAW

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Received November 3, 2017 – Accepted January 31, 2018

Toxigenic potential of different candidate fungi, isolated from rice straw feed of Degnala disease-affected bovines was analyzed along with species, age, gender and seasonal prevalence. Of 1,536 cases, 104 (6.77%) showed positive signs with a significant association ($p < 0.05$) between this disease and rice straw feeding, in buffaloes, and bovine aged over 1 year in the winter season. Complete blood count showed a marked increase in erythrocyte sedimentation rate and all white blood cells numbers, except lymphocytes in positive cases. There was a significant increase ($p < 0.05$) in alanine amino transferase, aspartate amino transferase and alkaline phosphatase in the liver function test. At the same time, an increased value of creatinine was noted in the renal function test. For isolation and screening of toxigenic fungi, rice straw samples ($n=40$) being fed to the positive cases were processed further, and 85 fungal isolates were found, mainly of *Aspergillus* (57), *Penicillium* (10), *Fusarium* (04), *Zygomycetes* (03), *Curvularia* (01) and unidentified (10). All isolated fungi were subjected for mycotoxin production and only 11 showed mycotoxin-producing capability (including *Aspergillus*, *Penicillium* and *Fusarium* isolates) analyzed by thin layer chromatography and quantified through high performance liquid chromatography. It is concluded that all the fungi contaminating rice straw feed of Degnala affected animals were not toxigenic. This work will help in establishing major mycotoxin-producing fungi leading to the probable cause of Degnala disease in bovine.

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SPOLIGOTYPING ANALYSIS OF *MYCOBACTERIUM TUBERCULOSIS* WITH TB-SPRINT TECHNOLOGY

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Received November 4, 2017 – Accepted February 6, 2018

Spoligotyping is a highly reproducible, fast polymerase chain reaction (PCR)-based approach for identification of different strains of *Mycobacterium tuberculosis* through genotyping of *Mycobacterium tuberculosis* complex (MTB). Molecular typing of MTB is helpful for understanding and controlling TB epidemics. Spoligotyping was performed on 166 strains of MTB collected from 25 districts of Khyber Pakhtunkhwa, Pakistan. Results were analyzed using online database, SITVIT2, developed by the Institute Pasteur de Guadeloupe, France and SPSS software V.15 (IMB Inc). Drug susceptibility test (DST) was performed on all strains using commercial liquid media in BACTECMGIT 960 instrument. Spoligotyping results showed that 145 (88%) strains displayed known patterns while 21 (12%) were new. Central Asian strain (CAS) was the predominant family (73%, $\chi^2=19.9$, $P=0.001$) followed by Beijing (5.4%) and T1 (4.2%). CAS1-Dehli was the major sub-family (82%) among the CAS family ($\chi^2=664$, $P=0.0001$). Furthermore, new spoligotyping patterns were clustered using the maximum parsimony (MP) test to find any evolutionary relationship with pre-published genotypes. Analysis showed that the majority of the strains with unknown pattern have an evolutionary link with CAS strain, and 9 (5.4%) of the unknown strains were epidemiologically linked and named CAS-KPK (Khyber Pakhtunkhwa). The present study demonstrated that CAS is a predominant strain of MTB prevailing in different areas of the Khyber Pakhtunkhwa province of Pakistan. Spoligotyping pattern of some strains could not match other reported pattern in the international database. Other tools, like MIRU/ VNTR, can be helpful to perform investigation of its epidemiological characteristics in future.

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NEUROPROTECTION OF ULINASTATIN ON TRANSIENT CEREBRAL ISCHEMIA VIA ANTIOXIDATIVE MECHANISMS

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Received December 11, 2017 – Accepted February 9, 2018

Ulinastatin [also called urinary trypsin inhibitor (UTI)] has beneficial effects on cerebral ischemic injury evoked by cardiac arrest (CA). However, the underlying mechanisms are unknown. The purpose of this report was to determine the involvement of antioxidative signal pathway of the hippocampus in effects of UTI in the process of neurological functions after transient cerebral ischemia. CA was induced by asphyxia followed by cardiopulmonary resuscitation in rats. Western blot analysis and ELISA were used to examine expression of Nrf2-antioxidant response element (ARE) and superoxide dismutases (SOD), and the levels of products of oxidative stress. In addition, the modified neurological severity score (mNSS) and spatial working memory performance were employed to assess neurological deficiencies in CA rats. Our results show that CA impaired Nrf2-ARE and SOD in the hippocampus CA1 region and amplified products of oxidative stress, namely 8-isoprostaglandin F₂ α (8-iso PGF₂ α) and 8-hydroxy-2'-deoxyguanosine (8-OHdG). Systemic administration of UTI largely restored Nrf2-ARE and SOD, and this also attenuated amplification of 8-iso PGF₂ α and 8-OHdG induced by cerebral ischemia and thereby alleviated neurological deficits with increasing survival of CA rats. Our data suggest that UTI improves Nrf2-ARE signals and inhibits products of oxidative stress in the hippocampus, which is linked to improvement of neurological deficiencies in transient cerebral ischemia. UTI plays a beneficial role in modulating cerebral ischemic injury via antioxidative mechanisms.

