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EDITORIAL

THE DIFFERENTIATION OF MAMMALIAN OVARIAN GRANULOSA CELLS – LIVING IN THE SHADOW OF CELLULAR DEVELOPMENTAL CAPACITY

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The mammalian cumulus-oocyte complex (COCs) promotes oocyte growth and development during long stages of folliculogenesis and oogenesis. Before ovulation, the follicle is formed by a variety of fully differentiated cell populations; cumulus cells (CCs) that tightly surround the female gamete, granulosa cells (GCs) and theca cells (TCs) which build the internal and external mass of the follicular wall. It is well documented that CCs surrounding the oocyte are necessary for resumption of meiosis and full maturation of the gamete. However, the role of the granulosa cells in acquisition of MII stage and/or full fertilization ability is not yet entirely known. In this article, we present an overview of mammalian oocytes and their relationship to the surrounding cumulus and granulosa cells. We also describe the processes of GCs differentiation and developmental capacity. Finally, we describe several markers of mammalian GCs, which could be used for positive identification of isolated cells. The developmental capacity of oocytes and surrounding somatic cells – a "fingerprint" of folliculogenesis and oogenesis

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EDITORIAL

MICROFLUIDIC VERSUS MOLECULAR ASSAYS - DIFFERENT APPROACHES IN ASSESSING OOCYTE DEVELOPMENTAL COMPETENCE

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In recent years, molecular techniques have brought about new solutions that focus on the developmental capacity of female oocytes and reproductive performance in the mammalian species. The developmental potency is the ability of oocytes to reach the MII stage following the long stages of folliculo- and oogenesis. The main proteins involved in this process belong to the connexin (Cx) family, which are responsible for the formation of gap junction (GJC) connections between the female gamete and surrounding somatic cells. The Cx are involved in bi-directional transport of small molecules and are therefore responsible for correct oocyte-somatic cell nutrition, proliferation, and differentiation. However, the application of certain molecular techniques often leads to destabilization or destruction of the materials of interest, such as cells or whole tissues. Therefore, the applications of microfluidic methods, which are non-invasive and quantitative, give new opportunities to further this area of biomedical research. Microfluidic research is based on real-time experiments that allow for control and/or observation of the results during each step. The purpose of this review is to present both positive and negative aspects of molecular-microfluidic methods while describing the role of connexins in oocyte developmental capacity.

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EDITORIAL

EXTRACORPOREAL SHOCK WAVES: PERSPECTIVES IN MALIGNANT TUMOR TREATMENT

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Progress in basic research led to the design of new generations of anticancer drugs with some notable achievements. Over the years, more and more powerful drugs have been developed with the purpose of increasing the rate of response to therapy. As molecular power of chemotherapeutic agents increased, unfortunately also toxicity and undesired side-effects increased. The search for new therapeutic strategies to be used in the management of cancer is one of the more promising strategies to reduce chemotherapy toxicity. Extracorporeal Shock Waves (ESW), widely used for the treatment of urolithiasis, have been reported to cause modifications of cell growth both *in vitro* and *in vivo*. They exert an agonist cytotoxic effect with several chemotherapeutic agents, such as cisplatin, doxorubicin, bleomycin, paclitaxel. Moreover, as it has been reported that their main mechanism of action is an increase in cell membrane permeability, ESW are also used to deliver oligonucleotides and other small particles to cells. Recently, it was found that certain dye compounds, in particular porphyrins, can achieve a cytopathogenic effect when the disease site is subjected to ultrasound irradiation. This technique is referred to as sonodynamic therapy. Based on the new knowledge regarding the interaction between ultrasound with bulk liquid, several studies have shown a synergic effect of ESW and porphyrins *in vitro*, thus opening a new perspective in sonodynamic therapy, able to overcome some drawbacks encountered during conventional anticancer drug treatment. Finally, current advances in bioengineering encouraged the application of nano-scale technologies to medicine. Nanobubbles, composed of an external shell and a gas core, can deliver chemotropic drugs and porfirins, to target tumour tissues in response to physical triggers, and ESW features make them an ideal alternative to ultrasound in combination with drug-loaded nanobubbles in delivery strategies.

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EDITORIAL

ENOX2 (or tNOX): A NEW AND OLD MOLECULE WITH CANCER ACTIVITY INVOLVED IN TUMOR PREVENTION AND THERAPY

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Cancer includes a number of related diseases due to abnormal cell proliferation that spreads to nearby tissues. Many compounds (physical, chemical and biological) have been used to try to halt this abnormal proliferation, but the therapeutic results are poor, due also to the side effects. It has been reported that ecto-nicotinamide adenine dinucleotide oxidase di-sulfide-thiol exchanger 2 (ENOX2), also known as tumor-associated nicotinamide adenine dinucleotide oxidase (tNOX), was found to be located on the cancer cell surface, essential for cancer cell growth. Capsaicin and other anti-oxidants are capable of inhibiting tNOX, causing apoptosis of cells, exerting anti-tumor activity. It is interesting that some authors reported that ENOX2 is present in the serum of cancer patients several years before the clinical symptoms of the tumor. However, this result has to be confirmed. In this article we discuss ENOX2 and its inhibition as a hope of improving cancer therapy.

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THE EFFECTS OF *P. GINGIVALIS* AND *E. COLI* LPS ON THE EXPRESSION OF PRO-INFLAMMATORY MEDIATORS IN HUMAN MAST CELLS AND THEIR RELEVANCE TO PERIODONTAL DISEASE

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Mast cells (MCs) are tissue-resident immune cells that participate in a variety of allergic and inflammatory conditions, including periodontal disease, through the release of cytokines, chemokines and proteolytic enzymes. *Porhyromonas gingivalis* (*P. g*) is widely recognized as a major pathogen in the development and progression of periodontitis. Here we compared the differential effects of lipopolysaccharides (LPS) from *P. g* and *E. coli* on the expression and production of tumor necrosis factor (TNF), vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein (MCP-1) by human MCs. Human LAD2 MCs were stimulated with LPS from either *P. g* or *E. coli* (1-1000 ng/ml). MCs were also stimulated with SP (2 μM) serving as the positive control or media alone as the negative control. After 24 h, the cells and supernatant fluids were collected and analyzed for β-Hexosaminidase (β-hex) spectrophotometrically, TNF, VEGF and MCP-1 release by ELISA and real-time polymerase chain reaction (PCR) for mediator gene expression, respectively. To assess the functional role of toll-like receptors (TRL) in mediator release, MCs were pre-incubated with either anti-TLR2 or anti-TLR4 (2 μg/ml) polyclonal antibody for 1 h before stimulation with LPS. When MCs were stimulated with SP (2 μM), there was a statistically significant β-hex release as well as release of TNF, VEGF and MCP-1. Stimulation of MCs with either type of LPS did not induce degranulation based on the lack of β-hex release. However, both types of LPS stimulated expression and release of TNF, VEGF and MCP-1. Although, *P. g* LPS induced significant release of TNF, VEGF and MCP-1, the effect was not concentration-dependent. There was no statistically significant difference between the effects of *P. g* and *E. coli* LPS. *P. g* LPS stimulated TNF through TLR-2 while *E. coli* utilized TRL-4 instead. In contrast, VEGF release by *P. g* LPS required both TRL-2 and TRL-4 while *E. coli* LPS required TLR-4. Release of MCP-1 was independent of TLR-2 or TLR-4. *P. g* LPS activates human MCs to generate and release TNF, VEGF and MCP-1 through different TLRs than *E. coli* LPS. MCs may, therefore, be involved in the inflammatory processes responsible for periodontal disease.

