Research Agenda
2009 - 2011

University of Health Sciences
Lahore

FOREWORD

The need for establishing a university dedicated to the health sciences had been long felt not only to stem the slide of medical education into mediocrity but to provide for the **development of human resources** committed to synthesizing the rapidly evolving technological advances with the traditional **humane** and compassionate dimension of **healthcare** delivery.

Research culture could not take strong root in our medical and health institutions. All these years we have been looking towards the West for solutions to our own health problems. UHS identified this gray area and revitalized the dying culture of research in medical and health sciences. It has gone **research intensive** and its ethos from the beginning has been to find **indigenous solutions** to our health problems akin to our needs.

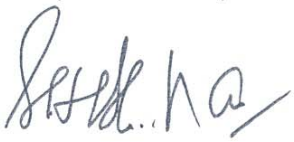
University has been promoting research not only at postgraduate but also at undergraduate level by extending all possible facilities to research scholars ranging from monetary support to the facilities for experimental research in laboratories. These scholars are interacting with eminent and renowned scientists of the world facilitated by video conferencing, internet connectivity and research journals of digital library, in addition to personal interaction.

The University introduced research activities in all its Undergraduate Programmes so as to acclimatize the budding Health Professional with the Research Methodology and how this force can singularly bring change not just in the Profession but also in every aspect of our existence.

Research is central to all reforms and innovations not only in the field of Medicine but in all areas of knowledge, skills and attitudes. UHS shall continue in its efforts to promote and facilitate Research and channel the Research Potential of Health Professionals along directions that shall bring lasting changes in Health Care Delivery not only in the region but the world over.

The University of Health Sciences (UHS) believes that the universities of the 21st century must broaden their commitment to develop **collaborative partnerships** with industry and governments for provision of health services, and training of tomorrow's leaders in addition to the university's more traditional role of creating and accumulating knowledge, while keeping a balance between the two. It can perhaps best be represented by an ellipse: a shape with two equal focuses in dynamic balance with one another. Remaining independent from economic society in order to promote basic research and the ongoing creation of new knowledge while being directly committed to collaborating with society, UHS has been able to tap into these unique resources which, through innovation and collaboration with partners can be put to direct use to improve society.

This document has outlined Research agenda of UHS in basic Medical Sciences, which I hope will be of immense value to postgraduate health professionals, current and perspective students of the University.



Prof. M. H. Mubbashar
Hilal-e-Imtiaz, Sitara-e-Imtiaz
MB, FRCP, FCPS Psych, FRC Psych, DPM
Vice Chancellor / Chief Executive,
University of Health Sciences, Lahore.

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1. ALLIED HEALTH SCIENCES

5.1 Cardiovascular Risk Factors in Hemodialysis Patients

Introduction: Cardiovascular disease is the leading cause of mortality in patients with end-stage renal disease (ESRD) and is attributed to a combination of traditional and non-traditional cardiovascular risk factors. Patients on hemodialysis (HD) are prone to atherosclerotic cardiovascular complications. Most of the traditional CVD risk factors, such as older age, diabetes mellitus, systolic hypertension, LVH, and low high-density lipoprotein (HDL) cholesterol, are highly prevalent in CKD. Serum concentrations of the cardiac troponins (cTn) T and I, specific markers of myocardial injury, are frequently elevated in haemodialysis patients. Hypertension, abnormal lipid particles, and thrombogenic proteins may contribute to the high prevalence of CVD in HD patients.

Material and Methods:

Study Design: Cross-sectional study

Sample Size: 60 samples will be collected from patients who are on maintenance dialysis therapy. 40 normal control samples will be collected. Cholesterol, Triglyceride, HDL and LDL will be performed on Metrolab 2300. Cardiac marker Trop T will be done on Vitros.

Methodology: 60 samples will be collected from patients who are on maintenance dialysis therapy. 40 normal control samples will be collected

Estimated Cost of the project: Approximately Rs. 35,000/-

5.2 Zinc and Copper Levels in Young Women Taking Oral Contraceptives

Introduction: The use of oral contraceptive agents is known to cause significant changes in metabolism. Changes in metal metabolism, such as that of sodium, potassium, calcium, magnesium, iron, zinc, and copper have been attributed to oral contraceptive usage .The regular intake of oral contraceptives has been demonstrated to increase plasma or serum copper levels and decrease serum zinc levels. These disturbed levels of zinc and copper can affect many metabolic pathways in the body.

High copper levels are toxic while Zinc deficiency during pregnancy can negatively affect both the mother and fetus. Objectives: (1) To assess levels of serum zinc and copper in young women taking oral contraceptives. (2) To compare levels of zinc and copper between women taking OCR and those who are not taking OCR.

Material and Methods:

Study Design: Cross-sectional study

Sample Size: 50 women, age range 20-35 years taking OCR will be included. 50 women who are not taking OCR will be included as a control group

Methodology: 50 women, age range 20-35 years taking OCR will be included. 50 women who are not taking OCR will be included as a control group: Measurement of Serum Zinc and Copper levels by Atomic absorption Spectrophotometer.

Estimated Cost of the project: Approximately Rs. 30,000/-

5.3 Effect of Induction Phase Chemotherapy on Growth Hormone in Children with Acute Lymphoblastic Leukemia.

Introduction: Acute Lymphoblastic leukemia is a malignant disease of lymphopoietic system. ALL is primarily a disease of childhood. The major mode of treatment is chemotherapy which is associated with many side effects. One of the side effects is growth hormone deficiency. Children with Growth Hormone Deficiency are normally present with short stature and a low growth velocity for age and pubertal stage. Objectives: (1) To determine the level of Growth hormone in patients of ALL before induction phase Chemotherapy (2) To assess the effect of induction phase chemotherapy on growth hormone.

Material and Methods:

Study Design: Longitudinal study

Sample Size: 30 children, age range 2-12 years, diagnosed with Acute Lymphoblastic Leukemia will be included in the study.

Methodology: First sample will be taken before the start of the chemotherapy. Second and third sample will be taken at 4^{th} and 6^{th} week after start of chemotherapy. Serum for GH levels will be tested by ELISA technique

Estimated Cost of the project: Approximately Rs. 25,000/-

5.4 Serum Trace Metals in Chronic Viral Hepatitis and Hepatocellular Carcinoma

Introduction: Trace metals are essential nutrients for normal growth and development. Both zinc and copper are involved in several hepatic enzyme system and are stored in large quantities in the liver. Decreased serum zinc or increased copper levels occur in patients with some acute and chronic liver diseases. Zinc supplementation has been suggested to be of therapeutic value in patients with cirrhosis and some other chronic liver diseases. Trace metal disturbance in liver diseases suggested that changes in liver cell pathology compounded by functional impairment may alter the metabolism of trace metals, in particular, zinc and copper. Serum zinc levels are significantly decreased in patients with chronic active hepatitis and hepatocellular carcinoma and copper levels are significantly elevated only in patients with hepatocellular carcinoma

Material and Methods:

Study Design: Cross-sectional study

Sample Size: 60 patients

Methodology: 60 patients are included in the study, 40 patients with chronic active hepatitis; of these 17 are anti-HCV positive and 23 are HBsAg positive. 20 patients of hepatocellular carcinoma are included. Analysis of serum zinc and copper with atomic absorption spectrophotometer

Estimated Cost of the project: Approximately Rs. 30,000/-

5.5 Lipid Abnormalities in Uremia, Dialysis, and Transplantation

Introduction: The institution of renal replacement therapy has sustained the lives of many patients with end-stage renal failure and has made it possible to study in depth

the metabolic abnormalities associated with the uremic state. An important consequence of chronic uremia is the development of lipid abnormalities, which continue to affect many patients on dialysis and after renal transplantation. There has been tremendous interest in lipid metabolism in chronic renal failure; especially after the provoking report of Lindner et al that dialysis accelerates atherosclerosis. Cardiovascular deaths still claim many lives on dialysis and after transplantation. Hyperlipidemia is the major lipid abnormality that occurs in dialyzed and undialyzed patients with uremia, also in kidney transplantation patients. Objectives: 1)To measure the lipid profile in uremic patients both on dialysis and without dialysis 2)Comparison of lipid profile of uremic patients with normal healthy persons without uremia.

