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1 Monoamine Oxidase-B Inhibitor Protects Degenerating Spinal Neurons and Enhances Nerve Regeneration and Functional Recovery in Sciatic Nerve Crush Injury Model

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Introduction: Monoamine oxidase (MAO) is a flavin adenine dinucleotide containing an enzyme which catalyzes the oxidation of amines. MAO-B is proposed to play an important role in the pathogenesis of neurodegeneration through the production of reactive oxygen species and neurotoxins. The present study was designed to outline the effects of the MAO-B inhibitor on sciatic nerve regeneration, neuroprotection of spinal neurons and sensory-motor functional recovery in the sciatic nerve crush injury model.

Methods: Male Wistar rats (4 months old) were assigned to (i) naïve (N), (ii) sham (S), (iii) sciatic nerve injured and treated with saline (I + saline) and (iv) sciatic nerve injured and treated with MAO-B inhibitor (I + MAO-B-I) groups (n = 10/group). In groups iii and iv, the injury was produced by crushing the sciatic nerve followed by treatment with saline or MAO-B-I (2.5 mg/kg) for 10 days. Behavioral tests were conducted from week 1 to week 7. At the end of the study, the sciatic nerve and lumbar spinal cord were studied by immunohistochemistry, light and electron microscopy. Data were analyzed with one-way ANOVA followed by Bonferroni’s multiple comparison tests.

Results: I + MAO-B-I treatment showed significant improvement in sensory and motor tests (hopping reflex, hot plate test, tail flick test, extensor postural thrust, foot position, toe spread test, mechanical hyperalgesia test) compared to the I + saline group (p < 0.05–0.001). The morphological study showed a significantly increased number of nerve fibers in sciatic nerve (p < 0.05), with a better myelination pattern in the I + MAO-B-I group compared to the I + saline group. Spinal cord ventral horns showed a significant increase in the number of NeuN-immunoreactive neurons in the I + MAO-B-I group compared to the I + saline group (p < 0.01).

Conclusions: MAO-B-I has a significant potential for protecting the degenerating spinal cord neurons and enhancing the regeneration of injured sciatic nerve following crush injury.

2 Thymoquinone Enhances Neurogenesis, Learning and Memory in Young Adult Rats Born to Diabetic Rats

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Introduction: Our earlier study and literature showed learning and memory deficits in young rats which were born to diabetic rats. The present experiment was aimed to study the effects of thymoquinone (TQ, a constituent of Nigella sativa seeds) on neurogenesis, learning and memory in the young rats born to the diabetic rats.

Methods: Diabetes was induced in pregnant rats on the
10th gestational day by streptozotocin (50 mg/kg i.p.). Pups born were fostered to normal mothers. They were divided into diabetic control (DC), and diabetic + TQ (D + TQ) groups when they were 40 days old. Age-matched rats born to normal pregnant rats were divided into normal control (NC) and TQ groups (n = 12 in each group). Rats in D + TQ and TQ groups were treated with TQ (10 mg/kg i.p.) from postnatal day (PND) 41 to PND 60. Rats in all 4 groups were subjected to the Morris water maze test, and they were perfused with 4% paraformaldehyde. Hippocampal tissue was processed for doublecortin (DCX; marker for new neurons) and glial fibrillary acidic protein (GFAP) immunostaining. Data were analyzed with one-way ANOVA. Results: Rats in the DC group showed significant learning and memory deficits compared to the NC group (p < 0.001). Treatment with TQ normalized learning and memory deficits in the D + TQ group. TQ alone did not show any effect on cognition compared to the NC group (p > 0.05). Numbers of new neurons and astrocytes were found to be significantly decreased in the DC compared to NC group (p < 0.01), and numbers of new neurons and astrocytes in the D + TQ group were not significantly different from those of the NC group (p > 0.05). TQ treatment alone increased neurogenesis in the TQ group compared to the NC group (p < 0.05). Western blot analysis of DCX and GFAP protein content confirmed the immunostaining data. Conclusions: Prenatal diabetes affects postnatal adult neurogenesis, learning and memory. TQ can enhance adult neurogenesis in the hippocampus of young rats born to diabetic mothers and thereby enhance the cognitive functions.

Introduction: Cataracts are an eye abnormality, often known as lens opacities. Congenital cataract is an early-onset inherited form of cataracts that is diagnosed at birth and considered the main reason for reversible blindness in children. Recently, many genetic studies have been conducted to unravel the molecular genetic background of the disease. Multiple genes were discovered to have a strong causative role in the development of the disorder. Here we genetically investigate autosomal dominant congenital bilateral cataract (ADCC) in a Kuwaiti multigenerational family.

