# CHAPTER 32

# MEDICAL SCIENCE
# MEDICAL BIOCHEMISTRY

## Doctoral Theses

01. BHAT (Musadiq Ahmad)
**Genetic Alterations to Understand the Biology of Epilepsy Syndromes.**
Supervisors : Dr. Alpana Saxena and Dr. Vinod Puri
Th 24085


*Abstract*
*(Verified)*

Juvenile Myoclonic Epilepsy (JME) and Lennox-Gastaut Syndrome (LGS) represent two important epileptic syndromes which manifest in the pediatric age group and are associated with lot of morbidity and interference with the quality of life. There is preliminary evidence that the pathogenesis of JME and cryptogenic LGS is primarily genetic in origin. It is clear that the nicotinic acetylcholine receptor genes (*CHRNA4*), sodium channel genes (*SCN1A* and *SCN2A*) and GABA receptor genes (*GABRA1*,*GABRD*and *GABRG2*) play critical roles in generation/propagation of action potentials in neurons/myocytes and in modulation of neuromuscular excitability. Alterations in the afore mentioned genes have been implicated in a number of epilepsies, however there are almost nil/scanty reports of their involvement in JME and LGS. In the present study, it was planned to elucidate the role of the nicotinic acetylcholine receptor, voltage gated sodium channel and GABA receptor genes in the pathophysiology of JME and LGS. In our study, it was observed that *CHRNA4* 839 C>T mutation leads to shorter duration of seizures and postictal period in JME. The *SCN1A* 2624C>T mutation correlates with decreased frequency of seizures in JME. The *SCN1A* 563A>T mutation was associated with an early onset of seizures in LGS. The *SCN1A* 3184A>G polymorphism was linked to a decreased risk of LGS development. *SCN2A* 56G>A polymorphism which causes Arg19Lys substitution at a moderately conserved residue was on the contrary associated with an increased susceptibility to LGS. *GABRA1* gene promoter hypermethylation was linked to increased frequency of seizures and decreased anti-epileptic drug efficacy in JME as well as in LGS. Also, GABRA1 965 C>A mutation correlated with decreased drug responsiveness in LGS. Further elucidation of *GABRA1* gene role by *in-vitro* methods demonstrated a clear correlation of its overexpression with increased neuronal cell viability and of its knock-out with increased neuronal apoptosis.


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02. GURU (Sameer Ahmad)
**Molecular Mechanisms of Blast Transformation in Imatinib Treated Chronic Myeloid Leukaemia Patients.**
Supervisors : Dr. Alpana Saxena and Dr. T. K. Mishra
Th 24081

*Abstract*
*(Verified)*

The side effects related to imatinib treatment of CML patients are many. But neutropenia and thrombocytopenia are more serious. The molecular mechanisms of these cytopenias are not well known, however, a link is suggested between platelet development and platelet derived growth factor receptor (PDGFR). This study is about the role of PDGFR in the development of imatinib associated thrombocytopenia. We characterized *PDGFRA* promoter methylation, polymorphisms, mutations, their influence on mRNA expression of *PDGFRA and PDGFRα protein* (Tyr754 – p- PDGFRα) phosphorylation/activation in CML patients on imatinib treatment and in vitro on k562 cells. Further, expression patterns of *PDGFRA* downstream molecules viz. *PI3K, AKT1* and *AKT2* gens were also studied in CML patients on treatment and in K562 cells. *PDGFRA* mRNA expression was found to be significantly down regulated in CML patients after imatinib treatment and in CML patients with thrombocytopenia. No increased risk of thrombocytopenia was found to be associated with *PDGFRA* promoter methylation in CML patients on imatinib treatment. However, *PDGFRA* +68GAins/del, +68GAins/ins and -909C/A polymorphism genotypes were found to associated increased risk for thrombocytopenia and decreased PDGFRA mRNA expression. No conclusive results for two *PDGFRA* 2525 A>T (D842V) and 1821T>A (V561D) mutations were observed in this study group. The mRNA expression of *PI3K and AKT2* genes was found to be down regulated in CML patients after imatinib treatment. PI3K mRNA expression was significantly reduced in thrombocytopenic CML patients compared to non-thrombocytopenic ones while the decrease in *AKT1 and AKT2* mRNA expression was not significant. Moreover, treatment of imatinib, curcumin and imatinib+curcumin led to decreased mRNA expression *of PDGFRA, PI3K, AKT1 and AKT2* genes and PDGFRα protein phosphorylation/activation in K562 cells compared to untreated K562 cells. In conclusion, this study emphasizes the role of *PDGFRA* mRNA expression, PDGFRα protein phosphorylation and its signalling components in imatinib induced thrombocytopenia in CML patients.