Material and Methods:

Study Design: Cross-sectional study

Sample Size: Study includes 80 patients, 20 controls without uremia

Methodology: Study includes 80 patients, 20 controls without uremia, 25 uremic patients on dialysis, 25 patients without dialysis and 10 patients with kidnetransplantation. Highly obese, pregnant and diabetic patients excluded from the study. Lipid profile will be measured by photometric analysis on Metrolab 2300.

Estimated Cost of the project: Approximately Rs. 15,000/-

5.6 Prevalence of Hepatitis C in Hemodialysis Patients

Introduction: Hepatitis C virus (HCV) infection is a major public health problem, with an estimated global prevalence of 3% occurring in about 170 million infected persons worldwide. Hepatitis C virus (HCV) infection is a significant cause of morbidity and mortality in hemodialysis (HD) patients. The reported prevalence of HCV among the HD population has varied greatly from 1.9 to 84.6% in different countries in recent years. Patients with renal disease are at an increased risk of acquiring HCV. The main reasons are: 1). The length of time on HD is generally believed to be associated with HCV acquisition in HD subjects. 2). The potential for exposure to infected patients and contaminated equipment. 3). Nosocomial patient-to-patient transmission of HCV among HD patients for which the main potential source is believed to be contaminated

hands and articles. Aims and objectives**:** To measure the prevalence of hepatitis C in patients on hemodialysis in Pakistani population.

Material and Methods:

Study Design: Cross-sectional study

Sample Size: 70 patients will be collected who are on chronic hemodialysis treatment. 30 controls will be collected

Methodology: 70 patients will be collected who are on chronic hemodialysis treatment. 30 controls will be collected. Liver function tests will be done by Metrolab 2300. Anti HCV screening will be done by ELISA

Estimated Cost of the project: Approximately Rs. 20,000/-

5.7 Evaluation of Bone Mineral Abnormalities in Children with Acute Lymphoblastic Leukemia after Induction Chemotherapy.

Introduction: Leukemia is the most common cancer in children and may affect virtually all organ system. Acute lymphoblastic leukemia (ALL), the most common malignancy in children, has an overall long-term survival of at least 75% In childhood acute lymphobastic leukemia skeletal changes are frequently found at the time of diagnosis and treatment, including: metafyseal lines, periostal reaction, lysis, sclerosis, osteoporosis and occasionally spontaneous fracture. Treatment with corticosteroid decreased bone formation and increased bone resorption with consequent net loss of bone minerals. The levels of bone mineral including calcium, magnesium, alkaline phosphatase and parathyroid harmone has been studied. Aims/objectives: Assessment of bone minerals in children treated for acute lymphoblastic leukaemia

Material and Methods:

Study Design: Cross sectional study

Sample Size: 30 samples will be collected from children treated for acute lymphoblastic leukemia

Methodology: 30 samples will be collected from children treated for acute lymphoblastic leukemia.Methods: Blood and urine levels of calcium and magnesium will be measured by standard spectrophotometryAlkaline phosphatase by standard biochemical methods. PTH by radioimmunoassay

Estimated Cost of the project: Approximately Rs. 40,000/-

5.8 Simplified Quantification of Urinary Protein Excretion in Children with Nephrotic Syndrome

Introduction: In Nephrotic Syndrome the amount of protein excretion is a reflection of activity of disease. The purpose of this study would be to assess the value of single voided random (spot) urinary protein to creatinine ratio in accurately predicting the 24-hour urinary protein excretion. Quantitative measurement of proteinuria by a 24-hour urine collection has been the accepted method of evaluation. If calculation of protein/creatinine ratio in a spot urine sample correlates well with the 24-hour urine protein (24-HUP) excretion than urinary protein to creatinine ratio can be used in place of 24-hour urinary protein.

Material and Methods:

Study Design: Cross sectional Study

Sample Size: 30 children with nephrotic syndrome and 10 control

Methodology: Protein and creatinine quantification in urine will be performed by metrolab 2300.

Estimated Cost of the project: Approximately Rs. 10,000/-

5.9 Assessment of Alteration in Serum Immunoglobulin Levels in Hepatitis C Infection.

Introduction: Hepatitis C virus has become a major worldwide problem because of the natural course of the disease to cirrhosis and then to hepatocellular carcinoma.

Hepatitis C virus is the most common cause of chronic hepatitis in North America, Europe and Japan.It is estimated to infect approximately 170 million individuals worldwide. The factors most strongly associated with infection are injection-drug use and receipt of a blood transfusion. Poverty, high-risk sexual behaviors having less than 12 years of education, and having been divorced or separated are linked to an increased risk of infection. Chronic HCV infection is associated with evidence of chronic liver injury. The host immune response to HCV infection is composed of both a non-specific immune response, including interferon (IFN) production and natural killer (NK) cell activity, and a virus-specific immune response, including humoral and cellular components. Hyperglobulinemia in patients with liver cirrhosis tends to affect all immunoglobulin subclasses.Hyperglobulinemia affecting the three main immunoglobulin classes was considered a hallmark of chronic active hepatitis. It has been studied that the levels of immunoglobulin IgM, IgG and IgA are increased in HCV patients. Aims/Objectives: To assess the immunoglobulin levels in HCV positive patients. Correlation of HCV infection with alteration of serum immunoglobulins.

Material and Methods:

Study Design: Cross sectional study

Sample Size: 50 samples of HCV positive patients will be collected.20 normal control samples will also be collected for comparison

Methodology: Serum IgA, IgG and IgM will be performed by ELISA.

Estimated Cost of the project: Approximately Rs. 70,000/-

5.10 Thyroid Dysfunction in Nephrotic Syndrome Patients

Study population: 30 patients of clinically diagnosed nephrotic syndrome and 10 normal controls. Lab investigation: Thyroid profile will be performed by EciQ vitrous using chemmiluminiscence technique.

Introduction: Thyroid function has been thought to be abnormal in nephrotic syndrome. This suggestion is based initially on the presence of hypo metabolism (low basal metabolic rate) and hypercholesterolemia. It has been demonstrated repeatedly that the concentration of protein bound iodine (PBI) may be abnormally low in patients with nephrotic syndrome because urine contains relatively large amounts of PBI. Low

binding globulins in serum and heavy proteinuria can be a cause of abnormal thyroid profile in patients of nephrotic syndrome. Objectives: To study the thyroid hormone status by measuring free tri-iodothyronine (FT_3), free thyroxin (FT_4) and thyroid-stimulating hormone (TSH) levels in nephrotic syndrome.

Material and Methods:

Study Design: Cross sectional study

Sample Size:

Methodology: Free tri-iodothyronine (FT_3), free thyroxin (FT_4) and thyroid-stimulating hormone (TSH) levels in nephrotic syndrome will be measured.

Estimated Cost of the project: Approximately Rs. 45,000/-

2. ANATOMY

5.1 Ethambutol Induced Optic Neuritis-Its Prevention by Natural Honey

Introduction: Ethambutol is an antituberculous drug. Its ocular adverse effects have been well established, it produces both bulbar and retro bulbar changes, later being more common i.e. optic neuritis. Its toxicity is dose related. At higher doses it is reported to alter the synaptic connections between horizontal cells and cones, leaving the rods unaffected. In optic nerve and optic chiasma it is responsible for vacuolation and cystic degeneration of axons with thinning out of the myelin sheath. In humans, Ethambutol induced optic neuritis produces symptoms of blurring of vision, decrease in visual acuity, bitemporal visual field scotoma defects, centrocaecal scotoma and disturbances in color perception.

It is reported that Ethambutol (EMB) induced toxicity is produced through an excitotoxic pathway which is strikingly similar to toxicity produced by glutamate. This mechanism of EMB induced toxicity as well as glutamate mediated toxicity in the optic nerve was classified as mitochondrial optic neuropathy induced by free radicals. Free radicals are produced as result of oxidative stress. Antioxidants are thought to protect the body against the injurious effects of free radicals by binding with them. Natural honey contains large amount of antioxidants so prompted the idea to investigate its protective role in preventing oculopathies induced by Ethambutol in albino rats.