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MEDICAMENTOUS THROMBOLYSIS DISGUISES PULMONARY THROMBOEMBOLISM AS A CAUSE OF DEATH

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Received November 22, 2017 – Accepted February 15, 2018

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We report the case of a 31-year-old female who was admitted to the emergency department with symptoms of cardiac arrest and ultimately died in spite of enormous resuscitation efforts. During resuscitation, pulmonary embolism was considered as a possible non-cardiac cause of cardiac arrest, and following its extremely unfavorable prognosis, the fatal outcome was not so surprising. However, since acute pulmonary emboli obstructing blood flow to a lobe or multiple lung segments was suspected, alteplase was indicated and administered. At the autopsy, no venous thrombosis in the vena cava, pelvic veins, or any of the lower extremity veins was documented; conversely, nor were specific signs of pulmonary thromboembolism (PTE) found macroscopically, until this was confirmed by histopathological staining which is not used as a routine diagnostic tool in forensic medicine. In this study, we conclude that PTE is generally overlooked as the principal diagnosis and the cause of death. Rightful postmortem diagnosis could lead to increased vigilance and a change in management in many of such cases, which could result in improved survival. Motivated by providing better awareness of PTE, this study aimed to illustrate unrecognized PTE and pathological findings that were masked by thrombolytic drugs.

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SUDDEN-ONSET UNILATERAL PTOSIS INDUCED BY PITUITARY MACROADENOMA, WITH FALSE-POSITIVE JOLLY AND NEOSTIGMINE TESTS

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Received January 24, 2018 – Accepted February 15, 2018

The development of ptosis as a consequence of pituitary tumor is an exceptionally rare occurrence. Here, we describe the case of sudden-onset unilateral ptosis induced by pituitary macroadenoma. The condition was characterized by false-positive Jolly and neostigmine tests. These findings mimic oculomotor nerve palsy and make the correct diagnostics rather challenging. The case points to the fact that patients with acquired ptosis need detailed neuroophthalmological examination.

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EXPRESSIONS AND CLINICAL SIGNIFICANCE OF FACTORS RELATED TO ACUTE CORONARY SYNDROME

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Received December 21, 2017 – Accepted February 8, 2018

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Acute coronary syndrome (ACS) is the acute stage of coronary artery disease, which remains a major cause of mortality and morbidity. It is essential to explore the role of matrix metalloproteinase -2 (MMP-2) and interleukin (IL)-18 and their association with disease in patients with severe ACS. Circulating MMP-2 and IL-18 levels were measured using enzyme linked immunosorbent assay (ELISA) in 94 subjects with acute coronary syndrome (ACS, n=38), stable angina pectoris (SAP, n=27) and healthy individuals (control group, n=29). We examined the correlations between the levels of MMP-2 and IL-18 and cardiac risk factors in ACS. Logistic regression analysis was performed to screen for factors that predict ACS. Both MMP-2 and IL-18 concentrations were increased in the ACS group compared to the SAP group or control group ($P<0.01$). Especially, MMP-2 and IL-18 were highly expressed in the patients with ST elevated myocardial infarction (STEMI). Both serum levels of MMP-2 and IL-18 in the single-, double- and triple-vessel lesion group were higher compared to the control group ($P<0.01$). MMP-2 levels were positively correlated with IL-18 ($r=0.639$, $P<0.01$), CK-MB ($r=0.47$, $P=0.003$) and hs-CRP levels ($r=0.583$, $P<0.01$). The logistic regression analysis showed that increases in MMP-2 levels may be a powerful predictor of ACS. Thus, the changes in levels of serum MMP-2 and IL-18 may be useful in the diagnosis of ACS and prediction of its prognosis.

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GLUCOCORTICOIDS IN COMBINATION WITH URSODESOXYCHOLIC ACID IN THE TREATMENT OF AUTOIMMUNE HEPATITIS

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Received August 19, 2017– Accepted February 16, 2018