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OBESTATIN DIRECTLY CONTROLS CHICKEN OVARIAN CELL FUNCTIONS

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The aim of the present *in-vitro* study was to examine the role of obestatin in the direct control of basic avian ovarian granulosa cell functions – proliferation, apoptosis and secretory activity. In addition, the effects of obestatin on hormone release by cultured ovarian granulosa cells and follicular fragments (containing both granulosa and theca cells) were examined. We identified the effect of obestatin addition (0.1, 10 or 100 ng/ml medium) on the accumulation of markers of proliferation (PCNA, cyclin B1, MAPK/ERK1,2) and nuclear (TdT) and cytoplasmic (bax, caspase 3) apoptosis, as well as the release of progesterone (P), testosterone (T) and estradiol (E) by cultured chicken granulosa cells. Furthermore, the action of obestatin addition (0.1, 10 or 100 ng/ml medium) on the release of P, T, E and arginine-vasotocin (AVT) by cultured fragments of chicken ovarian follicles was examined. The accumulation of proliferation and apoptosis markers was assessed by immunocytochemistry and SDS PAGE-Western immunoblotting. The release of hormones was determined by an EIA. It was observed that obestatin addition could inhibit the accumulation of proliferation markers (PCNA and cyclin B1, but not of MAPK/ERK1,2), promote the expression of nuclear (TdT) and cytoplasmic (bax, caspase 3) apoptosis markers and suppress P, T, and E release by cultured granulosa cells. In cultured ovarian follicular fragments, obestatin promoted P, T, and E, but not AVT, release. These observations represent the first demonstration that (i) obestatin can directly control avian ovarian cell proliferation, apoptosis and hormone release and (ii) the interrelationship between theca and granulosa cells can determine the characteristics of obestatin action on ovarian secretory activity.

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EVALUATION OF CD4$^+$ CD25$^+$ FoxP3$^+$ REGULATORY T CELLS DURING TREATMENT OF PATIENTS WITH BRUCELLOSIS

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Cell-mediated immunity (CMI) plays a critical role in the control of brucellosis. Regulatory T cells (Tregs) have a functional character in modulating the balance between host immune response and tolerance, which can eventually lead to chronic infection or relapse. The aim of this study was to assess the alteration of Tregs in cases of brucellosis before and after treatment. Thirty cases of acute brucellosis with the mean age of 41.03±15.15 years (case group) and 30 healthy persons with the mean age of 40.63± 13.95 years (control group) were selected and assessed. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood of all individuals. We analyzed the alteration of Treg cell count using flow cytometry for CD4, CD25, and FoxP3 markers. The level of CD4$^+$ CD25$^+$ FoxP3$^+$ Treg cells was increased in active patients compared with controls (2.5±0.99% vs 1.6± 0.84%, p= 0.0004), but it had declined in the treated cases (1.83±0.73%, p=0.02). The level of Tregs was elevated in three relapsed cases. The frequency of Tregs and Treg/Teff (effector T cell) ratio was correlated with inverse serum agglutination test (SAT) and, 2-mercaptoethanol (2-ME) titers as markers of treatment in brucellosis. Based on our findings, we suggest that regulatory cells, such as CD4+ CD25+ FoxP3+ Treg cells, may contribute to the development of infection processes involving immune responses in brucellosis, and evaluation of regulatory T-cell levels may be a potential diagnostic strategy for the treatment outcome in chronic and relapsed cases of brucellosis.

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ZNF185 INHIBITS GROWTH AND INVASION OF LUNG ADENOCARCINOMA CELLS THROUGH INHIBITION OF THE AKT/GSK3β PATHWAY

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Zinc finger (ZNF) proteins, a diverse family of proteins, have multiple biological functions in cancer. Increased expression of ZNF185 has been involved in the regulation of tumor growth and metastasis. However, the function and underlying mechanisms of ZNF185 in the tumorigenesis of lung adenocarcinoma (LAC) remain unclear. The protein expression of ZNF185 was examined in human LAC tissues by immunohistochemical assay. After lentiviral vector-mediated ZNF185 overexpression was infected into the LAC cell lines (A549 and LETPα-2), cell growth and invasive potential were respectively evaluated by MTT and Transwell assays. We found that the protein expression of ZNF185 was significantly downregulated in LAC tissues compared with the adjacent non-cancerous tissues (ANCT) (37.10% vs 58.06%, P=0.015), and was negatively correlated with the lymph node metastasis of the LAC patients (P=0.005). Furthermore, overexpression of ZNF185 reduced cell proliferation and invasion in LAC cells, followed by the downregulation of p-AKT, p-GSK3β, VEGF and MMP-9 expression. Taken together, our findings indicate that the decreased expression of ZNF185 is linked to the tumor metastasis in human LAC patients, and ZNF185 overexpression inhibits the growth and invasion of LAC cells through inhibition of the AKT/GSK3β signaling, suggesting that ZNF185 may represent a potential therapeutic target for the treatment of LAC.

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EPITHELIALIZATION AND STROMALIZATION OF PORCINE FOLLICULAR GRANULOSA CELLS DURING REAL-TIME PROLIFERATION - A PRIMARY CELL CULTURE APPROACH

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The process of oocyte growth and development takes place during long stages of folliculogenesis and oogenesis. This is accompanied by biochemical and morphological changes, occurring from the preantral to antral stages during ovarian follicle differentiation. It is well known that the process of follicle growth is associated with morphological modifications of theca (TCs) and granulosa cells (GCs). However, the relationship between proliferation and/or differentiation of porcine GCs during long-term *in vitro* culture requires further investigation. Moreover, the expression of cytokeratins and vimentin in porcine GCs, in relation to real-time cell proliferation, has yet to be explored. Utilizing confocal microscopy, we analyzed cytokeratin 18 (CK18), cytokeratin 8 + 18 + 19 (panCK), and vimentin (Vim) expression, as well as their protein distribution, within GCs isolated from slaughtered ovarian follicles. The cells were cultured for 168 h with protein expression and cell proliferation index analyzed at 24-h intervals. We found the highest expression of CK18, panCK, and Vim occurred at 120 h of *in vitro* culture (IVC) as compared with other experimental time intervals. All of the investigated proteins displayed cytoplasmic distribution. Analysis of real-time cell proliferation revealed an increased cell index after the first 24 h of IVC. Additionally, during each period between 24-168 h of IVC, a significant difference in the proliferation profile, expressed as the cell index, was also observed. We concluded that higher expression of vimentin at 120 h of *in vitro* proliferation might explain the culmination of the stromalization process associated with growth and domination of stromal cells in GC culture. Cytokeratin expression within GC cytoplasm confirms the presence of epithelial cells as well as epithelial-related GC development during IVC. Moreover, expression of both cytokeratins and vimentin during short-term culture suggests that the process of GC proliferation is also highly associated with porcine ovarian follicular granulosa cell differentiation *in vitro*.

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KRÜPPEL-LIKE FACTOR 2 SUPPRESSES GROWTH AND INVASION OF GASTRIC CANCER CELLS *IN VITRO* AND *IN VIVO*

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Krüppel-like factor 2 (KLF2), a novel tumor-suppressor gene, is implicated in diverse cellular processes, including cell growth, apoptosis, and invasion. However, the role and action mechanisms of KLF2 in gastric cancer (GC) need be further elucidated. The expression of KLF2 was investigated by immunohistochemical assay in human GC tissues, and lentivirus-mediated KLF2 overexpression was transfected into GC cells (AGS and HGC-27) for assessing cell proliferation and invasion, respectively indicated by MTT and Transwell assays. Subcutaneous GC tumor models were constructed for estimating tumor growth *in vivo*. As a result, the expression level of KLF2 was decreased in GC tissues compared with the para-carcinoma tissues (31.03% *vs* 53.45%, *P*=0.035), and negatively correlated with the lymph node metastasis in GC patients (*P*=0.02). Moreover, overexpression of KLF2 inhibited the cell proliferation and invasive potential and downregulated the protein expression of PCNA, Bcl-2 and MMP-9 in GC cells. The result *in vivo* showed KLF2 overexpression reduced the xenograft tumor growth. In conclusion, our findings indicate that KLF2 may function as a tumor suppressor involved in the progression of human GC.