Materials and methods:

Study design: Experimental study: Randomized controlled trial

Sample size: 32 albino rats of either sex

Methodology: The study will be conducted on thirty two adult albino rats of either sex. Animals will be randomly divided into four groups, A, B, C, and D, having eight rats in each. Group A will serve as control whereas group B, C, D will serve as experimental. Group B will be given EMB at dose of 100mg\kg\day orally for four weeks. Groups C and D will be given Natural Berry Honey at doses of 15mg\kg\day and 20mg\kg\day orally respectively along with oral administration of EMB at doses of 100mg\kg\day for

four weeks. The animals will be sacrificed after four weeks of experiment; their optic nerves along with eyeballs will be removed. Optic nerve will be processed for histological preparation and stained with Haemotoxylin and Eosin before examining under light microscope to record the observations on toxic effects of ethambutol in Group B and their prevention by Natural Honey at different doses in Group C & D respectively. The collected data will be analyzed using SPSS 16.0

Estimated Cost of the Project: 130,000/-PKR

5.2 The Effect of Monosodium Glutamate on the Purkinje Cells of Cerebellum of Adult Rat

Introduction: Mono Sodium Glutamate (MSG) is a salt which is used as a taste enhancer and preservative. It is sold in market under the name of Ajinomoto and is used commercially. Optimum amount of MSG added to food is 0.6%. It is being used indiscriminately, regardless of its toxic effects, in a variety of packed and restaurant foods. It is being used indiscriminately, regardless of its toxic effects, in a variety of packed and restaurant foods. MSG has specific glutamate receptors which are excitatory and produce excitotoxicity; these are scattered throughout body.

MSG has wide spread toxic effects on different organs including brain. Although toxicity of MSG had been extensively studied in the past on different organ systems, including brain in experimental set up; hallmark of toxic manifestations of MSG on central nervous system are the excitotoxic effects and disturbances in balance; there is hardly any study on the histological changes in the neuronal structure and number after MSG administration. Cerebellum is an important part of the nervous system with excitatory output ; In the presumption that it is highly susceptible to MSG toxicity and in view that there is hardly any work on direct effect of MSG on the cortical structure of cerebellum, the present study, therefore, is designed to see the effect of MSG on the structure and number of Purkinje cells of cerebellum, using adult Albino rats as an Experimental model.

Materials and Methodology*:* This study will be carried out on 30 adult albino rats and will be fed on normal rat diet and water at libitum ; they will be randomly divided in to 3 groups A, B and C .Each having 10 rats. Group A will serve as control where as B and

C are experimental groups .MSG obtained from local grocery store will be given orally, 6 gm /10ml of water in dosage of 3 and 6gm to groups B and C respectively .The control will receive equal amount of water daily, during the experimental period .The rats will be sacrificed on 16^{th} day. Cerebellum will be quickly removed and three mm^2 pieces of cerebellum will be processed for histological preparation; the Purkinje cells will be examined using light microscope and counted after calibrating eye piece reticule with linear stage micrometer at appropriate magnifications. The data will be entered and analyzed using SPSS 16.0.

Estimated Cost of Project: 60,000 Approximately

5.3 A Morphological Study of Normal Placentae and Its Anatomical Variations in Normal Pregnancies among Local Population.

Introduction: Placenta is a temporary organ that is intimately connected to two different people. It is the mirror image of the intrauterine life of a newborn and reflects the changed physiological condition and the stress faced by the mother during the pregnancy. The stress of normal pregnancy affects each person in a very different way so that normal variations are commonly seen. After delivery if the placenta is examined minutely it provides much insight into the prenatal health of the baby and the mother. It has been described as a multifunctional organ which is not only responsible for the nutrient exchange and protection of the fetus but also plays an important role in fetal programming which has great implications for health in later life. Most of the recent research shows that placenta can give specific indications about the degree of fetal compromise. Placental pathologists have played an important role in revealing the morphological findings that explains the underlying cause of the pathological events. Despite this, placenta remains an under examined organ. The objective of this study is to observe the structural morphology, both gross and microscopic of placentae and its anatomical variations in normal pregnancies in local population. The hypothesis is that there will be significant morphological differences in the placentae of normal pregnancies among the local population.

Material and Method: This study shall consist of 100 specimens of placentae. Fresh specimens fixed in formalin will be collected from Sheikh Zayed , Sir Gangaram and

Services Hospitals, Lahore. Study shall be conducted in the Department of Anatomy, at University of Health Sciences, Lahore.

Methodology: Each specimen will be examined for gross and histological parameters both qualitative and quantitative.
The results will be collected and analyzed statistically.

Estimated Cost of the Project: Total Cost = Rs. 111,794/-

5.4 Amphotericin-B Induced Nephrotoxicity in Rat-Its Protection by Nigella Sativa Extract.

Introduction: Drug induced nephrotoxicity is frequently observed with many drugs used in clinical practice. Kidneys are frequently exposed to drugs and their metabolites on account of their high vascularity and their major role in excreting these metabolites. Many of these nephrotoxins may overtax the renal parenchyma and produce various degrees of renal damage. A potent antifungal drug Amphotericin-B has been frequently used clinically against a variety of fungal species in treating invasive systemic fungal infections for more than 30 years. Nephrotoxicity is a frequently reported side effect and the major limitation to its therapeutic usefulness and ultimately proved to be the dose limiting factor in many patients particularly in immunocompromised. Studies have documented the generation of free radicals as the major contributors to the renal injury. Now a day's large number of herbs and medicinal plants proved to have beneficial therapeutic potentials. Seeds of Nigella sativa (KALONJI) have been employed for thousands of years as spice and food preservative. Many recent experimental studies have shown that administration of Nigella sativa extract prevent biochemical and histological changes of nephrotoxicity induced by certain nephrotoxic drugs used clinically. Most of its pharmacological actions are due to its potent antioxidant activity. However, in spite of the substantial body of literature on the antioxidant strength of Nigella sativa in various models of oxidative stress, there is hardly any information available on the prevention of Amphotericin-B induced nephrotoxicity by Nigella sativa. The present work is, therefore, designed to evaluate the protective effect of Nigella sativa extract on amphotericin-B induced nephrotoxicity.

Materials and Methods:

Study design: Experimental study, Randomized controlled trail

Sample Size: 30 adult albino rats

Methodology: This study will be carried out on 30 adult albino rats randomly divided into 3 groups each having 10 rats; group A will serve as control and will receive 10mg/kg/day intraperitoneally 5% dextrose for 4 days, Group B will be given Amphotericin-B 10mg/kg/day dissolved in 5ml of dextrose solution intraperitoneally for 4 days and group C will be given Amphotericin-B 10mg/kg/day dissolved in 5ml of dextrose solution intraperitoneally for 4 days followed by Nigella sativa extract 5 ml/kg/day orally for 7 days. Blood samples will be collected for assessment of serum creatinine and urea. Rats will be sacrificed and kidneys will be removed and evaluated both macroscopically and microscopically.

The data will be collected and analyzed by using SPSS 16.0.

Estimated Cost of the Project: 121,740/-

5.5 Teratogenic Effects of Bupivacaine on Heart Kidney and Brain in Albino Mice

Introduction: Local Anesthetics have been widely used in wound infiltration, nerve block, epidural, and intrathecal anesthesia. The presently used local anesthetics commonly employed in obstetrics cross the placenta readily. Teratogenic effects of general anesthetics have been studied in past but the local anesthetics hither to had not been given proper attention. In an in vitro study teratogenic effects of Lidocaine on neural tube had been reported. Another recent study revealed marked teratogenicity of cocaine in albino mice. Bupivacaine is a commonly used local anesthetic belonging to the amide group. Decreased pup survival in rats and an embryocidal effect in rabbits have been observed when bupivacaine hydrochloride was administered to these species in high doses during prenancy; further, it is reported that bupivacaine causes postnatal behavioral changes in new born. There is hardly any study in the animal experimental model to evaluate its teratogenic effect when given in therapeutic

doses. The present investigations are, therefore, designed to study the teratogenic effects of bupivacaine on heart and kidney, using adult mice as an experiential model.