Autoimmune hepatitis (AH) is usually manifested as chronic hepatitis in clinics; it may evolve to liver cirrhosis, hepatic failure, and even death if treatment is delayed. To investigate the clinical efficacy of glucocorticoids in combination with ursodesoxycholic acid in the treatment of glucocorticoids in combination with ursodesoxycholic acid, one hundred and twenty patients with AH who were admitted to the hospital from February 2014 and February 2016 were selected and randomly divided into an observation group and a control group using random number table. Patients in the control group were treated by glucocorticoids only, while patients in the observation group were treated by ursodesoxycholic acid and glucocorticoids. Patients in both groups were treated for six months. The clinical efficacy of the two groups was evaluated after treatment. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin (DBIL) and total bilirubin (TBIL) of the two groups both decreased after treatment ($P < 0.05$), the improvement of the level of liver function of the observation group was superior to that of the control group, and the difference was statistically significant ($P < 0.05$); after treatment, the levels of serum immunoglobulin G (IgG) and immunoglobulin m (IgM) of both groups significantly reduced after treatment, and the difference within groups before and after treatment had no statistical significance ($P < 0.05$). The reduction of the immunological indicators of the observation group was more remarkable after treatment, and the difference between the two groups had statistical significance ($P > 0.05$). The complete remission rate of the observation group was significantly higher than that of the control group; the incidence of adverse reactions was lower than that of the control group, and the difference had statistical significance ($P < 0.05$). Thus it can be concluded that glucocorticoids in combination with ursodesoxycholic acid has favorable efficacy in treating AH as it can promote the improvement of liver function and effectively reduce the dose of glucocorticoids and the incidence of adverse reactions. The therapy is of great clinical values.

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EVALUATING THE UTILITY OF AUTOANTIBODIES FOR DISEASE ACTIVITY AND RELAPSE IN GIANT CELL ARTERITIS

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Received August 1, 2017 – Accepted February 13, 2018

In patients with giant cell arteritis (GCA), autoantibodies against cytoskeletal elements, cardiolipin, neutrophil cytoplasmic antigens, ferritin, endothelial and smooth muscle cells have been reported, however no updated reviews are available evaluating their clinical utility. Methodology of detection is important, especially for quantitative assays, e.g. enzyme-linked immunoassays and multiplex bead-based immunoassays, while semiquantitative assays contribute valuable data on isoforms, epitope mapping and cellular localization. Most studies to date reporting on antiphospholipid antibodies in GCA have focused on anti-cardiolipin antibodies (aCL), while the highest prevalence of autoantibodies in GCA patients was reported for the anti-N-terminal peptides of the ferritin heavy chain (92%). Anti-neutrophil cytoplasmic antibodies were shown to be present in only a small percentage of GCA patients, decreasing after therapy, however in combination with aCL and antibodies against peptides of N-terminal ferritin heavy chain, they could represent an added value in detecting relapse in GCA patients.

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STATUS OF CATHELICIDIN LL-37, CYTOKINE TNF, AND VITAMIN D IN PATIENTS WITH PULMONARY TUBERCULOSIS

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Received December 30, 2017 – Accepted February 16, 2018

Development of *Mycobacterium tuberculosis* (*Mtb*) infection depends on the ability of the host to elicit the protective immune response to the pathogen. Cathelicidin plays a role in antibacterial innate immunity mechanisms. This peptide contributes to the barrier function of respiratory epithelium and takes part in controlling pulmonary bacterial infections. LL-37 (leucine-leucine-37) is involved in host defense and innate immune response to mycobacterial infections, as well. This study aims to evaluate the serum concentrations of LL-37 in individuals with active pulmonary tuberculosis (TB) and to determine whether any correlations between peptide LL-37, tumor necrosis factor (TNF) and vitamin D serum levels exist. A total of 46 adults with pulmonary TB were recruited for the study. Sixty-one controls were randomly selected as control group. Serum concentrations of cathelicidin LL-37, vitamin D (25(OH)D), as well as TNF, were measured using an enzyme-linked immunosorbent assay (ELISA) kit. The mean (\pm SEM) level of LL-37 was significantly higher in the TB group (7.45 ± 1.58) compared with healthy controls (1.41 ± 0.22) ($p < 0.001$). Mean serum concentration of TNF was significantly higher in the TB group (8.51 ± 1.92) compared with healthy controls (2.69 ± 0.19) ($p < 0.001$). There was no significant difference in mean serum levels of vitamin D between healthy (26.10 ± 1.74) and TB subjects (24.18 ± 1.95). No correlations between LL-37, TNF, and vitamin D levels in patients with TB were observed. Our results indicated that serum levels of peptide LL-37 during TB is raised significantly, and this observation is compatible with the general view of the important role of this cathelicidin in defense mechanisms against *Mtb* infection.