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VITAMINS D3 AND K2 MAY PARTIALLY COUNTERBALANCE THE DETRIMENTAL EFFECTS OF PENTOSIDINE IN *EX VIVO* HUMAN OSTEOBLASTS

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Osteoporosis is a metabolic multifaceted disorder, characterized by insufficient bone strength. It has been recently shown that advanced glycation end products (AGEs) play a role in senile osteoporosis, through bone cell impairment and altered biomechanical properties. Pentosidine (PENT), a well-characterized AGE, is also considered a biomarker of bone fracture. Adequate responses to various hormones, such as 1,25-dihydroxyvitamin D_3, are prerequisites for optimal osteoblasts functioning. Vitamin K_2 is known to enhance *in vitro* and *in vitro* vitamin D-induced bone formation. The aim of the study was to assess the effects of Vitamins D3 and K2 and PENT on *in vitro* osteoblast activity, to convey a possible translational clinical message. Ex vivo human osteoblasts cultured, for 3 weeks, with vitamin D_3 and vitamin K_2 were exposed to PENT, a well-known advanced glycoxidation end product for the last 72 hours. Experiments with PENT alone were also carried out. Gene expression of specific markers of bone osteoblast maturation [alkaline phosphatase, ALP; collagen I, COL Iα1; and osteocalcin (bone-Gla-protein) BGP] was measured, together with the receptor activator of nuclear factor kappa-B ligand/osteoproteregin (RANKL/OPG) ratio to assess bone remodeling. Expression of RAGE, a well-characterized receptor of AGEs, was also assessed. PENT+vitamins slightly inhibited ALP secretion while not affecting gene expression, indicating hampered osteoblast functional activity. PENT+vitamins up-regulated collagen gene expression, while protein secretion was unchanged. Intracellular collagen levels were partially decreased, and a significant reduction in BGP gene expression and intracellular protein concentration were both reported after PENT exposure. The RANKL/OPG ratio was increased, favouring bone reabsorption. RAGE gene expression significantly decreased. These results were confirmed by a lower mineralization rate. We provided *in vitro* evidence that glycoxidation might interfere with the maturation of osteoblasts, leading to morphological modifications, cellular malfunctioning, and inhibition of the calcification process. However, these processes may be all partially counterbalanced by vitamins D_3 and K_2. Therefore, detrimental AGE accumulation in bone might be attenuated and/or reversed by the presence or supplementation of vitamins D_3 and K_2.

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LETTER TO THE EDITOR

EXPOSURE TO ELECTROMAGNETIC FIELDS ABOARD HIGH-SPEED ELECTRIC MULTIPLE UNIT TRAINS

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High-speed electric multiple unit (EMU) trains generate high-frequency electric fields, low-frequency magnetic fields, and high-frequency wideband electromagnetic emissions when running. Potential human health concerns arise because the electromagnetic disturbances are transmitted mainly into the car body from windows, and from there to passengers and train staff. The transmission amount and amplitude distribution characteristics that dominate electromagnetic field emission need to be studied, and the exposure level of electromagnetic field emission to humans should be measured. We conducted a series of tests of the on board electromagnetic field distribution on several high-speed railway lines. While results showed that exposure was within permitted levels, the possibility of long-term health effects should be investigated.

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LETTER TO THE EDITOR

PERCUTANEOUS CORONARY INTERVENTION FOR POOR CORONARY MICROCIRCULATION REPERFUSION OF PATIENTS WITH STABLE ANGINA PECTORIS

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Percutaneous coronary intervention (PCI) has been extensively applied to repair the forward flow of diseased coronary artery and can achieve significant curative results. However, some patients with acute myocardial infarction (AMI) develop non-perfusion or poor perfusion of cardiac muscle tissue after PCI, which increases the incidence of cardiovascular events and the death rate. PCI can dredge narrowed or infarct-related artery (IRA) and thus induce full reperfusion of ischemic myocardium. It is found in practice that some cases of AMI still have no perfusion or poor perfusion in myocardial tissue even though coronary angiography suggests opened coronary artery after PCI, which increases the incidence of vascular events and mortality. Therefore, to explore the detailed mechanism of PCI in treating coronary microcirculation of patients with stable angina pectoris, we selected 140 patients with stable angina pectoris for PCI, observing the index of microcirculatory resistance (IMR) of descending branch and changes of myocardial injury markers and left ventricular systolic function, and made a subgroup analysis based on the correlation between clinical indexes, IMR and other variables of diabetic and non-diabetic patients, PCI-related and non-PCI-related myocardial infarction patients. The results suggest that IMR of anterior descending branch after PCI was higher compared to that before PCI, and the difference was significant ($P<0.05$); creatine kinase-MB (CK-MB), myohemoglobin and high sensitive troponin T were all increased after PCI, and the difference was also significant ($P<0.05$); brain natriuretic peptide (BNP) level became higher after PCI, with significant difference ($P<0.05$); left ventricular ejection fraction (LVEF) declined after PCI, and the difference before and after PCI was statistically significant ($P<0.05$). Moreover, subgroup analysis results of the three groups all demonstrated statistically significant differences. PCI can effectively increase microcirculatory resistance of patients with stable angina pectoris, especially those who develop both stable angina pectoris and diabetes. Patients with higher microcirculatory resistance before PCI are more likely to develop PCI-related myocardial infarction after PCI.

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LETTER TO THE EDITOR

ANALYSIS OF HIGH RISK FACTORS FOR ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY BILIARY METALLIC STENTING AFTER MALIGNANT DUODENAL STRICTURE SEMS IMPLANTATION

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The aim of this study was to explore the success rates and high risk factors for endoscopic retrograde cholangiopancreatography (ERCP) biliary metallic stenting after self-expandable metallic stent (SEMS) implantation in patients with malignant duodenal stricture. A retrospective cohort study was conducted. Forty-two unresectable patients with malignant duodenal stricture who received endoscopic SEMS implantation between February 2012 and February, 2015 in the Department of Digestive Diseases of Xijing Hospital were enrolled in the study. These patients underwent subsequent ERCP biliary metallic stenting due to malignant biliary stricture. The clinical and iconography materials of these patients were retrospectively analyzed. ERCP biliary metallic stenting was successfully carried out on 71.4% of patients with previous malignant duodenal stricture SEMS implantation. In type 1 duodenal strictures 88% success rate of ERCP guided biliary decompression was obtained *vs* 18.2% success rate in Type 2 duodenal strictures. In both type 1 and 2 duodenal strictures of a length greater than 3.5 cm, ERCP was 44.4% successful *vs* 89% successful for strictures less than 3.5cm. Multiple regression analysis revealed that duodenal stricture length ≥3.5 cm (OR, 9.85; 95% CI, 1.21-79.88) and 80 or 90 mm duodenal stent (OR, 17.03; 95% CI,1.99-145.81) were independent risk factors for the failure of ERCP (biliary drainage or biliary decompression) in the patients with previous SEMS implantation. Moreover, duodenal stents of 60 mm had a higher success rate of 88%, *vs* 18.2% in 80-90 mm stents. Nevertheless, the success rates of type III strictures were 100%, including synchronous and asynchronous implantation of SEMS implantation and ERCP biliary metallic stenting. For unresectable malignant duodenal stricture patients with SEMS implantation, subsequent ERCP biliary metallic stenting was safe and effective. The length of malignant duodenal stricture, longer duodenal stents and type II duodenal stricture were high risk factors for the failure of ERCP biliary metallic stenting.

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LETTER TO THE EDITOR

DYNAMICS OF MONOCYTE SURFACE RECEPTORS AFTER BURNS: A PILOT STUDY

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Previous studies suggested that monocytes may play a vital role in infection and sepsis following burn injury. The aim of this study was to determine whether burn injury had any effect on the levels of expression of monocyte cell-surface receptors at different phases post burn injury. Ten adult burn victims with burns of > 25% of the total body surface area were included in this study. Blood samples were collected on the first, third and seventh day post burn injury. The peripheral blood mononuclear cells were extracted, with or without lipopolysaccharide stimulation. The monocyte phenotypes of CD14, CD16, HLA-DR, CD163, TLR2 and TLR4 were characterized by flow cytometry. Six healthy volunteers were recruited as controls. The percentage of expressed CD14+ monocytes increased during the first day, and then decreased on the third and seventh day after burn injury. The percentages of CD14+ cells expressing CD16 and HLA-DR decreased on the first day, followed by an increase on the third and seventh day post burn. In comparison, the percentage of CD14+ monocytes expressing TLR2 and TLR4 was higher on the first day in burn patients than that of control participants, followed by no change on the third and seventh day post burn injury. There was no significant difference in the percentages of CD14+ expressing CD163 between the two groups. This study showed that the expression of the specific receptors on the surface of monocyte is affected by burn injury. The changes in the expression levels of these receptors may contribute to burn-induced infection susceptibility.