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03. JAFRI (Aiman Abbas)
**Studies on Molecular Mechanism of Anti Hyperglycemic Compound Isolated from Eugenia Jambolana in Type 2 Diabetes Mellitus.**
Supervisor : Dr. S.B. Sharma
Th 24084

*Abstract*
*(Not Verified)*

Background : In previous studies, Sharma et al. has already isolated an anti-hyperglycemic compound (FIIc) from the fruit pulp of *Eugenia jambolana*using HPLC and other chromatographic techniques. However, the effect of anti-hyperglycemic compound (FIIc) on the expression of GLUT-4, GLUT-8, PPAR gamma, Kv 1.3 Potassium Channel, IRS-1 and IRS-2 in type 2 diabetic rats has not been studied so far. Methods : 24 Male Wistar rats were taken and fed on High Sucrose Diet (HSD) for the development of type 2 diabetes for 30 weeks. Active compound FIIc was given to group C and Pioglitazone to group D at a dose of 20 mg/kg of body weight orally for 30 weeks respectively. Blood was drawn for the estimation of plasma glucose and serum insulin at week 0 and at week 30 from retro orbital plexus. At the end of the study, all the rats were sacrificed and organs including pancreas and skeletal muscles were isolated and stored at -80°C for expression studies. Real time PCR and

Immunohistochemistry was performed to quantify the expression levels of GLUT-4, GLUT-8, PPAR gamma, Kv 1.3 Potassium Channel, IRS-1 and IRS-2 among the four study groups. Results : After treatment with FIIc for 30 weeks we found a significant improvement in blood glucose and serum Insulin levels in group C rats compared to group B. The mRNA expression of GLUT-4, GLUT-8, PPAR γ, IRS-1 and IRS-2 were also found to be increased by many folds as compared to group B. Similarly, the expression of GLUT-4, GLUT-8 and PPAR γ at protein level were also found to be increased in group C rats as compared to group B. Conclusion : FIIc treatment for 30 weeks improves glycemic control and insulin sensitivity by increasing the expression of GLUT-4, GLUT-8, PPAR gamma, IRS-1 and IRS-2 at mRNA and at protein level.

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04. Mohd. Jahid

**Molecular Studies of Inflammatory Cytokine Genes in North Indian Rheumatoid Arthritis Patients.**

Supervisors : Prof. Rafat Sultana Ahmed and Rajnish Avasthi

Th 24079

*Abstract*
*(Not Verified)*

The present study was designed to study the promoter gene polymorphisms of cytokines in *TNFα-308*G/A, *IL1B-511*C/T and *IL10-1082*A/G and to assess their association with inflammatory markers, mRNA expression levels and serum cytokines levels in North Indian RA patients. A total of 214 controls and 187 RA patients were recruited according to the revised American College of Rheumatology, 2010 criteria. Genetic polymorphisms were analyzed by PCR-RFLP. Serum inflammatory markers and cytokines were estimated by ELISA. The mRNA expression of *TNF-α*, *IL-1B* and *IL-10* genes was measured by quantitative real time PCR. Dyslipidemia was observed in RA patients as compared to healthy controls. Rheumatoid factor (RF), anti-cyclic citrullinated protein (anti-CCP), C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were significantly higher in RA patients. For *TNF-α* gene polymorphism both the heterozygous (G/A) and homozygous mutant variants (A/A) were significantly higher in RA patients and associated with RA development. Levels of TNF-α, RF and CRP were higher in mutants. In *IL-1B* gene polymorphism both the heterozygous (C/T) and homozygous mutant variants (T/T) were significantly associated with RA. Serum IL-1β and anti-CCP levels were significantly higher in mutants. Further, *IL-10* gene polymorphism both the heterozygous (A/G) and homozygous mutant (G/G) variants were significantly associated with RA. Serum IL-10 and anti-CCP levels were significantly higher in mutants. The m-RNA expression levels of *TNF-α, IL-1B* and *IL-10* were higher in RA patients as compared to controls. Our results clearly indicate a significant association of *TNFα-308*G/A, *IL1B-*

*511*C/T and *IL10-1082*A/G promoter SNPs with the risk of RA in North Indian population and these could be used as markers of susceptibility to RA. Findings of the present study will be helpful for gene targeting and drug development.