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CORRELATION ANALYSIS OF *CD4* GENE POLYMORPHISM AND BLOOD ROUTINE INDEXES IN PIGS (*SUS SCROFA*)

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Received December 8, 2017 – Accepted February 19, 2018

The first two Authors made equal contributions to this article

Cluster of differentiation 4 (*CD4*) is an important molecule in the immune system of animals, which participates in the processes of T-lymphocyte differentiation, maturation, immune response and signal transduction. During this study, we adopted the direct sequencing of PCR products and time-of-flight mass spectrometry methods for the detection of single nucleotide polymorphisms (SNPs) in 382 Yorkshire pigs. The linkage disequilibrium of *CD4* gene polymorphisms and their genetic effects on blood routine indexes were also analyzed. The results showed that SNP was screened in intron 1, intron 6 and exon 10, respectively, of the porcine *CD4* gene, and each SNP locus was detected in Yorkshire pigs, which had three genotypes with moderate polymorphism. The three SNPs were in strong linkage disequilibrium ($r^2 > 0.8$), and constituted only two major haplotypes, AGT and CAC (both frequencies accounted for about 97%). The association analysis showed that *CD4* gene polymorphisms were significantly correlated with white blood cell, lymphocyte and monocyte count in Yorkshire pigs ($P < 0.05$). The white blood cell count and monocyte count in individual animals with CAC/CAC diplotype were significantly higher than those with AGT/CAC and AGT/AGT diplotypes ($P < 0.05$). The lymphocyte count in animals with CAC/CAC diplotype were significantly higher than those with the AGT/AGT diplotype ($P < 0.05$). This study indicates that *CD4* gene is significantly associated with partial blood routine indexes in pigs, and it can be considered as a candidate gene for the study of porcine disease resistance.

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IMPORTANCE OF BASOPHILS IN EOSINOPHILIC ASTHMA: THE MURINE COUNTERPART

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Received December 19, 2017 – Accepted February 19, 2018

Several experimental studies in mice showed that basophils participate in the initiation of Th2 adaptive immune response, in addition to the effector phase. However, the role of basophils in allergic airway inflammation is less clear. The aim of this experiment was to assess the importance of basophils in recruiting inflammatory cells and, in particular, eosinophils in a murine model of asthma induced by *Aspergillus fumigatus* allergens. Additionally, bronchial reactivity was evaluated. Basophil depletion resulted in a reduction of inflammatory cells in the airways and eosinophil recruitment was significantly impaired. Also bronchial reactivity seemed to be impaired in basophil-depleted mice, but the result was not statistically significant. According to these preliminary data, basophils seem to influence the local eosinophilic response of allergic asthma.

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CORRELATION BETWEEN CHILDHOOD ECZEMA AND SPECIFIC IgG ANTIBODY LEVEL

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Received April 21, 2017 – Accepted February 21, 2018

Eczema, a common pediatric dermatosis with unclear pathogenesis, can seriously affect the life quality of children due to its recurrence and long course. Recent study has found that food specific IgG (sIgG) might be involved in the course of eczema. To analyze the correlation between childhood eczema and sIgG and evaluate the role of avoiding taking intolerance food in the treatment of childhood eczema, this study enrolled 216 children with eczema who were admitted to the Taian Maternal and Child Health Care Hospital, Shandong, China, between August 2014 and October 2015. They were divided into an eczema group (N = 140) and an allergy group (N = 76). Eighty healthy children who were admitted to the Department of Children Healthcare in the same period were selected as a control group. Enzyme-linked immuno sorbent assay (ELISA) was used to detect the serum sIgG level. The result showed that the sIgG positive rates of children in the eczema group and allergy group were 91.4% and 93.4%, respectively, and the difference had no statistical significance ($P > 0.05$). However, the sIgG positive rates of children in the eczema group and allergy group were significantly higher than that in the control group ($P < 0.05$). Milk and eggs were the major allergy-causing food for children with sIgG positive rates higher than 70%. The sIgG test results revealed that eggs had the highest allergenicity, followed by milk, tomatoes and soybeans, and pork was not highly sensitive. Therefore, it can be concluded that sIgG positive rate of children with eczema is high, and examination of food sIgG antibody in serum is valuable in the diagnosis and treatment of childhood eczema.

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POSTERIOR HEMIVERTEBRA RESECTION IN COMBINATION WITH SCREW ROD INTERNAL FIXATION IN THE TREATMENT OF PEDIATRIC CONGENITAL SCOLIOSIS

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Received June 7, 2016 – Accepted February 21, 2017

Congenital scoliosis, a commonly seen disease occurring in children, can not only affect the growth, but also can uglify the individual which can severely affect the health and quality of life of children. To investigate the efficacy of posterior hemivertebra resection in combination with screw rod internal fixation in the treatment of congenital hemivertebra scoliosis, 115 patients were randomly divided into an observation group and a control group. Patients in the observation group were treated by posterior hemivertebra resection in combination with screw rod internal fixation, while patients in the control group were treated by posterior hemivertebra resection only. The surgical evaluation indicators, postoperative improvement of scoliosis and incidence of complications were recorded. The results demonstrated that the observation group had longer average operation time and less average blood loss compared to the control group, and the differences had statistical significance ($P<0.05$); the correction efficacy of the observation group was superior to that of the control group ($P<0.05$); the incidence of postoperative complications in the two groups had no significant difference, but the incidence of correction loss of the observation group was much lower than that of the control group ($P<0.05$). In conclusion, posterior hemivertebra resection in combination with screw rod internal fixation is a highly efficient and safe treatment which can significantly relieve the clinical symptoms and cause few complications.