Methods: Several members of a multigenerational Kuwaiti family suffering from ADCC were recruited from Al-Bahar Eye Centre. Clinical examination and assessment were completed. DNA was extracted from both affected and unaffected members. Genetic linkage analysis was performed using Affymetrix Gene Chip Human Mapping 250K Arrays. Whole-exome sequencing using a NextSeq Illumina platform was performed. Results: Genomewide linkage analysis results of the investigated ADCC family revealed 2 high logarithmic odds ratio scores (2.4 and 1.75) for 2 distinguished loci, 22q13.31 and 3q22. Whole-exome sequencing (WES) resulted in the discovery of many novel single-nucleotide polymorphisms and mutations that were predicted to play a role in cataractogenesis. The most prominent mutations were 2 novel
deletions in the CELSR1 gene and CRYAA gene. The CRYAA gene encodes for α-crystallin A protein of the lens. Other mutations in this gene have been implicated with cataract. On the other hand, the CELSR1 gene encodes a cadherin receptor and has never been linked to cataract, but we suspect that it played an important role in the manifestation of congenital cataract. **Conclusions:** ADCC is considered a relatively rare disorder. More research in this field is encouraged in order to reach a better understanding of the disease’s development. WES proved to be a very successful tool for the discovery of disease-causing mutation and led us to the discovery of 2 novel deletions in the CELSR1 and CRYAA genes. **Funding Agency:** Yes.

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(-)-Epigallocatechin-3-Gallate Enhances Learning and Memory and Adult Neurogenesis in Streptozotocin-Induced Diabetes Rats

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**Introduction:** Diabetes mellitus is a chronic disease characterized by high levels of glucose in the blood. (-)-Epigallocatechin-3-gallate (EGCG), found in green tea, proved to be responsible for its beneficial effects. The objective of this study was to examine the effects of EGCG on learning and memory and adult hippocampal neurogenesis in streptozotocin (STZ)-induced diabetes rats. **Methods:** Male Wistar rats (3 months old) were divided into vehicle control (VC), EGCG, diabetic (DI), and diabetic + EGCG-treated (DI + EGCG) groups (n = 12 in each group). Diabetes was induced in DI and DI + EGCG groups with STZ (40 mg/kg i.p.). EGCG and DI + EGCG rats were treated with 50 mg/kg of EGCG (i.p.) for 3 weeks. The VC group was injected with 1 ml of saline for 3 weeks. Learning and memory were assessed in all animals during the 3rd week. Rats were perfused with 4% paraformaldehyde for immunohistochemical analysis of neurogenesis and astrogliosis. Data were analyzed with one-way ANOVA and Bonferroni’s test. **Results:** Learning and memory test showed a significant memory deficit in DI rats compared to VC and EGCG groups (p < 0.001). In contrast, memory was significantly improved in the DI + EGCG group compared to DI animals (p < 0.001). Doublecortin (DCX, a marker for new neurons) and glial fibrillary acidic protein (GFAP, a marker for astrocytes) showed a significant decrease in neurogenesis and number of astrocytes in DI compared to VC and EGCG groups (p < 0.01). Neurogenesis was found to be significantly increased in the DI + EGCG compared to the DI group (p < 0.001). Western blot analysis of DCX and GFAP protein content confirmed the immunostaining data. **Conclusions:** EGCG enhances astrocyte number which provides neurotrophic support for neurogenesis, thereby enhancing neurogenesis and cognitive function. We conclude that EGCG has a beneficial role in minimizing the effects of diabetes on the hippocampal neurogenesis. **Funding Agency:** Kuwait University Research Grant No. YM01/14.
Graduate Research Award for Medical Residents

1 Performance of CarbR GeneXpert® Assay against Culture and PCR for the Detection of Carbapenemase-Producing Enterobacteriaceae in Rectal Swabs

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Introduction: Carbapenemase-producing Enterobacteriaceae (CPE) have emerged as a global threat around the world. CPE have the propensity to spread easily between humans (hand carriage, contaminated food and water). These emerging pathogens cause difficult-to-treat infections with high morbidity and mortality. This study was undertaken to evaluate the performance of the CarbR GeneXpert assay (CGXA) against culture and PCR in the detection of CPE from rectal swabs. Methods: A total of 100 non-repetitive rectal swabs, in duplicates, were collected from patients in the adult intensive-care unit (ICU), pediatric ICU and a surgical ward. They were investigated simultaneously by culture and the CGXA. The culture method was by direct inoculation on a MacConkey agar plate on which a 10-μg meropenem disk was placed and incubated in air at 37 °C for 24 h. After overnight incubation, isolates identified as CPE were confirmed by PCR performed using established primers. The CGXA was performed according to the manufacturer’s protocol. Five isolates with known metallo-β-lactamase genes were included in the assay. Results: The sensitivity and specificity were calculated using the PCR assay as the reference test standard. The sensitivity and specificity of the CGXA were 80 and 98.9%, respectively. The prevalence of CPE colonization in our high-risk population was 5 with 80% identified as NDM-1-positive. A recent travel history was significantly associated with CPE colonization (p < 0.005). The turn-around time from specimen to result was 1 h compared with culture and subsequent PCR of 30 h. Conclusions: With such a performance, the CGXA should readily be incorporated into any busy routine clinical microbiology laboratory. The rapid detection of CPE harboring blaKPC, blaVIM, blaIMP, blaNDM or blaOXA-48 genes directly from rectal swabs within 1 h should assist in timely decision-making on contact precautions and early detection of outbreaks within the hospital.