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LETTER TO THE EDITOR

ROSUVASTATIN ALLEVIATES THE DEVELOPMENT OF MONOCROTALINE-INDUCED PULMONARY HYPERTENSION IN RATS

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Statins can increase endothelial function through enhancement of the expression and activity of endothelial nitric oxide synthase (eNOS). The aim of this study is to evaluate the effect of rosuvastatin on the number of circulating endothelial progenitor cells (EPCs) and endothelial expression of eNOS in monocrotaline-induced pulmonary hypertensive rats. Sixty Sprague-Dawley (SD) rats were divided into three groups of 20: control (group A), pulmonary hypertension (PAH) + rosuvastatin group (group B), and PAH (group C). Monocrotaline (MCT; 60 mg/kg) was injected (intraperitoneally) to induce PAH. Rats in group B received rosuvastatin [10 mg/(kg. day)] for 2 weeks. Peripheral blood (5 mL) was aspirated from the femoral artery of each rat before and after 2 weeks of treatment. Mononuclear cells were isolated and subcultured to obtain EPCs. Small and moderately sized pulmonary arteries were collected 2 weeks later for histological analyses. eNOS gene expression in endothelial cells of pulmonary arteries were then determined at mRNA and protein levels. eNOS expression at mRNA and protein levels and the number of circulating EPCs were reduced significantly in groups B and C compared with group A ($P < 0.05$), and a significant difference between group B and group C ($P < 0.05$) was observed. Vascular remodeling in small and moderately sized pulmonary arteries was attenuated markedly in group B compared with group C. These results suggest that rosuvastatin can ameliorate the remodeling of pulmonary arteries in MCT-induced PAH rats by increasing the number of circulating EPCs and eNOS upregulation.

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LETTER TO THE EDITOR

HEPATIC FIBROSIS AND SUPERSONIC SHEAR IMAGING IN PATIENTS WITH DIFFERENT ETIOLOGICAL CHRONIC HEPATIC DISEASES

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The first two authors contributed equally to this work

The objective of the present study was to investigate whether hepatic fibrosis difference of supersonic shear imaging (SSI) value existed in patients with different etiological chronic hepatic diseases. Retrospective analysis was used to study chronic hepatitis. All the subjects were diagnosed by shear wave elastography and percutaneous liver biopsy. The shear moduli were analyzed to check whether any difference existed between groups. For the chronic hepatitis B, autoimmune hepatitis and fatty hepatitis, the shear moduli in S0 stage were (8.50±3.1)kPa, (9.41±2.5)kPa, (8.97±3.8)kPa; the shear moduli in S1 stage were (9.54±3.0)kPa, (10.42±5.1)kPa, (9.51±4.6)kPa; the shear moduli in S2 stage were (11.77±4.8) kPa, (13.25±5.6)kPa, (11.03±6.0)kPa; the shear moduli in S3 stage were (14.96±6.1)kPa, (19.03±7.8) kPa, (15.38±7.8)kPa; the shear moduli in S4 stage were (20.36±7.5)kPa, (24.99±9.5)kPa, (19.53±5.6)kPa. Shear wave elastography could measure the different etiological chronic hepatic diseases.

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LETTER TO THE EDITOR

BODY COMPOSITION IN HEALTHY OLDER PERSONS: ROLE OF THE RATIO OF EXTRACELLULAR/TOTAL BODY WATER

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The aim of this study was to identify the best prognostic parameters for quickly assessing fluid volume status in the context of nutritional status and water balance in older persons and to facilitate decision-making of the general practitioner (GP). This pilot study was conducted with 142 volunteers aged 60 years or older who were Polish students of the University of the Third Age. Inclusion and exclusion criteria for the study were defined. Assessment tools included: the Mini Nutritional Assessment questionnaire (MNA®) and the anthropometric measurements. Weight and body composition analysis were determined by Bioelectrical Impedance Analysis (BIA) using the Tanita MC-780 multi frequency segmental Body Composition Analyzer. According to the MNA scale, 89.2% of the sample was well-nourished and 10.8% were at risk of malnutrition. A total of 47.1% participants had normal body mass index, 20.6% were overweight, and 32.3% were obese. The BIA showed that females had more fat mass (FM) compared to males (35.84% *vs* 23.90%), while men had more free fat mass (FFM) and total body water (TBW; 61.16% *vs* 45.22% and 53.31% *vs* 45.22% respectively). There were no statistically significant differences in FM, FFM, and TBW by age. The ratio of Extracellular to Total Body Water (ECW/TBW) was higher in women than in men (46.76% *vs* 43.66%). Of all measures, only ECW/TBW increased significantly with age and sex, especially after 65 years. We propose that ECW/TBW may be used as the first, simple, and fast indicator of water volume status in the context of nutritional status and water balance in older subjects. Systematic control of the ECW/TBW by GP or nurse may increase senior independence, resulting in longer self-maintenance at home and reduced hospital admissions.

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LETTER TO THE EDITOR

EXPRESSIONS OF NDRG1, VEGF AND Ki-67 IN CONDYLOMA ACUMINATUM

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The objective of this study was to explore the expressions and significance of NDRG1 (N-myc downregulated gene family 1), VEGF (vascular endothelial growth factor) and Ki-67 in lesions of Condyloma Acuminatum (CA). Immunohistochemistry was adopted to measure the expressions of NDRG1, VEGF and Ki-67 in 48 cases of CA and 18 normal skin controls. The positive rates of NDRG1, VEGF and Ki-67 were 63. 83.33% (40/48), 93.75% (45/48) and 85.42% (41/48) in the CA tissues, and 27.78% (5/18), 94.44% (17/18) and 61.11% (11/18) in the controls, respectively. The intensities of the expressions of NDRG1, VEGF and Ki-67 in CA tissues were significantly higher than those in the controls. There were significant differences both in the positive rates and the expression intensities of NDRG1, VEGF and Ki-67 between the two groups (P <0.05). The Spearman's Rank-Order Correlation analysis indicated that the expressions of NDRG1 protein and VEGF protein were positively correlated by the Spearman's Rank-Order Correlation analysis (r = 0.346, P=0.016). For the CA tissues with high expressions of NDRG1 and VEGF, NDRG1 and VEGF influenced both the occurrence and development of CA.

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LETTER TO THE EDITOR

EFFECT OF TUMOR NECROSIS FACTOR-INDUCED PROTEIN 8 ON T-CELL-MEDIATED IMMUNITY IN MICE AFTER THERMAL INJURY

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Tumor necrosis factor-induced protein 8(TNFAIP8), the first identified member of the TNFAIP8 family, shares considerable sequence homology with members of this family. It is expressed in a wide variety of human normal tissues, with relatively higher levels in lymphoid tissues and placenta. The present study was designed to examine the effect of TNFAIP8 on T-cell-mediated immunity secondary to burn injury. Sixty male mice were randomly divided into four groups as follows: sham burn group, burn group, burn with TNFAIP8-siRNA transfection group, and burn with negative control transfection group, and they were sacrificed at designated time points. CD4+ T cells were isolated using MACS microbeads. T-Cell proliferation was analyzed with MTT assay, and IL-2, soluble IL-2R, IL-4, interferon-γ (IFN-γ) were determined with enzyme-linked immunosorbent assay kits. It was found that CD4+ T lymphocyte proliferative activity was significantly down-regulated when TNFAIP8 gene was silenced by siRNA in mice at 24 h post burn. Down-regulation of TNFAIP8 can significantly decrease expression levels of IL-2 and soluble IL-2R at 24 h after thermal injury. These results demonstrated that TNFAIP8 appeared to be involved in the immune regulation of CD4+ T lymphocytes, and the decreased expression of TNFAIP8 could affect T lymphocyte functions after thermal injury.

