Abstracts of Theses Approved for the MSc and PhD Degrees at the Faculty of Medicine, Health Sciences Center, Kuwait University, Kuwait

MSc Degree

1 Vitamin D and Its Association with Multiple Sclerosis in Kuwait
Anwar Saad Al-Enezi
Department of Pathology, Faculty of Medicine, Kuwait University, Kuwait

Introduction and Objectives: Vitamin D deficiency is associated with several complex disorders including multiple sclerosis (MS). Several factors influence vitamin D levels and its optimal multifunction maintenance. Our objective was to assess quantifiable variables influencing vitamin D level and metabolism in MS patients from Kuwait. Methods: This is a case-control study involving 50 Kuwaiti MS patients and 50 healthy control Kuwaiti individuals recruited at Dasman Diabetes Institute’s neurology clinic. Plasma vitamin D levels were determined using an enzyme-linked immunosorbent assay. Vitamin D receptor (VDR) variants were assessed using Taqman genotyping assays. Skin pigmentation indices were ascertained using a hand-held spectrophotometer. Statistical analyses included Mann-Whitney rank sum test, χ² test, and Student t test. A p value <0.05 was considered significant for all tests. Results: We found overall vitamin D levels to be deficient in both groups, and supplement use to be common practice. VDR variants Taq-I (rs731236) GG genotype and Bsm-I (rs1544410) CC genotype were associated with MS risk (p = 0.0008, p = 0.003; respectively). VDR SNP Apa-I (rs7975232) AA genotype was associated with low disease progression (p = 0.003). VDR variant Fok-I (rs2228570) GG genotype was associated with higher constitutive melanin indices in both cohorts (p = 0.04). Conclusions: Several quantifiable variables related to vitamin D are associated with MS risk and clinical course, suggesting a possible clinical immunomodulatory application for vitamin D supplementation in Kuwaiti MS patients as a mode of palliative/management therapy.

Dr. Rabeah Al-Temaimi (Supervisor)
Dr. Ahmed Al-Serri (Cosupervisor)

2 Impact of Prenatal Dexamethasone Administration on the Expression of p73 Gene Variants in Fetal Brain
Mai Abdullah Abul
Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait

Synthetic glucocorticoids (GCs), such as dexamethasone (Dex), are clinically prescribed to pregnant women at risk of preterm delivery or bearing fetuses at risk of congenital adrenal hyperplasia. Prenatal Dex administration was associated with several neuropsychological and behavioural disorders during infancy and adulthood. The long-lasting impact of prenatal GCs on fetal brain development is well known; however, the mechanisms through which prenatal GCs induce these long-lasting disturbances have not been discovered yet. Recently, prenatal GC exposure was found to induce fetal brain growth restriction and changed the expression of one of the genes that are involved in neural cell death, survival, and differentiation programmes: p73. The p73 gene encodes for 2 protein variants; the pro-apoptotic protein (TAp73), and the anti-apoptotic protein (ΔNp73). It is not clear how synthetic GCs modulate this gene and which neural brain cell types (neurons or glia) are affected. In this study, the impact of prenatal Dex on fetal brain development will be discovered by detecting the expression and the neural location of p73 gene/protein variants. Pregnant dams received daily intraperitoneal injections of either
Dex (0.4 mg/kg, n = 6) or pyrogen-free saline (n = 6) from gestation day (GD) 14 until GD21. Fetal brains were collected and snap frozen in liquid nitrogen, then stored at −80°C until used for detection of TAp73 and ΔNp73 mRNAs and proteins by RT-PCR and Western blot, respectively. Another group of fetal brains was immersed in 10% buffered formalin solution, then paraffin embedded to be used in an immunohistochemistry study. Prenatal Dex administration during critical periods of fetal brain development (GD14–GD21) significantly reduced fetal body as well as brain weight. Prenatal Dex increased TAp73 protein expression; this enhanced TAp73 expression was associated with pronounced neural cell death as revealed by increased cleaved caspase-3 immunoreactive cells. Furthermore, Dex significantly reduced the number of mature neurons co-expressing TAp73 protein. The increased expression of TAp73 and the concurrent increase in neural cell death and reduction in the number of differentiated neurons may be responsible for the restricted fetal brain development in Dex-treated dams.

Dr. Abdeslam Mouihate (Supervisor)
Dr. Maie Dawoud Al-Bader (Cosupervisor)

3 Effects of Oxygen Glucose Deprivation and Recovery on Polarization of Microglia in Primary Culture and Effects on Astrocytes of these Processes
Rawan Muneer Ishak Barakat
Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait

Activation of microglia/macrophages following cerebral ischemia may be beneficial or detrimental for the survival of brain cells. This ambiguity was explained by findings that ischemia induces transformation of the microglia/macrophages into two different phenotypes, a process often referred to as polarization into M1 and M2 phenotypes. To which extent these processes depend on paracrine signaling from other cells of the neurovascular unit (NVU) is not clear. The aim of this in vitro study was to explore whether polarization of these cells into the two phenotypes could occur in the absence/low abundance of other NVU cells and to assess the effect of microglia/macrophage-derived cytokines on astrocytes’ viability during anoxia. Primary cultures of rat microglia/macrophages were exposed to 2 h of oxygen glucose deprivation (OGD) and then incubated further under normal conditions, which was considered as a recovery period. Expression of phenotype-specific markers at transcript and protein levels and secretion of phenotype-specific cytokines were explored at different time points of the recovery period by real-time PCR, Western blotting and immunohistochemistry, respectively. The expression of the proliferating cell nuclear antigen and caspase-3 was investigated by immunohistochemistry to assess cell proliferation and apoptosis, respectively. The gene expression of MTA1 increased on 19 dg, and its cytoplasmic protein expression increased at 21 dg in the labyrinth zone in the DEX group. In the basal zone, an increase in its cytoplasmic expression was on 19 dg and a decrease in nuclear expression at 21 dg in the DEX group. The MTA2 gene expression decreased in the labyrinth zone at 19 and 21 dg in the DEX group. The MTA2 protein expression increased in the cytoplasm of the basal zone and in the labyrinth zone at 19 dg in the DEX group. The MTA3 gene expression decreased on 19 and 21 dg in the labyrinth zone of the DEX group and at 21 dg in the basal zone of the DEX group. In the basal zone its protein expression decreased in the nuclear fraction at 21 dg and increased in the cytosolic fraction at 19 dg in the DEX group. Cell proliferation showed a decrease and cell death showed an increase in the DEX group. IUGR is associated with molecular changes in MTA expression in different placental zones and cell fractions. This could be linked to decreased cell proliferation and increased cell death.