Materials and Methods:

Study design: Experimental randomized controlled trial

Sample Size: 32 albino mice (24 adult female and 8 male albino)

Methodology: This study will be carried out on 24 adult female and 8 male albino mice of BALB/c strain, 6-8 weeks old weighing 30 ± 0.22g which will be procured from NIH Islamabad and maintained in the Animal House of the University of Health Sciences Lahore. Three female and one male will be housed together for mating. Pregnancy will be confirmed by presence of the vaginal plug. Twenty four pregnant mice will be divided into three groups, having eight mice each. The control group A will receive daily intraperitoneal injection of normal saline from day 7 to 14 of gestation. The experimental group B will be subjected to daily single intraperitoneal injections of 25 mg/kg Bupivacaine while group C will receive 125 mg/kg bupivacaine in same manner. Mice will be sacrificed on 18 day of gestation and will be observed for the gross abnormalities and heart and kidneys shall be prepared for microscopic examination for evidence of any abnormality.

The data will be collected and analyzed using SPSS 16.0

Estimated Cost of the Project: 74,230

5.6 Perinatal Histogenesis of Pancreatic Islet α and β-cells an Experimental Study.

Introduction: Pancreas consists of exocrine and endocrine components, the later being composed of aggregate of cells called islets of langerhans. Beta and Alpha cells are the most abundant cell types secreting insulin and glucagon, respectively, for maintaining normoglycemia.

Diabetes mellitus is a condition which results from both absolute or relative deficiency and peripheral resistance to insulin; the condition results on account of deranged structure and

function of beta cells which is reported to be increasing, involving 4% of the adult population, the world over; islet tissue, is, therefore, being studied from various aspects in recent years. Due to successes in organ transplants, pancreas and islets are being extensively evaluated for use as a transplant material for the cure of diabetes mellitus. The current study is, therefore, designed to investigate perinatal growth and development of alpha and beta cells of the islet tissue in order to evaluate fetal tissue as possible transplant material for treatment of diabetes mellitus.

Materials and Methodology: In this study adult normal female rats will be taken, mating will be allowed and pregnancy confirmed with the observation of vaginal plug. Pregnant rats will be divided into three groups, A, B and C. The animals of group A will be sacrificed on day 20 of gestation and their fetuses extracted for their pancreatic tissue whereas the pups from the rats of groups B and C shall be used 2 and 7 days postnatally to examine their islet histologically. Ten pancreata will be taken for each group, making a total of 30 samples.

Methodology: The tissue will be fixed in 10% formalin for 48 hours and processed in the usual way for paraffin infiltration and embedding; the blocks shall be cut to obtain 4 µm thick sections; these will be stained with H&E, gomori's chrome alum hematoxylin phloxine and aldehyde fuchsin for alpha and beta cells. The number of the islets and development of alpha and beta cells and their relative proportion shall be observed at different stages of perinatal development.

Statistical Evaluation: The results will be evaluated using SPSS 16.0 software on computer. Mean ± S.D will be given for normally distributed quantitative variables and one way ANOVA will be applied. Median ± IQR and krushkal-wallis H test will be given for non-normally distributed quantitative variables. Frequencies and percentages will be given for qualitative variables. A P-Value≤ 0.05 will be considered statistically significant.

Estimated cost of the project: Rs.69, 000

3. BIOCHEMISTRY

5.1 Basic Development Need Program (BDNP)

Introduction: There is a dire need to broaden the horizon of research because a researcher should foresee 10-15 years ahead. Pakistan is the most populous country accounting for 30% of the regional population in EMR, where WHO supports enormously for disadvantaged Population. Our 70% population lives in rural areas which are neglected from Health point of view. We can work on Primary Health Care which is a core Policy of WHO. National Health Surveillance according to WHO approved guidelines includes:

1. Family History to know racial/hereditary factors.
2. Access to safe drinking water.
3. Sanitation.
4. Population growth in terms of family planning
5. Prevalence of communicable & non communicable diseases.
6. Dietary habits, life style, dietary induced metabolic disorders.
7. Malnutrition etc.

As a result of complete uniform Health Survey, we would be able to find out our current Health Status, our own Reference Standards, our own valuable original data. In the 2nd Phase, after identifying the weaknesses, & further to reform & strengthen the Health Status, many new strategies can be initiated like,

1. Creating Awareness against the identified weaknesses.
2. Many new technological projects based on preventive, curative & investigational Basis can be started. This would also help to establish inborn screening programs.

5.2 Genetic Variants in the CYP2C19 Genes in Helicobacter Pylori Infected Cases.

Introduction: This study is important to patient care because for many drugs there are inter-individual differences in drug metabolism and drug response. These differences significantly alter the safety and success of therapy. Genome Variations are differences in the sequence of DNA among individuals. It has been estimated that the genomes of

non related people differ about 0.1% of the genome. These Genome variations include both polymorphisms and mutations.

Helicobacter Pylori is spiral bacterium infecting half of world's population. It is responsible for a spectrum of diseases in alimentary canal. The proton pump inhibitor Omeprazole is primarily inactivated by CYP2C19 enzyme and thus its metabolism is subject to genetic polymorphism.

Study Design: Cross sectional analytical

Sample Size: 50 subjects will be included in this study.

Material and Methodology: Genetic Variants of CYP2C19 associated with poor metabolism (PM) phenotype is to be determined. DNA will be extracted from peripheral blood leukocytes using genomic DNA purification kit based on salting out method. DNA will be quantified and qualified spectrophotometrically and by gel electrophoresis. Genotyping of CYP2C19 gene variant will be carried out by the use of Polymerase chain reaction (PCR) and restriction fragment length Polymorphism (RFLP) assay using specific primers, followed by sequencing by DNA sequencer, which will make it possible to screen simultaneously for detection of Variant alleles.

Estimated cost: Rs. 100,000/-

5.3 Relationship between Obesity, Lipid Profile and Osteoarthritis

Introduction: Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy. The incidence of obesity is about 10-20% in men and 10-25% in women. It is one of the most important risk factors for osteoarthritis (OA) in knee(s). OA, also known as degenerative arthritis is a group of diseases and mechanical abnormalities entailing degradation of joints, including articular cartilage and the subchondral bone next to it. OA can cause a crackling noise (called "crepitus") when the affected joint is moved or touched, and patients may experience muscle spasm and contractions in the tendons. Occasionally, the joints may also be filled with fluid. Humid and cold weather increases the pain in many patients.

However, the relationship between obesity and OA in hand(s) and hip(s) remains controversial and needs further investigation. The inability to control food intake and to engage in consistent exercise may account for repetitive episodes of weight gain. With the result the evidence is emerged in recent years that excessive weight can be considered as a potential modifier of osteoporosis risk. Due to increased fat mass, the following consequences will be met; psychosocial dysfunction, obstructive sleep apnea, and osteoarthritis are common in obesity. The purpose of this study is to investigate the impact of obesity and lipid profile on the incidence of osteoarthritis (OA) in a general population.

Study Design: Cross sectional analytical

Sample size: Serum and synovial fluid of 50 subjects will be collected for analysis.

Material and Methodology:

To measure the obesity we will perform the multiscan computerized tomography (CT). Bioelectric impedance analysis (BIA) will be done for

- Body mass index (BMI)
- Weight-for-height tables
- Waist circumference
- Waist-to-hip ratio
- Other parameters
  - Serum Calcium, Phosphorous and uric acid.
  - Biochemical and microscopic analysis of synovial fluid.
  - Serum lipid profile will be performed to relate obesity.
  - To measure the osteoarthritis, the patients will be selected who have already diagnosed by MRI scan.