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LETTER TO THE EDITOR

PHYTOPHARMACOLOGY OF *TRIBULUS TERRESTRIS*

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***Tribulus terrestris* is an annual herb which belongs to the *Zygophyllaceae* family. This plant has been used in traditional medicine for the treatment of various diseases for hundreds of decades. The main active phytoconstituents of this plant include flavonoids, alkaloids, saponins, lignin, amides, and glycosides. The plant parts have different pharmacological activities including aphrodisiac, antiinflammatory, antimicrobial and antioxidant potential. *T. terrestris* is most often used for infertility and loss of libido. It has potential application as immunomodulatory, hepatoprotective, hypolipidemic, anthelmintic and anticarcinogenic activities. The aim of the present article is to create a database for further investigation of the phytopharmacological properties of this plant to promote research. This study will definitely help to confirm its traditional use along with its value-added utility, eventually leading to higher revenues from the plant.**

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LETTER TO THE EDITOR

EFFECTS OF 10-HYDROXYCAMPTOTHECIN ON DIFFERENTIATION OF RAW264.7 CELLS INTO OSTEOCLASTS

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This study was designed to investigate the effect of 10-hydroxycamptothecin (10-HCPT) on osteoclast formation. RAW264.7 cells were cultured *in vitro* with 100 ng/ml receptor activator for nuclear factor-κ B ligand (RANKL) and 30 ng/ml recombinant macrophage colony stimulating factor (M-CSF), and 10-HCPT with different solubilities were added. After five-day cultivation, tartrate-resistant acid phosphatase (TRAP) staining was used to observe the number of osteoclasts. mRNA expression of osteoclast-specific genes, such as TRAP, cathepsin K (CTSK) and matrix metalloproteinase protease 9 (MMP-9), was detected by real-time Polymerase Chain Reaction (PCR). The effect of 10-HCPT on the proliferation activity of RAW264.7 cells was detected using Cell Counting Kit-8 (CCK-8). CCK-8 detection showed that 10-HCPT with a certain concentration (1 ng/ml to 5 ng/ml) had no effect on cell proliferation (P>0.05); 10-HCPT could inhibit the generation of osteoclasts. With the increase of the concentration of 10-HCPT, the number of osteoclasts generated from cells cultured with 10-HCPT [1 ng/ml (86±11.14), 2 ng/ml (66.67±7.51), 5 ng/ml (27.67±6.51)] was much lower than that of the control group (145±8.19), and the difference was statistically significant (all P=0, P<0.05); mRNA expression of osteoclast-specific gene TRAP [1 ng/ml (24.38±0.68), 2 ng/ml (20.09±1.86), 5 ng/ml (6.23±0.53)], CTSK [1 ng/ml (10.08±0.81), 2 ng/ml (7.30±0.30), 5 ng/ml (3.20±0.56)] and MMP-9 [1 ng/ml (43.54±6.96), 2 ng/ml (28.28±5.83), 5 ng/ml (11.07±2.53)] was much lower than that of the groups added with RANKL and M-CSF only (all P=0, P<0.05), and with the increase of concentration of 10-HCPT, the expression of osteoclast-specific genes showed a decreasing tendency. All the findings suggest that 10-HCPT can inhibit the formation of osteoclasts by reducing the expression of osteoclast-specific genes such as TRAP, CTSK and MMP-9.

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LETTER TO THE EDITOR

RADIOTHERAPY OF THE NECK AND CAROTID STENOSIS

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The first choice of treatment for neck cancer is often radiotherapy. Therefore, we aimed to investigate the microinflammation after radiotherapy of the neck and the incidence of carotid stenosis. This study reports on patients treated with radiotherapy as part of the treatment for laryngeal cancer in the Department of Radiation Oncology, The Second Hospital of Jilin University, Changchun, P.R. China. Sixty-two males and nine females were treated with radiotherapy between 2006 and 3012. The carotid diameter was determined by measuring carotid intima-media thickness (IMT) in the common, external and internal carotid artery. Microinflammatory conditions were assessed by high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and tumor necrosis factor–alpha (TNF-α). Other studied risk factors included age, treatment modalities, radiation dose and energy, the height of the radiation field, and follow-up time. Carotid stenosis was detected in all of the 71 patients. It was mainly clinically unsuspected; 19 patients had sustained a vascular event (14 TIA, 5 CVI) at a median of 3.11 years (range 2.3–5.6 years) following RT. In four of five CVI patients, CVI occurred on the side of the irradiation. Eleven patients who suffered vascular incident had severe stenosis of the carotid artery and 6 had moderate (31-49% of the lumen). Only two patients with mild stenosis on the irradiated side suffered TIAs. Serum hs-CRP levels in carotid stenosis were 9.4 (±SD=5.97) mg/ml, IL-6 = 12.8 (±SD=2.62) pg/ml and TNF-α = 15.4 (±SD=4.49) ng/ml. The clinical detection of asymptomatic carotid stenosis is challenging, and current recommendations regarding the follow-up period should be scrutinized.

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LETTER TO THE EDITOR

USE OF THREE-DIMENSIONAL COLOR POWER DOPPLER IN IMAGING OF LIVER CANCER

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We aimed to assess the role of three-dimensional color power Doppler (3D-CPD) imaging in diagnosis of liver cancer. First of all, we performed 2D- and 3D-CPD imaging on 96 cases of liver tumors with a total of 106 lesions to examine the characteristics of the vascular distribution patterns of the tumors, and in turn, compare the sensitivity and specificity. Also, with the use of three-dimensional volumetric measurement, we calculated the volume of tumors, quantity of intra-tumor blood vessels, and the ratio of the quantity of intra-tumor blood vessels to tumor volumes (vascular index, VI). Finally, we statistically analyzed the vascular index between benign and malignant tumors. We found that the sensitivity/specificity were 21.3%/100% for 2D-CPD, and 81.3%/100% for 3D-CPD, based on the use of type-III of blood vessels for diagnosis of the malignant lesions. In 3D-CPD, the type-III of blood vessels along with VI of > 0.3/cm^3 can be used as the criteria for the diagnosis of liver cancer since we found that average VI was 0.38/cm^3 in 75 malignant tumors, but 0.18/cm^3 in 31 benign tumors ($p<0.05$). The sensitivity and specificity in determining malignancy in the liver based on VI > 0.3/cm^3 were 78.7% and 87.1%, respectively.

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LETTER TO THE EDITOR

AN ETHNOBOTANICAL STUDY OF MEDICINAL PLANTS WITH NARCOTIC, SEDATIVE AND ANALGESIC EFFECTS IN WEST OF IRAN

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The first step for identification of medicinal plants and their therapeutic effects is to determine their use by local people, traditional medicine books and personal experiences. The aim of this study was to document the medicinal plants used as analgesic, sedative or narcotic agents by local residents of Dehloran, Iran. Interviews conducted with 53 informants (38 male and 15 female) revealed that a total of 32 medicinal plants belonging to 22 families are used in Dehloran as narcotic, sedative and analgesic agents. The most utilized plant families were *Asteraceae*, *Rosaceae* and *Fabaceae*. Approximately 74% of the utilized plants was attributed to herbs, followed by trees (13%) and shrubs (13%). Sixty-six percent of the medicinal plants used in the study area were perennial and the rest were annual or biannual. The most widely used plant parts were flowers (34%) followed by leaves (24%) and fruits (14%). Thirty-nine percent of the medicinal plants were used as sedatives, 39 percent as analgesics, and 24% as narcotics. Recommended plants in this study can be good candidates for further clinical and laboratory trials on diseases that are associated with pain, suffering, stress and depression. They also can be used to develop new sedative, narcotic and analgesic drugs.

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LETTER TO THE EDITOR

INVESTIGATION INTO EARLY POSTOPERATIVE INFLAMMATORY SMALL BOWEL OBSTRUCTION BY APPLYING GASTROINTESTINAL DECOMPRESSION

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The objective of this study was to investigate early postoperative inflammatory small bowel obstruction (EPISBO) by applying gastrointestinal decompression to relieve abdominal distension. Thirty-six cases of patients were randomly divided into two groups: a control group (20 cases) and an observation group (16 cases). Routine continuous gastrointestinal decompression was assigned to the control group, while gastrointestinal decompression with dynamic and profound adjustment of the gastric tube and abdomen movement was assigned to the observation group, to induce abundant gastric juice and gas, and significantly relieve abdominal distension. A test was performed for each of the two groups to observe the relief time of the abdominal distension and the difference of abdominal girth of 5 cm before and after gastrointestinal decompression. Compared with the control group, the patients in the observation group with abdominal distension had earlier pain relief. More patients in the observation group had a difference of abdominal girth of 5 cm before and after gastrointestinal decompression. In gastrointestinal decompression, the method of dynamic and profound adjustment of the gastric tube and abdomen movement improve the effect of the gastrointestinal decompression, which relieves abdominal distention and promotes the postoperative recovery of organ functions.