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THREE-VESSEL-TRACHEA VIEW IN THE DIAGNOSIS OF FETAL CARDIAC GREAT VESSEL MALFORMATION

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Received June 21, 2017 – Accepted February 21, 2018

Fetal cardiac great vessel malformation is attracting increasing attention in the prenatal ultrasonic diagnosis of fetal congenital heart disease. To investigate the clinical diagnostic values of three-vessel-trachea view (3VT view) in the ultrasonic diagnosis of this malformation, the present study analyzed the echocardiographic examination results of 77 fetuses with great vessel malformation, retrospectively analyzed the echocardiographic characteristics in the three-vessel-trachea view, and followed up the enrolled cases. The results suggest that great vessel malformation had characteristic manifestations, such as abnormal arrangement order, inner diameter, blood flow direction and branch. Color Doppler flow imaging found V, O, C, U, Ioo and oVo structures. There were 20 cases of blood vessel position abnormality, 38 cases of abnormal blood vessel diameter, and 19 cases of abnormal number of blood vessels. The detection rate of abnormal blood vessel diameter was 95%, which was the highest; the detection rate of abnormal blood vessel position was 97.4%, and that of abnormal number of blood vessels was 84.2%. It is concluded that the 3VT view can indicate fetal cardiac great vessel malformation. The 3VT view is beneficial to timely prenatal diagnosis, relief of body pain and improvement of quality of birth.

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EFFECT OF BRANCHED CHAIN AMINO ACIDS ON PERIOPERATIVE TEMPERATURE, GLUCOSE LEVEL AND FAT METABOLISM IN PATIENTS WITH GASTROINTESTINAL TUMORS

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Received December 21, 2017 – Accepted February 6, 2018

This study aimed to investigate the effect of branched chain amino acids (BCCAs) on perioperative temperature, glucose and fat metabolism in patients with gastrointestinal tumors. Fifty-six patients undergoing gastrointestinal tumor surgery were included in the study and randomly divided into two groups of 28 patients each: an experimental and a control group. During surgery, the experimental group received 5.64mL·Kg⁻¹·h⁻¹(4KJ·Kg⁻¹·h⁻¹) of BCCAs intravenously, through an infusion pump, and the control group received an equal volume of NaCl 0.9%. Vital signs were continuously monitored during the operation. Nasopharynx temperature levels of glucose, insulin, free fatty acid and ketone bodies in the blood were determined 30 min before anesthesia (t 0), after anesthesia and before surgery (t 1), 30 min after the start of surgery (t 2), 2 h after start of surgery (t 3) and 1 h after the end of surgery (T4). Patients' shivering intensity (Wrench grading) and pain degree [Visual analogue scale (VAS)] were estimated 1 h after the endotracheal tube was removed. Nasopharynx temperature was decreased ($p < 0.05$) in both groups after anesthesia induction, while 1 h after the tube was removed it was higher in the experimental group than the control group ($p < 0.05$); compared with pre-surgery values, blood glucose levels were increased during surgery in both groups, but the experimental group had a lower increasing trend compared to the control group, though without statistical significance ($p > 0.05$). Insulin levels were significantly different between the two groups at all time-points during surgery ($p < 0.05$). However, the rising trend of the experimental group was more dramatic during the period from t 0 to t 3. One hour after surgery (t 4), the insulin levels varied, but still at higher levels than pre-surgery, with a significant difference ($p < 0.05$); levels of free fatty acids had a downward trend in both groups, and levels in the experimental group continued to decline until 1 h after surgery. Patients who received branched chain amino acids had less temperature decrease during surgery. Moreover, blood glucose levels were not increased, which limits fat mobilization and leads to production of ketone bodies, reduces the shivering and its intensity after surgery.

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ABILITY OF COMPUTED TOMOGRAPHY TO PREDICT RIGHT HEART STRAIN ON AN ECHOCARDIOGRAM IN PATIENTS WITH ACUTE PULMONARY EMBOLUS

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Received January 14, 2018 – Accepted February 19, 2018

Patients with submassive pulmonary embolism (PE) resulting in right heart strain (RHS) have an increased risk of mortality compared to those with a preserved right ventricular function. This study aimed to investigate the predictive value of computed tomography pulmonary angiogram (CTPA) findings of right heart strain in patients with computed tomography (CT)-proven PE for the diagnosis of right heart strain by echocardiogram (ECHO). The institutional review board (IRB) approved retrospective chart review of the adult emergency department patients diagnosed with an acute PE between 2012 and 2016. A total of 128 patients diagnosed with RHS by CT who had received an ECHO during their hospitalization were included in the study. Descriptive statistics were run for the variables of interest. The majority of patients (101 patients) with reported findings of RHS on CT had similar findings on ECHO. In our cohort, a finding of enlarged right atrium (RA) on CT was 100% predictive of RHS diagnosis on ECHO, whereas having interventricular septal bowing alone on CT was the least predictive of RHS on subsequent ECHO (61%). The 2 remaining subgroups: right ventricle (RV) enlargement alone and RV enlargement with either interventricular septal bowing/hepatic vein blood reflux or both lies somewhere in between, with 80% of these patients showing strain on ECHO. We found that signs of RHS on CT are predictive of strain on an ECHO (78%) and RA enlargement in any combination was the most predictive finding of RHS on ECHO (100%). Future prospective randomized investigations are needed to confirm such findings.