Estimated Cost: Rs. 90,000/-

5.4 Estimation of Health Hazards Due to Pesticide Residues in Marketed Fresh Fruits and Vegetables.

Introduction: Pesticides are applied worldwide to a broad variety of crops to control pests and prevent disease in order to increase agricultural production. The increasing

use of pesticides on fruits and vegetables puts the consumers on greater risks of acute as well as chronic toxicity of these chemicals through direct exposure, ingestion and by increasing soil and water contamination.

Owing to their hazardous effects on consumers, concentration of pesticides must always be below maximum residue limit (MRL). In the developed countries, the eatables are monitored for the levels of pesticide residues. But, the developing countries like Pakistan are far behind yet.

In this study, tomato, apple and cucumber samples will be collected randomly from four main vegetable markets of Lahore and will be analyzed to evaluate the content of nine pesticides. The analysis will be done using Liquid Chromatography- mass Spectrometry (LC-MS) system. The detected residue levels will be looked for the compliance with maximum residue limits (MRL) set by FAO/WHO Codex Alimentarius Commission (CAC).

- The data collected by this study will be helpful as a reference point for future monitoring as well as for providing a basis for taking preventive measures to minimize the risks to human health.
- It will pave way for improving our exports of eatables if found safe.
- If found unsafe, the study will be helpful in making new strategies for improving the quality and safety of foods.

The objectives of this study are

1. Quantitative assessment of selected pesticides in the fresh tomatoes, apples and cucumbers being sold in the four large markets of Lahore city.
2. Comparison of the detected residue levels with the accepted maximum residue limits (MRLs) as adopted by FAO/WHO Codex Alimentarius Commission (CAC).

5.5 Role of Exercise and Dietary Intervention to Alleviate the Osteoporosis of Post- Menopausal Women

Introduction: Osteoporosis is defined as defined by WHO in women as bone mineral density 2.5, standard deviation below the peak bone mass (20 years old healthy female average) as measured by Dual energy X-ray absorptiometry (DXA); the term “established Osteoporosis” includes the presence of fragility fracture. It is most

common in women after menopause when it is called post menopausal osteoporosis. The underlying mechanism in all cases of osteoporosis is an imbalance between bone resorption and formation.

In post- menopausal women the lack of estrogen is responsible for this imbalance. Osteoporosis in post- menopausal women can be prevented with life style changes i.e. diet rich in Calcium and Vitamin D.

The objective of the study is that osteoporosis can be managed if dietary and life style issues could be overcome.

Study Design: cross sectional analytical

Sample size: 100 subjects will be included for this study.

Material and Methodology:

1. The study will include following parameter
2. Bone mineral density by using DXA
3. Serum level of Calcium and Vitamin D
4. Urinary deoxypyridinoline, a biochemical marker for bone resorption
5. Subjects labelled as with osteoporosis will be monitored for exercise and dietary control to see the improvement.

This study can provide the status of osteoporosis in our locality and due to lack of effective therapy dietary control may help for management of osteoporosis.

Estimated Cost: Rs. 80,000/-

5.6 Prevalence of Environmental Asthma Due to Dust Storm in Multan

Introduction: Asthma is a clinical syndrome characterized by episodic reversible airway obstruction, increased bronchial reactivity and airway inflammation. Asthma results from complex interaction among inflammatory cells their mediators, airway epithelium and smooth muscle nervous system. In genetically susceptible individuals, there interactions can lead the patients with asthma to symptoms of breathlessness,

wheezing, cough and chest infection. Risk factors include a family history of allergic disease, the presence of IgE, viral respiratory illness, exposure to aeroallergens, cigarette smoke, obesity and lower socio-economic status. Antigen presenting cells in airway capture and process the antigen and then present it to helper T-cell which in turn become activated and secret cytokines. These cytokines induce the formation of T_{H1} and T_{H2} which promote B-cell IgE production and eosinophil recruitment. Then IgE binds on the receptor on mast cells and basophils. This exposure of allergen IgE secrets cytokines like IL3, IL4, IL5, and TNF-α from mast cells. The purpose of this study is to find out the prevalence of allergic asthma and causative allergen in the dusty environment of Multan.

Study design: Randomize cross sectional

Sample size: Clinical data of 100 patients will be taken and EDTA blood and serum will be collected.

Material and Methodology:

The subjects will be selected for the study by taking clinical history and the following parameters will be determined.

1. Allergic skin reaction test
2. Serum IgE level
3. Eosinophil count in whole blood

This study will help to screen the environmental factors which are causative agents for asthma and also to make strategies in order to overcome these causative agents.

Estimated Cost: Rs. 70,000/-

4. BIOMEDICAL ENGINEERING

5.1 "Gait Analysis Lab" For the Monitoring Of Patients with Implants

Introduction:

Basic Types of Implants:

The implants which are of our interest come under the category of "Orthopedic Fracture Fixation". These fixators could be of internal or external type. Following figure illustrates such fixators in detail.

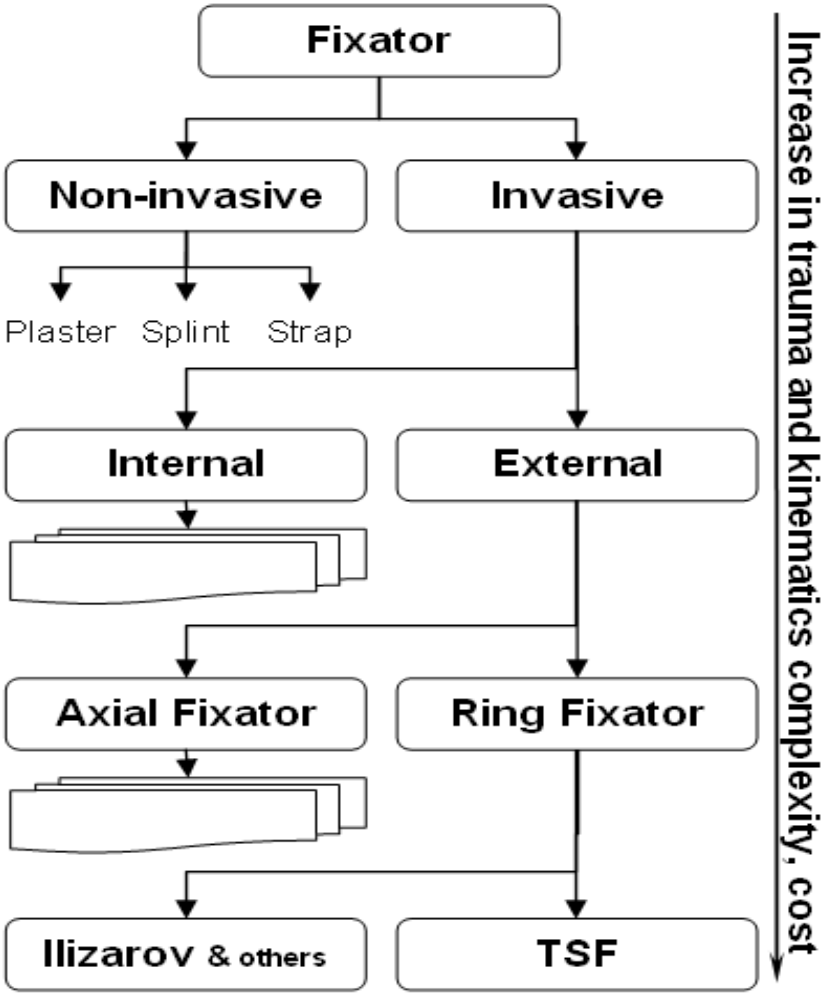

Figure 1. Types of orthopedic fracture fixation

Figure 1 presents a flow chart for the types of fixation used to date. As one progresses down the chart the cost of treatment, complexity of kinematics of fixation and severity of the injury increases.

The fixators can be mainly divided into two groups: Invasive and non invasive. Non-invasive stabilization methods are usually used for treating relatively simple bone fractures. The invasive fractures can be grouped into two types internal and external.