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05. PANDEY (Apoorva)

**Role of Innate Immune Response Mechanism in Development of Bleomycin Induced Lung Fibrosis.**

Supervisor : Dr. S. K. Bansal

Th 24078

*Abstract*
*(Not Verified)*

Pulmonary fibrosis is a devastating disease causing irreversible damage to the lung architecture. It is characterized by excessive deposition of extracellular matrix components in the lung. Pulmonary fibrosis comprises of two main phases- cellular and fibrotic. In the cellular phase, damage to the lung tissue causes release of inflammatory mediators (cytokines, chemokines, growth factors, etc) leading to recruitment of various inflammatory cells like neutrophils, lymphocytes etc. Under the influence of inflammatory mediators, fibroblasts proliferate, migrate and activate into my fibroblasts. My fibroblasts secrete ECM components at the site of injury, which constitutes the fibrotic phase. Fibrosis occurs when the balance between proteases/anti-proteases gets disrupted. Bleomycin is an anti-tumor antibiotic extracted from *Streptomyces verticillis*. It has been used in the treatment of skin tumors, Hodgkin's lymphoma etc. Pulmonary fibrosis has been recognized as a complication of bleomycin therapy. The pathologic end stage of the toxic reaction, pulmonary fibrosis, has been described in both humans and animals. The present study describes the changes in the lung histopathology and function (sRaw and sGaw), lung collagen and LMW Hyaluronanquantification, CD68, NFκBp65, active TGF-β1 levels, TLR- 2, TLR-4, TGF-β1, MMP-9, TIMP-1, TIMP-3 mRNA and protein levels following the administration of bleomycin to rats on 0, 7, 14 and 28 days. Intratracheal administration of bleomycin successfully established pulmonary fibrosis in rat model. The innate immune response apparently triggers sterile interstitial inflammation via LMW-HA activation of the TLR-2, 4 and NFκBsignalling after bleomycin injury. The downregulation of the TLR-2,4 mRNA levels, upregulation and activation of TGF-β1 and development of protease/antiprotease imbalance with reversal of the MMP-9/TIMP-1,3 ratios in favour of TIMPs may appear to be the major mechanism that may lead to the progression of aberrant parenchymal remodeling to the fibrosis. The present study elaborates the role of the TLR-NFκB-TGF-β1-MMP/TIMP pathway in the immunopathogenesis of pulmonary fibrosis.

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06. SUMI (Mamta Pervin)

**A Genetic Study to Evaluate Clinical Implication of Thrombomodulin and Associated Proteins in Coronary Artery Disease.**

Supervisors : Dr. Alpana Saxena and Dr. Girish MP

Th 24289

*Abstract*
*(Not Verified)*

Title : A genetic study to evaluate clinical implication of Thrombomodulin and associated proteins in Coronary Artery Disease Multigenetic interaction have

been implicated in Coronary Artery Disease (CAD) though exact effects on genetic variations is yet to be elucidated. To evaluate clinical implication of Thrombomodulin and associated proteins in CAD. The study included hundred angiographically confirmed CAD patients along with hundred age and sex matched healthy controls. Polymorphisms and mutations were investigated by PCR-RFLP and AS-PCR. Quantitative Real Time PCR was carried out for the measurement of mRNA expression. THBD mRNA expression was higher in CAD patients compared to healthy control subjects along with THBD C1418T and G-33A gene polymorphisms were associated with higher risk of CAD. A significant increase in ERK 1 mRNA expression was observed in CAD patients compared to healthy controls. ERK2 expression was also higher in CAD patients, but the difference did not reach statistical significance. Substantial and significant decreased expression of NFkB gene was seen in CAD patients as compared to healthy controls, implicating NFkB to act as protective for CAD. Moreover reduced expression of NFkB conferred significant risk for multi vessel involvement. HFE C282Y mutation did not show any significant differential association among CAD patients and control subjects with no effect on disease severity. Single Nucleotide Polymorphisms in other genes like ESR1 -397T>C, ESR1 -351A>G, Connexin 37 C1019T, Chemokine receptors CX3CR1 V249I, CX3CR1 T280M, MTHFR C677T and Cystathionine beta synthase CBS T833C polymorphisms were all differentially associated with increased risk of CAD except CX3CR1 T280M polymorphism failed to demonstrate any significant association. Thrombomodulin C1418T and G-33A gene polymorphisms were associated with higher risk of CAD. MAPK (ERK1 and ERK2), NFκβ, HFE, ESR1, Connexin 37, Chemokine receptors CX3CR1, MTHFR and Cystathionine beta synthase CBS polymorphisms were all differentially associated with increased risk of CAD.

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