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DETECTION OF HAND-FOOT-MOUTH DISEASE AND ITS SPATIAL-TEMPORAL EPIDEMIOLOGICAL CHARACTERISTICS WITH GIS PLATFORM

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Received December 21, 2017 – Accepted February 8, 2018

The aim of this study is to investigate the main category of hand-foot-mouth (HFM) virus and analyze the distribution characteristics and susceptible population of HFM disease in China. Infants who have had HFM disease for less than 7 days were selected from the First Affiliated Hospital of Guangxi Medical University, Guangxi, China. Various specimens were collected from the infants, and EV71 and CA16 nucleic acid detections were performed using fluorescence quantitative assay. The positive results of the specimens were compared to determine the components of the pathogen. Moreover, the data of the target cases were analyzed based on Geographic Information System (GIS) to obtain the spatial-temporal epidemiological features of HFM disease in China. The detection rate of HFM virus in the throat swab, feces, bleb fluid and cerebrospinal fluid were 75%, 81.13%, 85.71% and 25%, respectively, indicating that the detection rate of virus in the bleb fluid was the highest. When the detection was based on more than one specimen, it was found that the positive rate was higher compared to detection based on a single specimen. The positive detection rate of EV71 in the target specimens was significantly higher than that of CA16 and mixed infection. Moreover, CA16 infection was usually accompanied by EV71 infection. As to spatial-temporal distribution, hand-foot-mouth disease broke out in the South of China in April, then spread to the north, and diminished in July. There was a notable difference in the number of cases between different provinces. EV71 and CA16 are the main viruses inducing HFM disease, especially EV71. Fluorescence quantitative polymerase chain reaction with high sensitivity can be used to detect the copy number of viruses, which is applicable to the early diagnosis of HFM disease. The incidence of HFM disease is notably different according to the influence of time, geographical space, gender and the living conditions of the children. Early diagnosis and treatment based on scientific methods are needed to reduce the incidence of severe diseases and avoid death.

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ROLE OF FUNGAL-INDUCED STRESS IN BIOCHEMICAL AND PHYTOCHEMICAL PROFILING OF *PEGANUM HARMALA*

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Received July 27, 2017 – Accepted December 20, 2017

The current study was designed to evaluate bioactive compounds under the influence of biotic stress on seedlings of *Peganum harmala*. Biologically active compounds were determined by using different techniques. The HPLC and GC-MS analyses detected the significant amount of phenolic acids and active biological compound. Total protein content, activity of proteases, α -amylases, catalases and peroxidases were observed to be accelerated under fungal stress. The seedling extracts exhibited prominent antifungal and antimicrobial activity against selected strains. The present study showed that *P. harmala* is a good candidate to be used in natural therapies and medicine.

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EXTRACORPOREAL SHOCKWAVE THERAPY *VERSUS* EXERCISE PROGRAM IN PATIENTS WITH LOW BACK PAIN: SHORT-TERM RESULTS OF A RANDOMISED CONTROLLED TRIAL

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Received September 20, 2017 – Accepted January 23, 2018

The physiotherapy treatment of low back pain (LBP) with physical stimulation offers different possibilities of application. Until now, the physical therapies used in LBP are laser therapy, ultrasonotherapy and currents. We conducted a clinical trial in order to verify whether shockwave therapy, which is very effective in treating tendinopathies and fracture consolidation delays, leads to clinical and electromyographic improvement in patients affected by LBP. We randomized thirty patients affected by LBP treated with shock waves (shockwave group) or a standard protocol characterized by rehabilitative exercises (control group). At one and three months, the patients treated with shockwave therapy showed clinical improvement measured by VAS scales ($p=0.002$; $p=0.02$), and disability evaluated with Roland scales ($p=0.002$; $p=0.002$) and Oswestry ($p=0.002$; $p=0.002$). At three months, the patients treated with shock waves, showed a significant improvement in terms of values of amplitude of the sensory nerve conduction velocity (SNCV) of the plantar medialis nerve (left: $p=0.007$; right: $p=0.04$), the motor nerve muscular conduction (MNCV) of the deep peroneal nerve (left: $p=0.28$; right: $p=0.01$) and recruitment of motor units of finger brevis extensor (left: $p=0.02$; right: $p=0.006$). In the control group, there was a trend to increase the clinical and electromyographic results without statistical significance. The preliminary results suggest a good applicability of shockwave therapy in the treatment of LBP, in accordance with the anti-inflammatory, antalgic, decontracting effects and remodeling of the nerve fiber damage verified in previous studies conducted on other pathological models. Future research will allow us to verify the integration of this therapy into a rehabilitation protocol combined with other physical therapies.