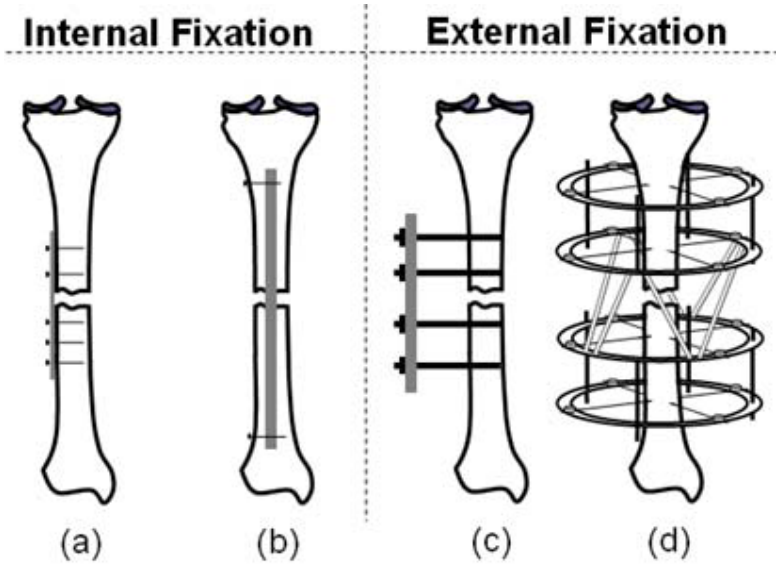

Figure 2. Examples of invasive fracture fixation methods. a) plate and screws, b) intramedullary nail fixed with screws inside the bone, c) unilateral bar connected to bone via half-pins, d) TSF (Taylor Spatial Frame) ring fixator with two accessory rings and connection to bone via 8 fine wires.

Material & Methods:

Equipment Required:

Availability of a suitable lab is the basic requirement of this project and following is the detail of the basic equipment required for it.

- 3D motion capture analysis system containing a 6 camera, optical , 3D motion capture/analysis system.
- Two force platforms with multiple mounting configuration.
- An 8 channel telemetered electromyography system
- A treadmill capable of measuring vertical ground reaction forces, stationary cycles.
- A balance evaluation system
- A system with isokinetic dynamometers for strength testing.

Figurative Illustration of such systems:

Figure: The Biodex Balance System with standard primary components and adjustment mechanisms and a gait trainer.

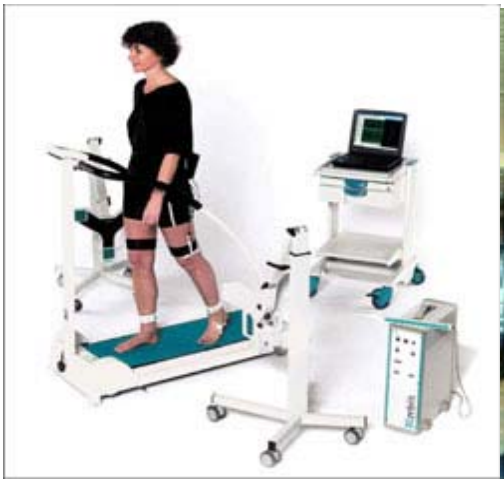

Figure: Wingate

Figure: Complete Gait

Figure: Litegait

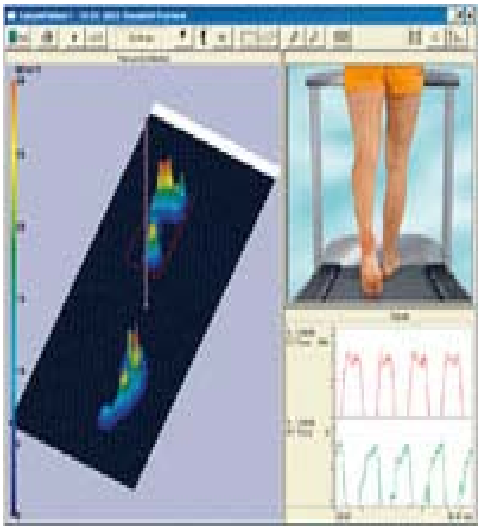

Figure: Gait Analysis

Figure: Floor-gait-plate

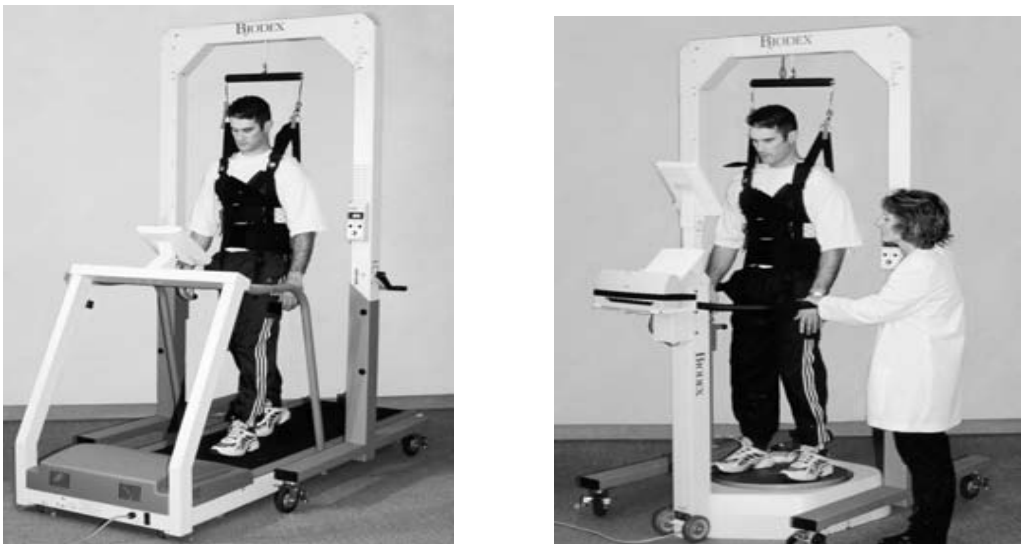

Figure: Standard un-weighing system for treadmill and balance evaluation system

Study Design: Study would be made with the patients having same type of implant in the body lying in a specific age group. Comparison of such patients would be made with a control group comprising of healthy persons without any implant. These age groups can be further divided into further three groups; for example from age 12 to 20, from age 21 to 45 and from age 45 to 65.

Sample size: At least 5 patients would be needed in one group for one specific implant and for one specific age group in addition to the control group.

Methodology: Patients would be contacted having a surgical procedure for an implant in the recent past and would be invited to come in the lab for such type of analysis. A team of engineers and medical doctors would analyze their gait and useful suggestions would be given to the patients for improving their gait after comparison with the control group.

Estimated cost of the project: Cost of the project is itself negligible once we have the required lab with all necessary facilities. The estimated cost of the "Gait Analysis Lab" from Velamed Company in Germany as obtained on 28th May, 2008 was 142,139 Euro which is approximately 15 million Pak rupees.

5.2 Study the Biomechanics of Human Beings Suffering from an Acquired or Genetic Disease

Similar equipment can be used to study the biomechanics of human beings either suffering from an acquired or genetic disease and are not able to walk in a proper manner. Patients suffering from Parkinson's disease, Polio, Alzheimer's disease and many others exhibit such problems. This lab can play a very important role in improving there body motions which can provide much ease to them. Data from local patients can be calculated and published internationally. Such type of lab will also play a vital role in rehabilitation of patients and can generate some funding for the university as well.

Further details are the same as of Project 1. (See above)

5.3 Interaction of Different Materials with Human Cells to Enhance the Quality of Implanted Materials

Introduction: Interaction of different materials with human cells is also one of the best research areas related to Biomedical Research Field. Different types of implanted materials would be selected and would be checked for their real time interaction with human cells in an in-vivo environment. Depending on the results some changes in the parent material can be suggested. Such as to increase or decrease the percentage of a specific material in an alloy.

Multi-Centered Research Project: This project will be carried out as a multi-centered research project in which we will require assistance for the preparation of metallic alloys according to the required percentage.

Material & Method:

Equipment Required:

Availability of a suitable lab is the basic requirement of this project and following is the detail of the basic equipment required for it. As mentioned earlier this is a multi-centered research project, the basic equipment which we would require on our end is as follows

- CO_2 Incubators.
- -60 ^{o}C Refrigerators for chemical storage.
- Cell Culturing Lab.