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LETTER TO THE EDITOR

IN VITRO EFFECT OF IL-17D ON HUMAN OVARIAN CARCINOMA CELLS AND INHERENT IMMUNITY

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This study explored the expression of interleukin 17D (IL-17D) secreted by human ovariancarcinoma cells and the effect of exogenous IL-17D transfection on MICA, which is the ligand of NKG2D, on the surface of ovary carcinoma cells. Human ovarian papillary serous adenocarcinoma cell line SKOV3, empty vector control cell line SKOV3/vector, exogenous human IL-17D stable-transfected cell line SKOV3/IL-17D, as well as cisplatin (CDDP)-resistant cell SKOV/CDDP were cultured; ovarian adenocarcinoma cell line OVCAR-3, empty vector control cell line OVCAR3/vector and OVCAE3/IL-17D were observed under a microscope. In the study, methyl-thiazolyl-tetrazolium (MTT) method was used to detect the inhibition rate, resistance index and proliferation of SKOV3 and SKOV3/CDDP. It was found that the expression of IL-17 D in SKOV3/CDDP was much higher than that of its parent cell line SKOV3; IL-17D might be correlated to the drug resistance of cells; the proliferation of SKOV3 transfected with IL-17D was significantly accelerated, indicating IL-17D may be effective in promoting the growth of oncocyte.

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LETTER TO THE EDITOR

CLINICAL EFFECT OF MECHANICAL FRAGMENTATION COMBINED WITH RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR ARTERY THROMBOLYSIS ON ACUTE CEREBRAL INFARCTION

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This study aims to explore the clinical effect of mechanical fragmentation combined with recombinant tissue plasminogen activator (rt-PA) artery thrombolysis on acute cerebral infarction (ACI). One hundred and thirty-two cases of ACI patients were randomly divided into an experimental group (66 patients) and a control group (66 patients). The experimental group was treated with mechanical fragmentation combined with rt-PA artery thrombolysis method, while the control group was treated with only the rt-PA artery thrombolysis method. All the patients had their basic information recorded. A computational analysis on National Institutes of Health Stroke Scale (NIHSS) scores, curative effect and bleeding data was carried out. The results showed that in the experimental group the curative effects were better and there were fewer complications. Accordingly, we conclude that mechanical fragmentation combined with rt-PA artery thrombolytic method is a safe and reliable therapy with significant curative effects. It improves the NIHSS scores of the patients markedly and reduces the incidence of subsequent pneumonia.

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LETTER TO THE EDITOR

VITAMIN D AND MICRO-INFLAMMATORY STATE IN HEMODIALYSIS PATIENTS A MINI REVIEW AND META-ANALYSIS

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There is growing evidence that vitamin D (VitD) plays a role in the pathophysiological mechanism of every patient undergoing hemodialysis, and this role is significantly altered in a microinflammatory state. However, it is unclear whether supplementation dosage or route of administration should be altered due to this state. Thus, the objective of our mini review and meta-analysis was to re-consider supplementation of VitD in HD patients exhibiting micro-inflammatory state. Pubmed, Web of Science and Google Scholar were searched up to January 19, 2016. We included studies that evaluated supplementation in HD patients with micro-inflammatory state. One reviewer extracted data and one reviewer verified the data accuracy. We qualitatively summarized the main results and meta-analyzed data on comparable outcomes across studies. The main outcome measures were serum levels of VitD. Ten eligible studies were published between 2002 and 2016, involving a total 1,239 patients. Average vintage of hemodialysis was 35.36 (±31.08) months. We identified a high degree of clinical diversity (interventions and outcomes) and methodological heterogeneity (sample size and risk of bias) in included trials. The studies we reviewed provide some weak evidence to support VitD supplementation in patients on hemodialysis exhibiting micro-inflammatory state. We recommend that future trials focus on our main outcome measures (that is variable comparable across studies).

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LETTER TO THE EDITOR

COMPARISON OF EFFECTS OF ^{18}F-FDG PET-CT AND MRI IN IDENTIFYING AND GRADING GLIOMAS

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The first two authors contributed equally to this study as co-first authors

Glioma is the most common type of brain tumor. Malignant gliomas tend to have an increasingly higher incidence and are difficult to treat. Therefore, an accurate diagnosis of the grade of glioma before surgery is very important for planning surgery and determining prognosis. To compare the values of ^{18}F-deoxyglucose positron emission tomography/computer tomography (^{18}F-FDG PET-CT) and magnetic resonance imaging (MRI) for identifying and grading gliomas, we selected 70 patients who were diagnosed as having a primary glioma or suspected glioma at the People's Hospital of Liaocheng in Shandong, China, and divided them into an observation group, which was examined by ^{18}F-FDG PET-CT and a control group, which was examined by MRI. Image analysis, visual semi-quantitative analysis and qualitative analysis, follow-up and pathological results of the two groups were compared. Specificity, accuracy and sensitivity of brain MRI and PET-CT in grading the gliomas were calculated, and the results obtained were processed by *Chi*-squared test. Standard uptake value (SUV), $SUV_{correct}$ and L/WM (SUV_{max} ratio of a lesion to normal white matters in the opposite side) of FDG in the different grades of glioma were analyzed by single-factor variance analysis. Postoperative pathological detection confirmed 47 cases of glioma; the sensitivity, specificity and accuracy of PET-CT in grading glioma were all higher than those of MRI ($P<0.05$); the correlation between SUV and glioma grade, between $SUV_{correct}$ and glioma grade, and between L/WM and glioma had significant difference ($P<0.05$). Thus, it was concluded that ^{18}F-FDG PET-CT performs better in diagnosing gliomas than MRI and is also more suitable for identifing different grades of glioma.

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LETTER TO THE EDITOR

DETECTION AND STUDY OF PLASMA D-DIMER CHANGE IN PATIENTS WITH ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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The purpose of this study was to observe the change in plasma D-dimer of patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD). The patients were divided into three groups, i.e., AECOPD group, stable COPD group (COPD kept stable after treatment) and a healthy control group. The content of plasma fibrinogen (FIB) and D-dimer of all research subjects was detected and the difference between groups was analyzed. Moreover, pulmonary functions of patients in the AECOPD group and the stable COPD group, including forced expiratory volume in 1 second (FEV1%) and forced vital capacity rate of 1 second (FEV1/FVC), and blood gas (oxygen partial pressure (PO_2) and partial pressure of carbon dioxide ($PaCO_2$), were detected; and the differences between the two groups and the possible correlation were analyzed. Compared to the COPD stable group and the control group, the AECOPD group had a statistically significant higher content of plasma FIB and D-dimer ($p < 0.05$); the content of plasma FIB and D-dimer of the COPD stable group was much higher than that of the healthy control group, but the difference had no statistical significance ($p > 0.05$); the content of D-dimer of AECOPD patients was in a negative correlation with FEV1 and PO_2 ($p < 0.05$) and in a positive correlation with PCO_2 ($p < 0.05$). It can be concluded that D-dimer is correlated to the severity of AECOPD; hence, it can be used as an evaluation index for the severity of AECOPD.

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LETTER TO THE EDITOR

NON-SURGICAL PERIODONTAL MANAGEMENT IN SCLERODERMA DISEASE PATIENTS

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The aim of the present study is to investigate the periodontal status of people with scleroderma and their response to non-surgical treatment protocol aimed at controlling the evolution of the disease. The response to non-surgical periodontal treatment was tested on patients belonging to a scleroderma group and a control group: the data show an improvement of the periodontal conditions of all these patients in response to treatment. When compared on the same diagram, a slight remission of the periodontal disease was obtained in both scleroderma and healthy patients. This highlights the benefit to soft tissues produced by non-surgical periodontal treatment also in patients affected by systemic diseases.