2 Recurrent Meningitis and Brain Abscess due to Infected Fibrin Glue Sealant following Functional Endoscopic Sinus Surgery

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Background: The most frequently affected sites of cranial complications of functional endoscopic sinus surgery (FESS) are the boundaries between the anterior and posterior ethmoid roof, the frontal recess and the cribriform plate. Serious complications after FESS are rare, one of them being cerebrospinal fluid (CSF) leak while other less often reported complications are frontal lobe injury, subarachnoid hemorrhage, pneumocephalus and meningitis. We report a case of recurrent meningitis with brain abscess in a patient who underwent FESS for chronic sinusitis 2 years ago. Case Report: A 22-year-old Kuwaiti male patient underwent FESS in 2013 for chronic sinusitis, nasal polyp and deviated nasal septum. During surgery CSF leakage occurred, which was repaired with fibrin glue sealant. In late 2015, he was admitted with fever, headache and photophobia. Biochemical tests and cytology of a CSF sample indicated pyogenic meningitis. However, culture of CSF did not yield growth of any microorganism. The patient was discharged a week later after having been successfully treated with ceftriaxone, vancomycin and dexamethasone although MRI of the brain showed ethmoidal fungal sinusitis extending to the left cribiform plate and the floor of the anterior cranial fossa reaching the left frontal lobe. Ten days later, he was readmitted with a similar presentation, and this time CSF culture grew Pseudomonas aeruginosa. He received therapy with piperacillin/tazobactam to which he responded. Repeat MRI of the brain revealed a brain abscess, which was empirically treated by adding gentamicin and metronidazole to the regimen. Revised ESS revealed infected fibrin glue. Conclusion: Despite the huge development in surgical instrumentation and surgical abilities, serious post-ESS complications can still occur. To the best of our knowledge, our patient represents a unique case who developed a delayed complication of recurrent meningitis and brain abscess, not reported earlier in the literature.
Best Young Researcher Award for Basic Sciences

Association of the I Allele of the Common ACE I/D Polymorphism with Type 2 Diabetes Mellitus among Kuwaiti Cardiovascular Disease Patients

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Introduction: The D allele of the common angiotensin-converting enzyme (ACE) I/D gene polymorphism (rs4646994) predisposes to type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). However, results on which allele predisposes to disease susceptibility remains controversial in Arab populations. This study was performed to evaluate the association of the common ACE I/D gene polymorphism with both T2DM and CVD susceptibility in a Kuwaiti population.

Methods: We genotyped the ACE I/D polymorphisms by direct allele-specific PCR in 183 healthy controls and 400 CVD patients with diabetes (n = 204) and without (n = 196). Statistical analyses comparing between the different groups were conducted using the R statistic package ‘SNPassoc’.

Results: Two genetic models were used, the additive and codominant models. The I allele was found to be associated with T2DM (odds ratio = 1.84, p = 0.00009) after adjusting for age, sex and body mass index. However, there was no association with CVD susceptibility (p > 0.05).

Conclusions: The ACE I allele is found to be associated with T2DM; however, no association was observed with CVD. The inconsistency between studies is suggested to be attributed to genetic diversity due to the existence of subpopulations found in Arab populations.

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Best Young Researcher Award for Clinical Sciences

Does Vitamin D Deficiency Increase the Risk of Fractures in Children?

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Introduction: Vitamin D deficiency is the most prevalent nutritional deficiency globally. It has been linked with a wide spectrum of conditions and diseases (e.g. rickets in children). Its classic role involves calcium (Ca) regulation, and it is known to affect bone quality and health. This study aimed to assess vitamin D levels in children with fractures compared to healthy children.

Methods: A prospective case-control study was conducted of children (boys <14 and girls <12 years old) in Al-Razi Orthopaedic Hospital in Kuwait. Data collected were serum 25-hydroxyvitamin D (25[OH]D) levels and a bone profile (calcium, magnesium, albumin, alkaline phosphatase). The levels used were; insufficiency <75 nmol/l, deficiency <50 nmol/l and severe deficiency <25 nmol/l. A \( \chi^2 \) test was performed to examine the relation between vitamin D levels and fractures. A multivariate and a bivariate logistic regression model was made to compare fractures with age, gender, sport activity, supplementation and daily milk intake.

Results: It was a convenience sample that included 188 subjects (104 fractures and 84 controls). The \( \chi^2 \) analysis for the relation between these fractures and vitamin D levels was not significant, \( \chi^2(2, n = 189) = 14.14, p = 0.33 \). Our null hypothesis (vitamin D does not increase fracture risk) was not rejected. In the logistic regression model, we found that children who do not meet the reference daily intake (RDA) for milk will have a 6.68 times greater risk of getting a fracture compared with those who meet the RDA.

Conclusions: No statistically significant relation was established between vitamin D levels and fractures.