- Laser Scanning Microscopes
- Latest computers along with appropriate softwares.

Study Design: A specific biomaterial would be obtained already being used in the construction of the implants and would be tested for its interaction with human cells to simulate the exactly in-vivo environment that it would face after being implanted in the human body. That would be our control group.
Percentage of different materials would be varied and then the biomaterial would again be tested for its suitability to be implanted in the human body.

Sample Size: Sample size is not very large. We will change the percentage of one component material of an alloy turn by turn to study its suitability.

Methodology: Cell culturing of human cells would be conducted on the biomaterial and then studied with the help of Laser scanning microscope and computer simulation softwares. Results could be shared with the companies currently producing various types of biomaterials and thus some funding can also be generated for the organization. Some projects from the companies already manufacturing biomaterials can also be obtained for such lab equipped with appropriate equipment.

Estimated cost of the project: Again the cost of the project is itself negligible once we have the required lab with all necessary facilities. The estimated cost of such biomaterial interaction lab is very huge because of the “Laser Scanning Microscope” which itself costs more than 70 million Pak rupees.

5.4 Different Protein Coatings Used to Enhance the Cell Adherence with the Implant

Introduction: Different protein coatings are used to enhance the cell attachment with implanted material. Such protein coatings are Fibronectin, Laminin, Concavalin A, both polymers of poly-lysine and extracelluar matrix gel. Human cells are being cultured by using these protein coatings and then these cells cultured on the substrate material are studied under a Laser Scanning Microscope. 3-D images are obtained from LSM of the real word cell adhesions on the substrate. By using these images in

an appropriate soft ware like Matlab, data is calculated to check the maximum adhesion obtained.

Experiments can be performed to use different types of protein coatings or different proteins mixed together with different percentages to achieve the maximum cell adhesion and results can be published.

Material & Method:

Equipment Required:

Availability of a suitable lab is the basic requirement of this project and following is the detail of the basic equipment required for it.

- CO_2 Incubators.
- -60 ^{o}C Refrigerators for chemical storage.
- Cell Culturing Lab.
- Laser Scanning Microscopes
- Latest computers along with appropriate softwares.

Study Design & Sample Size: Different protein coatings would be selected that may have some improving effect on the cell adhesion with the implanted material. Such protein coatings are Fibronectin, Laminin, Concavalin A, both polymers of poly-lysine and extracelluar matrix gel. The control group for this project would be the material itself without any protein coating. So that at the end results of the control group can be compared with the results obtained after using different protein coatings.

Methodology: By using control group and other selected protein coating human cells would be cultured on them. One example of the human cells used for this purpose is of HEK (Human Embryonal Kidney) cells. After proper cell growth on the protein coatings the slides would be studied under a Laser Scanning Microscope to obtain 3-D images and with the help of right software cell adhesion would be calculated.

Again a group of engineers and health professionals would be required to carry out such project.

Estimated cost of the project: Same lab is required for this project as in project 4.

5.5 To Improve the Design of Stents and Heart Valves By Simulating the Stresses Induced after their Implantation in the Human Body by Using State of the Art Computer Softwares.

Introduction: It is the duty of biomedical engineers to play their role in improving the designs of the stents and heart valves. This can be achieved by using stress analysis softwares that can accurately predict the stresses induced to the flow of the blood, if any change is being produced in the valve or the stunt. Engineering background is necessary for a student interested in such type of project. Once the design is finalized then prototype of a heart valve can be prepared and tested in animals for its suitability.

Material & Method:

Equipment Required:

A computer lab is required for such a project with state of the art stress analysis softwares. Specialized softwares are available for that purpose. One of them is CFD-ACE + software.

Methodology: This software is capable of helping the students a lot in designing of grafts, stents and filters. Catheter design and the analysis of its functioning is also one of the major advantages of this software. Simulation can be done of different heart valves like mitral and aortic heart valves both natural and prosthetic.

Estimated cost of the project: This is essential software for this research project and estimated cost of this software alone is around 1.7 million. So the estimated cost of such “Stress Simulation Lab” is around 2.5 million.

5.6 Distribution of Stresses after a Metallic Implant Within the Human Body in Case of Severe Bone Fracture by Using Soft Wares and in Reality with the Help of “Gait Analysis Lab”.

Introduction: This project is actually a merger of the “Gait Analysis Lab” under project 1 and then the “Stress Simulation Lab” as mentioned in project 5. Many such types of projects can be constructed once the required facilities are there in the University campus.

Material & Method:

Equipment Required:

This project would be carried out in both the labs mentioned above. So the basic equipment requirement is the same as of project 1 and 5.

Methodology: Designing of various Orthopedic implants will be conducted in this project and afterwards studying the stresses which they will induce in the human body after being implanted in the body. Such designs can be made to minimize the stresses produced in the body. All of this work would be carried out in the "Stress Simulation Lab" and once the design has been finalized, prototype can be made to implant in the human body and then study their performance with the help of Gait Analysis Lab.

Again a group of engineers with a very good back ground of principles of mechanics along with doctors who are expert in human biomechanics would be needed for such a project.

5.7 Characterization of Different Biomaterials used for Implantation for Human Body.

Introduction: Characterization of any material is the most important issue before using it to construct either an implant or any device. In case of a biomaterial it is more than important as it can cause us the life of a patient if the material introduced in the human body gets some reaction from the patient and generates some toxic waste products

A series of various research projects can be performed by the students on this equipment related to characterization of various biomaterials depending upon their specific implementation.

Material & Method:

Equipment Required:

The specialized equipment needed for this project is the mini-bionix system This specialized equipment is specifically engineered to perform various functions related to the characterization of the soft biomaterial.

Figure: 858 Mini Bionix Test System

Following are the various tests that can be performed on a biomaterial related to its characterization on this specialized equipment.

- **Testing Biomechanical Constructs**:

This equipment is capable of testing various biomechanical constructs. Such as:

- Fatigue Certification Studies
- Bone, joint and soft tissue studies
- External prosthesis studies
- In vitro biomaterials studies
- Studies of other medical and dental devices where material and structural properties of the device or device tissue interface must be known.

- **Characterization of Biomaterial Properties:**

Following are various characterization properties that can be verified with the help of this equipment.

- Yield and ultimate strength.
- Creep and visco elastic characteristics.
- Fracture toughness and fracture mechanics.
- Modulus of Elasticity

- Poisson's Ratio
- Wear Characteristics
- Co-efficient of thermal expansion
- Response characteristic

Methodology: Characterization of a material is the most important step before its finalization for the manufacture of prototype. Above mentioned characteristics that are very important for the selection of a biomaterial would be tested on this equipment and then the combined results would help us in the final selection of the material for any specific job purpose.

Estimated cost of the project: The estimated cost of this project is negligible if we have this mini bionix test system which costs around 20 million Pak rupees.

5.8 Planning and Designing of the Required Labs like "Gait Analysis Lab", "Stress Analysis Lab" Along with a "Biomedical Workshop".

Introduction: One important question is what would be the role of the BME students before the establishment of such labs? What I would suggest is, the future students can play their role in the following way.

Methodology: The new students can be a part of planning and designing such labs and will have some hands on experience for such activities; meanwhile there would be an opportunity for them to get different trainings for various sophisticated equipments installed in such labs. A complete "Biomedical Workshop" is the most mandatory for this program just like an operation theater in a teaching hospital. Where students should have the liberty to get hands on training on various available equipments or can design their own projects. This research agenda is inclined more towards establishment of labs because this is a new department and currently don't have any labs of its own.

5. HAEMATOLOGY

5.1 Prevalence of Alloantibodies in Multipara Females and Multitransfused Patients

Introduction: Alloantibodies are produced when the erythrocytes from one person are infused into another person. Antigens on the infused cells may be recognized as foreign by the recipient's lymphocytes, stimulating the production of antibodies. These antibodies react only with the antigens on infused cells but not with patient's own erythrocytes. The development of alloantibodies complicates transfusion therapy in patients. The factors for alloimmunization are quite complex. It involves 3 main contributing elements; RBC antigenic differences between donor & recipient; recipient's immune status and the effect of the allogenic blood transfusions on the recipient's immune system.