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ORAL LICHEN PLANUS

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Received December 21, 2017 – Accepted February 12, 2018

Oral Lichen planus (OLP) is the most frequent mucosal localization of Lichen planus, affecting about 1-2% of the population. It is associated with skin lesions in 60-70% of cases, while occurring as the only manifestations in 15-25% of patients. Six clinical forms of OLP are identified: reticular (the most common), plaque, papular, atrophic, vesicles/bullous and erosive. The evolution is chronic, especially in the erosive form. A 1% incidence of squamous-cell carcinoma has been reported, thus considering OLP as a potential premalignant condition. The etiopathogenesis is still not completely understood: genetic (HLA-DR2), immunologic (T cell-mediated) and infectious (association with viral hepatitis C, differences in oral microbiota in OLP, and bacteria internalization into infiltrating T cells and oral epithelial cells) are considered the main predisposing or provoking factors. Management is based on the severity of the lesions; topical steroids are the first-line therapy and oral glucocorticoids are used for severe erosive lesions.

0393-974X (2018)

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COMPARISON OF CORONAL LEAKAGE OF DIFFERENT ROOT CANAL FILLING TECHNIQUES: AN *EX VIVO* STUDY

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Received October 6, 2017 – Accepted February 5, 2018

The aim of this study was to compare the quality of the coronal seal, using an *in vitro* bacterial invasion test, of three different root canal filling systems. Twenty-seven freshly extracted mandibular premolars were selected and divided into three experimental groups (G1, G2 and G3 n=7) and two control groups (Ct+ and Ct- n=3). All teeth in the experimental groups were prepared using NiTi Mtwo rotating instruments and then the endodontic treatments were completed using the three-tested warm guttapercha root filling techniques: Microseal (G1), Thermafil (G2) and System B (G3). All root filling techniques were performed using the same endodontic sealer (Pulp Canal Sealer). Three teeth were instrumented and not filled, serving as positive controls (Ct+) and the last three teeth, with intact crowns and no endodontic treatment, served as negative controls (Ct-). All samples were mounted in a two-chamber apparatus and exposed to *Enterococcus faecalis* performing a bacterial infiltration test. All samples were observed for a maximum period of 60 days checking for turbidity of the BHI broth on a daily basis recording when contamination occurred. A quantitative evaluation of the bacterial CFU/ml was performed using the URO-QUICK™ system. On day 32 an overall value was recorded of contamination of 42.85% for group G1, 71.42% for G2 and 42.85% for G3; after 60 days, the final contamination result was 85.71% for group G1, and 100% for both G2 and G3 groups. Considering the number of contaminated samples at the end of the observation period, the three techniques showed no statistically significant differences. The study highlighted the bacterial permeability of gutta-percha/seal barrier, underlining the importance of an effective coronal restoration to ensure a durable seal after root canal treatment.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

EFFECT OF BIOPHYSICAL THERAPY ON ARTICULAR PAIN IN A PRIMARY CARE SETTING COMPARED TO IBUPROFEN AND PLACEBO: A RANDOMIZED CONTROLLED TRIAL

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Received November 4, 2017 – Accepted February 7, 2018

Articular pain is one of the most frequent complaints practitioners face in their daily work. With an aging population, many patients have multiple comorbidities that are associated with the presence of chronic diseases, while others experience allergies, side effects or do not respond to standard medications or procedures. Therefore, there is an urgent need for new effective and safe strategies to manage articular pain, especially in its chronic manifestations. This randomized controlled trial was designed to assess the efficacy of a single therapy session using a biophysical procedure matched with a common non-steroidal anti-inflammatory drug (ibuprofen) and placebo. Biophysical therapy was performed using a Med Select 729 device. One hundred fifty patients (mean age 56 ± 15.6 years) diagnosed with acute or chronic articular pain at different locations were randomized into 3 groups and the Numeric Pain Rating Score (NPRS) was used to measure pain at baseline, after one week, one month, and three months. While no difference in NPRS was observed at baseline among the 3 groups, a statistically significant difference was observed at all subsequent time points, respectively, after one week ($p < 0.05$), one month ($p < 0.001$), and three months ($p < 0.01$), for both ibuprofen and biophysical groups vs placebo. Biophysical treatment of articular pain was shown to be as effective as a conventional non-steroidal anti-inflammatory treatment over a period of 3 months compared to placebo and could, therefore, represent an integrative, safe and long-lasting therapy to be considered for the management of acute and particularly chronic articular pain in current medical practice.