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LETTER TO THE EDITOR

STANDARDIZATION PROCEDURE FOR THE NASAL NITRIC OXIDE MEASUREMENT METHOD USING NIOX MINO® AND THE TIDAL-BREATHING TECHNIQUE WITH VELUM-CLOSURE

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Nitric oxide (NO) is a molecule that performs many functions in the human body. The entire respiratory tract can produce NO, but the highest production occurs in the upper respiratory tract, in the paranasal sinuses in particular. The aim of the present study was to assess a new nasal NO (nNO) measurement method using the Niox MINO Nasal® device (Aerocrine AB, Solna, Sweden) and a special procedure, in order to compare the nNO values obtained in 32 healthy subjects with the values found in the international literature. The measured normal nNO values were equal to 426.76±143.27 ppb, with a 95% confidence interval [160.22-733.30]. Males had an average nNO value equal to 446.76±133.63 [178.64 – 714.02], whereas in females the average value was 403.80±154.90 [94.00 – 713.60]. This study allows us to confirm that we have been able to establish the normal range of nitric oxide quantity produced in the nasal/sinus cavities of healthy individuals using the Niox MINO Nasal® device and tidal-breathing with velum-closure manoeuvre.

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LETTER TO THE EDITOR

LIGHTS AND SHADOWS OF DENTAL IMPLANTS: FOCUS ON MUCOSITIS AND PERIMPLANTITIS AND THEIR BIOLOGICAL MARKERS

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The increase in oral rehabilitation by means of dental implants has required an evolution of the related managing protocols and correct updating of the skills of dental professionals. Postsurgical management of the clinical case is aimed to stabilize the obtained results and preserve them from adverse conditions: a healthy implant prosthesis is maintained thanks to the huge number of consolidated protocols of oral hygiene. This practice plays a decisive role in the prevention of perimplant pathologies, forming a strong basis to ensure long implant life and avoid unnecessary and painful new surgical procedures. Furthermore, dental companies, in order to satisfy the new needs of professionals in oral hygiene, have produced new instrumentations and targeted drugs, in agreement to the cutting-edge scientific literature, thus creating a new market attracting huge interests in healthcare. The purpose of this topical review is to briefly comment on the state of the art of post-surgical dental implant management. This research is aimed to report the current protocols available to reduce the risk of oral diseases and prevent the progression of perimplant complications. Special focus has been dedicated to the most effective surgical and non-surgical protocols for treating mucositis and perimplantitis.

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LETTER TO THE EDITOR

CYTOKINE GENOTYPE DISTRIBUTION IN PATIENTS WITH PERIODONTAL DISEASE AND RHEUMATOID ARTHRITIS OR DIABETES MELLITUS

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The association between oral and systemic health has highlighted the importance of periodontal health and treatment, with the consequence that dental assessment and attention to oral hygiene have assumed an increasingly important part in the clinical management of patients with diabetes mellitus and rheumatoid arthritis. The aim of this work was to assess genotype frequencies in polymorphisms of genes of IL-1α-889 and IL-1β-511 in a case-controlled study population of patients affected by periodontal disease and rheumatoid arthritis or diabetes mellitus.

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LETTER TO THE EDITOR

THYROID AND SHOULDER DISEASES: THE BASES OF A LINKED CHANNEL

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The association between thyroid disorders and musculoskeletal diseases has long been suspected, but it is still debated whether they have a role in the pathogenesis of shoulder diseases. *In vivo* and *in vitro* studies describe the role of thyroid hormones in bone, cartilage and tendon biology. Retrospective studies and case reports suggest that thyroid diseases should be considered as risk factors and hold prognostic value in some of the most common causes of shoulder pain. Thus, it is advisable to search for underlying thyroid disorders in these patients. The pathophysiologic mechanisms by which thyroid hormone imbalance affects the onset, progression and response to treatment of these diseases are yet to be thoroughly defined and demand further studies.

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LETTER TO THE EDITOR

A SOLITARY UTERINE RELAPSE IN T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA: CT FEATURES AND PATHOLOGIC CORRELATION

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T-cell Acute Lymphoblastic Leukemia (T-cell ALL) is a rare haematological neoplasia, that affects children and less commonly adults. Female genital tract and particularly uterus involvement in acute ALL is rare. This report presents the CT features of a 64-year-old woman with uterine relapse of T-cell ALL, occurring 11 months after the diagnosis, as a second, unique relapse of disease. The patient was asymptomatic when a CT examination showed a homogenous thickness of the uterine wall in comparison with the previous CT examination. Histology from biopsy specimens, obtained through hysteroscopy, confirmed T-cell ALL localisation (TdT+, CD10+, CD3c+ and CD2+). The uterus could be a site of relapse in patients suffering from ALL. Even though an MRI examination could better demonstrate the disease in cases of suspected female genital tract involvement by ALL, the comparison of differences between a present and a previous CT examination is sufficient to suspect the diagnosis.

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LETTER TO THE EDITOR

ENDOMETRIOSIS AND GLANZMANN'S THROMBASTHENIA

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Glanzmann's thrombasthenia (GT) is a rare bleeding syndrome characterized by deficiency or defect of platelet aggregation complex. The pathogenesis of endometriosis is controversial but the strongest evidence leans towards retrograde menstruation. GT probably predisposes to endometriosis. The management of women affected by this disease can be difficult due to the risk of bleeding complications, especially during surgical treatment. We describe the cases of three sisters affected by endometriosis and GT, referred to our Department, who received different therapeutic management.

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LETTER TO THE EDITOR

CENTRAL APELIN-13 ADMINISTRATION MODULATES HYPOTHALAMIC CONTROL OF FEEDING

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The 77 amino prepropeptide apelin has been isolated from bovine stomach tissue and several smaller fragments, including apelin-13, showed high affinity for the orphan APJ receptor. The distribution of apelinergic fibers and receptors in the hypothalamus may suggest a role of apelin-13 on energy balance regulation, albeit the studies reporting the acute effects of apelin on feeding control are inconsistent. Considering the possible involvement of apelinergic system on hypothalamic appetite controlling network, in the present study we evaluated in the rat the effects of intrahypothalamic apelin-13 injection on food intake and the involvement of orexigenic and anorexigenic hypothalamic peptides and neurotransmitters. Eighteen rats (6 for each group of treatment) were injected into the ARC with either vehicle or apelin-13 (1-2 μg/rat). Food intake and hypothalamic peptide and neurotransmitter levels were evaluated 2 and 24 h after injection. Compared to vehicle, apelin-13 administration increased food intake both 2 and 24 h following treatment. This effect could be related to inhibited cocaine- and amphetamine-regulated transcript (CART) gene expression and serotonin (5-hydroxytryptamine, 5-HT) synthesis and release, and increased orexin A gene expression in the hypothalamus.

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LETTER TO THE EDITOR

USE OF SODIUM HYALURONATE AND SYNTHETIC AMINO ACID PRECURSORS OF COLLAGEN FOR THE SYMPTOMATIC TREATMENT OF MUCOSITIS IN PATIENTS UNDERGOING HAEMATOPOIETIC STEM CELL TRANSPLANTS

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Oral mucositis (OM) may occur in up to 100% of patients undergoing condition regimen to hematopoietic stem cell transplant (HSCT). From the patient's perspective, OM is one of the most debilitating side effects of transplantation. It is commonly thought that oral hygiene can modify the incidence and severity of oral mucositis, therefore professional oral health care (POHC) is recommended prior to conditioning regimen for HSCT. A new strategy for the treatment of OM is sodium hyaluronate (SH) combined with amino acid precursors of collagen (Aas) (Mucosamin®). SH is a mucoaderent polymer acting as a mechanical barrier and pain reliever. Furthermore, it allows prolonged contact of the product with the mucous membrane. In this study, a total of 68 adult patients due to undergo HSCT for allogenic and autologous transplant were enrolled at the Stem Cell Transplant Unit. The patients were divided into two groups. One group was treated with POHC before HSCT and applications of Mucosamin® during the recovery after transplantation. The second group served as controls, with the usual treatment of Clorexidine 0.20% adopted by the department. After HSCT the same clinician, an expert in oral medicine trained for the clinical trial, evaluated symptoms of the patients' mucositis of both groups every day. The treated patients developed less severe OM, therefore Mucosamin® seems to have a protective role against the more severe phases of mucositis. The maximum OM pain, measured with the VAS scale, was higher in patients who did not use Mucosamin®. In the treated group OM resolved sooner than in the control group.