Dr. Maie Dawoud Al-Bader (Supervisor)
Dr. Abdeslam Mouihate (Cosupervisor)

4 Intrauterine Growth Restriction Is Associated with Changes in Placental Expression of Metastasis Tumor Antigens in Rats
Mariam Fahad Alqaryyan
Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait

Molecular mechanisms underlying placental formation in normal and intrauterine growth restriction (IUGR) placentas are not clearly known. Since metastasis tumor antigen (MTA) 1 and MTA2 promote cell proliferation while MTA3 suppresses proliferation, we hypothesized that during normal placental development there would be an increase in MTA1 and MTA2 expression and a decrease in MTA3 expression, which might be reversed with IUGR. Pregnant Sprague-Dawley rats received daily intraperitoneal injections of either dexamethasone (0.4 mg/kg; DEX group), or saline (control group) starting from 14 days of gestation (dg) to either 19 or 21 dg. Gene and protein expression of MTA1, MTA2 and MTA3 in the basal and labyrinth zones of the placenta were investigated by real-time PCR, Western blotting and immunohistochemistry, respectively. The expression of the proliferating cell nuclear antigen and caspase-3 was investigated by immunohistochemistry to assess cell proliferation and apoptosis, respectively. The gene expression of MTA1 increased on 19 dg, and its cytoplasmic protein expression increased at 21 dg in the labyrinth zone in the DEX group. In the basal zone, an increase in its cytoplasmic expression was on 19 dg and a decrease in nuclear expression at 21 dg in the DEX group. The MTA2 gene expression decreased in the labyrinth zone at 19 and 21 dg in the DEX group. The MTA2 protein expression increased in the cytoplasm of the basal zone and in the labyrinth zone at 19 dg in the DEX group. The MTA3 gene expression decreased on 19 and 21 dg in the labyrinth zone of the DEX group and at 21 dg in the basal zone of the DEX group. In the basal zone its protein expression decreased in the nuclear fraction at 21 dg and increased in the cytosolic fraction at 19 dg in the DEX group. Cell proliferation showed a decrease and cell death showed an increase in the DEX group. IUGR is associated with molecular changes in MTA expression in different placental zones and cell fractions. This could be linked to decreased cell proliferation and increased cell death.

Dr. Maie Dawoud Al-Bader (Supervisor)
Dr. Abdeslam Mouihate (Cosupervisor)

Abstracts
Molecular Characterization of Methicillin-Resistant Staphylococcus aureus Isolated in Kuwait Hospitals
Samar S.S. Boswihi
Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

As the epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) is constantly changing globally, determining the prevailing MRSA clones in a local health care facility is important for the better management of infections. This study investigated antibiotic resistance patterns, carriage of virulence factors, clonal composition and distribution of MRSA isolates in Kuwait’s hospitals using a combination of antibiogram and molecular typing methods. In total, 400 nonrepeat MRSA isolates obtained between 1992 and 2010 in 13 public hospitals were characterized. The isolates were resistant to kanamycin (74.2%), erythromycin (69.5%), tetracycline (66.7%), gentamicin (61%), ciprofloxacin, (61%), fusidic acid (53.5%), clindamycin (41.5%) and high-level mupirocin (5.2%), and carried aphA3, aacA-aphD, ermA, ermC, mupA, tetK, tetM, fusC and far1. Molecular typing revealed 31 different MRSA clones consisting of ST239-MRSA-III (52.2%), ST22-MRSA-IV (9.2%), ST80-MRSA-IV (7.5%), ST5-MRSA-II/IV/VI (6.5%), ST30-MRSA-IV (3.5%), ST241-MRSA-III (2.7%), ST6-MRSA-IV (2.2%), ST36-MRSA-II (2%) and ST772-MRSA-V (2%). The isolates differed in the carriage of genes for enterotoxins, PVL, tst1, arginine catabolic mobile element (ACME) and exfoliate toxins. The number of clones increased from 1 (ST239-III-t037) in 1992 to 30 in 2010 including ST8-IV-t008 [PVL+] [ACME+] (USA300), ST772-V (Bengal Bay clone) and ST2816 identified for the first time in Kuwait. The study revealed that the MRSA isolates belonged to diverse clones that changed in numbers and diversity over time. Although ST239-MRSA-III remained the dominant MRSA clone over time, the newly emerged clones consisted mostly of community-associated methicillin-resistant S. aureus (CA-MRSA).

Dr. Edet E. Udo (Supervisor)
Prof. Noura Al-Sweih (Cosupervisor)

Supported by the College of Graduate Studies and Research Sector (grants YM 02/12 and SRUL02/13), Kuwait University.