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CLINICAL COURSE OF SEVERE COLITIS: A COMPARISON BETWEEN CROHN'S DISEASE AND ULCERATIVE COLITIS

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Received June 12, 2017 – Accepted February 21, 2018

Few data are available about the clinical course of severe colonic Crohn's disease (CD). The aim of this study is to describe the clinical course of severe Crohn's colitis in a patient cohort with isolated colonic or ileocolonic CD, and to compare it with the clinical course of patients with severe ulcerative colitis (UC). Thirty-four patients with severe Crohn's colitis were prospectively identified in our cohort of 593 consecutive hospitalized patients through evaluation of the Crohn's Disease Activity Index score and the Harvey-Bradshaw Index. One hundred sixty-nine patients with severe ulcerative colitis were prospectively identified in our cohort of 449 consecutive hospitalized patients through evaluation of the Lichtiger score and the Truelove-Witts score. We evaluated the following data/aspects: response to steroids, response to biologics, colectomy rate in acute, colectomy rate during follow-up, megacolon and cytomegalovirus infection rate. We did not find significant differences in the response to steroids and to biologics, in the percentage of cytomegalovirus infection and of megacolon, while the rate of colectomy in acute turned out to be greater in patients with severe Crohn's colitis compared to patients with severe UC, and this difference appeared to be the limit of statistical significance (*Chi-squared* 3.31, $p = 0.069$, OR 0.39); the difference between the colectomy rates at the end of the follow-up was also not significant. In the whole population, by univariate analysis, according to the linear regression model, a young age at diagnosis is associated with a higher overall colectomy rate ($p = 0.024$) and a higher elective colectomy rate ($p = 0.022$), but not with a higher acute colectomy rate, and an elevated ESR is correlated with a

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ETIOLOGICAL PERIODONTAL TREATMENT WITH AND WITHOUT LOW LEVEL LASER THERAPY ON IL-1 β LEVEL IN GINGIVAL CREVICULAR FLUID: AN *IN VIVO* MULTICENTRIC PILOT STUDY

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Received January 31, 2018 – Accepted March 13, 2018

Cytokine proteins may have important roles during different human physiological and pathological processes. In the oral cavity, the bone loss and periodontal tissue pathology was related to inflammatory process activation. The aim of the present study was to assess the effects of etiological periodontal therapy with and without the use of Low Level Laser Therapy (LLLT) on clinical periodontal parameters and interleukin(IL)-1 β level in gingival crevicular fluid (GCF) from chronic periodontitis (CP) patients. Thirty non-smoker CP patients were selected from the Foggia University Dental Clinic and other 2 private dental clinics. All patients were divided into two homogeneous randomized groups: 15 patients were treated with only scaling and root planing (group 1) and 15 patients with scaling and root planing etiological treatment and LLLT (group 2). In all sites, at baseline before treatment, the periodontal pocket depth (PPD) and bleeding on probing (BOP) were measured. In the PPD sites, the GCF samples were collected from 30 deep (≥ 5 mm) and shallow (≤ 3 mm) sites and IL-1 β were evaluated at baseline, after 10 days and 1 month. In all the samples at baseline, the IL-1 β concentration in GCF and BOP rate were significantly higher at deep PPD sites than at the shallow ones. After 10 days in all samples no PPD improvement was observed in the BOP rate but the IL-1 β level was statistically significantly improved ($p < 0.005$) in group 2 compared to group 1. At 10 days and 1 month, in all deep PPD sites, PPD and BOP improvements were observed. At same time, IL-1 β levels were lower and statistically significantly ($p < 0.005$) improved in group 2 compared to group 1. The results confirmed that the periodontal etiology treatment of deep PPD sites with or with-out associated LLLT promotes periodontal health. Etiological treatment associated with LLLT, improves BOP and inflammation in periodontal disease. Moreover, the IL-1 β concentration changes in GCF suggest these cytokines as a predictable marker of gingival inflammation in chronic periodontitis patients.

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IL-1 HAPLOTYPE ANALYSIS IN PERIODONTAL DISEASE

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Received March 13, 2018 – Accepted March 20, 2018

Numerous studies have established statistical associations of the IL-1 gene cluster polymorphisms with various inflammatory diseases. Deriving from that, the present study was intended to determine whether single-nucleotide polymorphisms (SNPs) in these gene are also associated with periodontal disease in a Linkage disequilibrium analysis. This investigation also created two haplotype blocks, both consisting of two different SNPs. Recent theoretical analyses indicate that research with an interpretation of periodontal disease as a complex, oligogenic disorder, with IL-1 genetic variation contributes an important but not exclusive influence on disease risk. Further studies are needed to confirm these results and to understand the mechanisms behind the observed association between IL-1 SNPs and periodontal disease.