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LETTER TO THE EDITOR

PROBIOTIC MIXTURE SUPPLEMENTATION IN THE PREVENTIVE MANAGEMENT OF TRINITROBENZENESULFONIC ACID-INDUCED INFLAMMATION IN A MURINE MODEL

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Inflammatory bowel diseases (IBD) are characterized by inflammatory conditions of the intestine. Probiotic bacteria (PB) can have beneficial effects in several gastrointestinal disorders. The objectives of this study were: (i) to provide an acute experimental IBD model induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) in CD-1 mice, and (ii) to assess the preventive effects of Citogenex (*Lactobacillus casei* and *Bifidobacterum lactis*) supplementation on intestinal tissues and microbiota. Mice were inoculated intrarectally with saline, ethanol or different TNBS solutions. 1%TNBS induced clinical signs of colitis ($P < 0.01$) and histological damage ($P < 0.01$). Based on these results, mice were pre-treated with Citogenex or saline for 1, 2 or 3 weeks before 1%TNBS treatment. Probiotic pre-treatment determined a reduction of clinical signs ($P < 0.05$), histological alterations of colitis ($P < 0.05$) and increased beneficial bacteria ($P < 0.05$). This study confirms that TNBS-induced colitis in CD-1 mice is useful for studying the mechanisms involved in IBD pathogenesis, and pre-treatment with Citogenex prevents the intestinal damage induced by TNBS.

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LETTER TO THE EDITOR

AGE-RELATED ULTRASTRUCTURAL AND MONOAMINE OXIDASE CHANGES IN THE RAT OPTIC NERVE

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The aim of this paper is to study the morphology and the distribution of the monoamine oxidase enzymatic system in the optic nerve of 4 month-old Wistar (young) and 28 month-old Wistar (old) rats. The optic nerve was harvested from 20 young and old rats. The segment of optic nerve was divided longitudinally into two pieces, each 0.1 mm in length. The first piece was used for transmission electron microscopy. The second piece was stained with histochemical reaction for monoamine oxidase. The age-related changes in the optic nerve of rats include micro-anatomical details, ultrastructure and monoamine oxidase histochemical staining. A strong decrease of the thin nerve fibers and a swelling of the thick ones can be observed in optic nerve fibers of old rats. Increased monoamine oxidase histochemical staining of the optic nerve of aged rats is well demonstrated. The increase of meningeal shealth and the decrease of thin nerve fibers of the optic nerve in old rats are well documented. Morphological, ultrastructural and histochemical changes observed in optic nerve fibers of the old rats show a close relation with aging.

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LETTER TO THE EDITOR

NEWBORN SCREENING OF INHERITED METABOLIC DISORDERS: THE ITALIAN SITUATION

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Starting from an international overview of the current status of screening programs, the present paper focuses on the legal situation in Italy and the great differences among Italian regions. Since the introduction of tandem mass spectrometry (MS/MS) in the '90s the paradigm "one spot-one disease" changed. Only recently, some regions issued legislative acts to promote expanded newborn screening with MS/MS. This approach raises medico-legal and ethical issues because a fast neonatal diagnosis of an inborn error of metabolism (IEM) could increase chances of an early treatment and reduce disabilities, therefore citizens ought to have the same access to care countrywide. Enacting a mandatory standard for a disease screening panel using MS/MS and a few centers specialized in diagnosis, treatment and follow-up of patients affected by IEM (inborn errors of metabolism) can reduce legal and ethical issues.

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LETTER TO THE EDITOR

IgG4-RELATED CRANIO-SPINAL HYPERTROPHIC PACHYMENINGITIS INVOLVING THE INTERNAL AUDITORY CANAL

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Immunoglobulin G4 (IgG4)-related hypertrophic pachymeningitis, which is a focally or diffusely thickened dura mater and lymphoplasmacytic infiltration with increased IgG4 bearing plasma cells, is a rare disease, and cases involving the whole cervical spine are even rarer. Here, we describe a case of probable IgG4-related hypertrophic pachymeningitis involving the whole cervical spine and the auditory canals in an 18-year-old male. The patient, who had a history of paresthesia and had previously experienced weakness, presented with generalized tonic seizures. A decompressive laminectomy on cervical vertebrae was performed as a matter of urgency, removing intradural fibrous material. The patient responded well to treatment and was discharged walking independently, with no strength deficit to any of the 4 limbs, and with normal blood tests.

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LETTER TO THE EDITOR

NUTRACEUTICAL APPROACHES TO HOMOCYSTEINE LOWERING IN HYPERTENSIVE SUBJECTS AT LOW CARDIOVASCULAR RISK: A MULTICENTER, RANDOMIZED CLINICAL TRIAL

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Although the role of homocysteine (HCys) in secondary cardiovascular prevention has been scaled down, hyper-homocysteinemia remains a risk factor for cerebrovascular events. The aim of this study was to investigate the efficacy of nutraceuticals in lowering HCys serum levels versus a conventional vitamin supplementation in hypertensive subjects at low cardiovascular risk. One-hundred and four patients (mean age 62.8±14.5 years, 63.5% males), 52 for each treatment group, were enrolled. The study recruited patients with stage 1 essential hypertension and hyper-homocysteinemia (HCys ≥15 μmol/L), without a history of cardiovascular and cerebrovascular disease. They were sequentially randomized to receive a combined nutraceutical containing 400 μg folate-6-5-methyltetrahydrofolate, 3 mg vitamin B6, 5 μg vitamin B12, 2.4 mg vitamin B2, 12.5 mg zinc and 250 mg betaine (Normocis400®) once daily for two months, or supplementation with highly dosed folic acid (5 mg/day) (control group). Differences in serum HCys values were compared by ANOVA for repeated measures. A significant HCys reduction in comparison to baseline was found in both groups at the end of the study treatment, from 21.5□8.7 to 10.0±1.7 μmol/L for Normocis400® subjects (p<0.0001), and from 22.6±6.2 to 14.3±2.8 μmol/L for controls (p<0.0001). HCys reduction was significantly higher among patients treated with Normocis400® (p<0.035). The ideal HCys level (i.e. <10 μmol/L) was reached in 55.8% of cases in the Normocis400® group, and it was significantly higher than in controls. No side effects were observed in either treatment group. Randomized clinical trials are ongoing to test the effect of folate, B6, and B12 supplementation in primary prevention of cardiovascular and cerebrovascular events. In the meantime, especially when the ideal HCys level is far from being reached, Normocis400® appears to be safe, well tolerated and effective in reducing HCys levels.

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

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LETTER TO THE EDITOR

NEONATAL OXIDATIVE STRESS DEPENDS ON OXYGEN BLOOD PRESSURE IN UMBILICAL ARTERY

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With advancing gestation, partial pressure of oxygen (pO_2) and pH fall significantly. Hypoxia is a main factor inducing free radical generation and thereby oxidative stress (OS). Placental and fetal tissue response when oxygen becomes restricted is complex and partially known. We tested the hypothesis that changes in umbilical artery and vein blood gas concentrations modulate OS occurrence in the newborn. Seventy umbilical artery and vein plasma samples were collected from healthy term newborns immediately after delivery. F2 Isoprostanes (F2-Isop) were measured in all samples as reliable markers of lipid peroxidation. Significantly lower pCO_2 and higher pO_2 and pH were found in umbilical vein than in artery, as expected. A positive correlation was detected between pH and pO_2 only in umbilical artery (p=0.019). F2-Isop levels were no different between artery and vein in cord blood. Significant correlations were found between F2-Isop and pCO_2 (p=0.025) as well as between F2-Isop and pH in umbilical vein (p=0.027). F2-Isop correlated with pCO_2 (p=0.007) as well as with pO_2 values (p=0.005) in umbilical artery blood. Oxidative stress (OS) in newborns depends on oxygen concentrations in umbilical artery. OS biomarkers significantly correlate with pO_2 and in umbilical artery but not in umbilical vein. In normoxic conditions fetal-maternal gas exchanges occurring in placenta re-establish normal higher oxygen levels in umbilical vein than artery, with a normal production of free radicals without any deleterious effects.

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