Antibody screening test should be included in the panel of pretransfusion tests for safer transfusion, and it is particularly mandatory for the patients with multiple transfusions and pregnant women because many complications like hemolytic transfusion reaction, Immune hemolytic anemia, and hemolytic disease of newborn can be caused by them.

Study Design: It will be an Analytical Cross sectional study.

Sample size: A total of 60 Samples will be obtained from Multipara females**,** patients of Thalasemia and of chronic renal failure.

Methodology: Antibody screening will be carried out by using 'Three cell panel' and antibody detection will be done by 'Eleven cell panel'.

Approximate Cost of the Project: Rs: 70,000

5.2 Frequency of Aspirin Resistance in Patients with Coronary Artery Disease in Pakistan

Introduction: Aspirin resistance refers to patients who are taking aspirin but do not display an adequate degree of platelet inhibition. Aspirin resistance is an emerging clinical entity. However the data available on aspirin resistance in Asian population is scarce. Aspirin has been shown to have variable antiplatelet activity in individual

patients. The purpose of the study is to evaluate the frequency of aspirin resistance in patients with stable coronary artery disease (CAD) in Pakistan.

Study Design: It will be an analytical cross-sectional study.

Sample Size: A total 100 patients more than 21 years of age, with established coronary artery disease for at least two months and on aspirin therapy for more than seven days will be included in the study.

Methodology: Responders will be assessed on the basis of ADP induced platelet aggregation using Chronolog 490-2D platelet aggregometer.
Frequency of aspirin non-responders will be calculated and correlation will be discussed with clinical risk factors. A significant proportion of Pakistani population may have aspirin resistance. Better knowledge of this aspirin resistance in Pakistan will indicate the need for other treatment strategy to be used in patients with aspirin resistance.

Approximate Cost of the Project: Rs: 1,00,000

5.3 Evaluation of Anaemia in children

Introduction: Anemia is defined as hemoglobin concentration below established cut off levels. It is a widespread public health problem. Although, estimates of prevalence of anemia vary widely. It can be assumed that significant proportions of young children and women of childbearing age are anemic.

Anemia is an indicator of both poor nutrition and poor health. General symptoms and signs of anemia are irritability with excessive crying, poor cognitive function, a decline in psychomotor development and pallor.

Multiple causes exist, but with a thorough history, a physical examination and limited laboratory evaluation a specific diagnosis can usually be established. The use of the mean corpuscular volume (MCV) to classify the anemia as; microcytic, normocytic or macrocytic is a standard diagnostic approach. The most common form of microcytic

anemia is iron deficiency caused by reduced dietary intake and chronic blood loss caused by worm infestation particularly in developing countries. The reticulocyte count will help to narrow the differential diagnosis; however, additional testing may be necessary to rule out hemolysis, hemoglobinopathies, membrane defects and enzymopathies. Macrocytic anemia may be caused by a deficiency of folic acid and/or vitamin B12, hypothyroidism and liver diseases.

The most prevalent and preventable form of microcytic anemia is iron deficiency anemia. Other causes are anemia of chronic diseases, sideroblastic anemia and thalasemia trait.

Diagnosis of microcytic anemia is confirmed by measuring serum iron, TIBC, serum ferritin and serum transferrin receptor. This will differentiate between iron deficiency anemia and anemia of chronic diseases. Diagnosis of thalasemia trait is made by RBC indices and RBC count and confirmed by electrophoresis. Sideroblastic anemia is rare in children and diagnosis is confirmed by bone marrow aspiration.

Macrocytic anemias in children are relatively uncommon, but are usually caused by a deficiency of vitamin B12, folate or intrinsic factor. Other possible causes include chronic liver disease, hypothyroidism and myelodysplastic disorders. Folic acid deficiency is usually due to increase requirement, prematurity, inadequate dietary intake, goat's milk, scurvy and malabsorption.

Determining a diagnosis of normocytic anemia in a child can be clinically difficult. First, obtain a reticulocyte count to determine whether there is decreased production or increased destruction of red blood cells. With decreased red cell production the reticulocyte count will be depressed relative to the hemoglobin concentration. Depending on the severity of the anemia, the evaluation may ultimately warrant a bone marrow aspiration.

A study will be designed for Screening of children (2 to 5 year) and categorization of anemic children on the basis of MCV / MCH and evaluation of Hypochromic microcytic, Normochromic normocytic and macrocytic anemia. Further testing will be done to elucidate the causes of a particular type of anemia.

Expected outcome: Most common causes of anemia will be determined. Treatment strategies can be planned accordingly to benefit maximum number of children and prevention of treatable causes can be undertaken.

Material and Methods:
Study design: Cross- sectional study

Sample size: 200 childrens

Methodology: At least 200 children were selected; the tests which are performed are Complete blood counts (CBC), transferrin, and ferritin, Hemosidrin and transferrin receptors

Estimated cost: Rs.150,000

5.4 Evaluation of Acute Leukemia on the Basis of Immunophenotyping and Fusion Genes Analysis

Introduction: The disease process in acute leukemia is characterized by the uncontrolled proliferation of immature white cells or blasts. The diagnosis is made by morphology from bone marrow smears including cytochemistry, the detection of immunological markers, cytogenetic analysis and molecular genetic methods. Acute leukemias are broadly classified into Acute myeloid and Acute lymphoblastic.

The pathogenesis of acute leukemia in many patients is linked to oncogenic fusion proteins, generated as a consequence of chromosome translocations or inversions.
Many different translocations have been described in AML, the most frequent being the t(9;11), t(15;17), t(8;21), and inv(16), which, taken together with their variants, account for 20–30% of AML cases. These recurring translocations are now the basis for classification of patients with AML.

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease in which the malignant clone arises from lymphatic progenitors in the bone marrow or lymphatic system. Chromosomal karyotype and translocation in cytogenetic studies have considerable

diagnostic and prognostic value. Since the discovery of the first fusion gene, BCR-ABL, resulting from a t(9;22) translocation, many fusion transcripts that occur in ALL, such as t(12;21), t(4;11), and t(1;19), have subsequently been detected. Research has shown that normally-fused translocated genes play a crucial role in the development and function of bone marrow cells. It has therefore been suggested that the fusion genes may be closely correlated with the onset of leukemia. Advances in molecular genetics have demonstrated that many fusion genes are difficult to detect with conventional karyotyping and highlight the value of molecular genetics in the diagnosis and treatment of leukemia

Molecular studies have shown that these structural chromosomal rearrangements create a fusion gene encoding a chimeric protein. Most of them can be detected by RT-PCR including complex and cryptic cytogenetic variants.

Twenty years ago, classification of leukaemia was proposed according to their morphological features. (F.A.B. defined classification). This classification is based on cell morphology of peripheral blood and bone marrow smears with the addition of simple cytochemical techniques which has now, been found insufficient to make a diagnosis of acute myeloid leukemia (AML) or acute lymphoblastic leukaemia (ALL). Besides, in a minority of cases of acute leukemia the blast cells show nonspecific features of either AML and ALL on morphology and cytochemical stains which not only leads to diagnostic dilemmas but is also responsible for administration of wrong chemotherapeutic agents which is misinterpreted as resistant disease and results in wastage of resources. Immunophenotyping and genetic analysis is being used regularly, as part of diagnostic protocols in several countries.

A study will be designed for evaluation of Acute Leukemias on the basis of Immunophenotyping and Fusion gene analysis in our population.

This study will provide an insight into the chromosomal abnormalities leading to production of fusion genes as an etiology of acute leukemias in our population.

It will provide guidance to develop a strategy for diagnosis of Acute Leukemia in our set up. Fusion gene analysis if adopted as a regular parameter for diagnosis of Leukemia will serve as a useful tool for administration of specific therapy according to the genetic abnormality and detection of Minimal Residual disease (MDR)

Material and Methods:

Study design: Cohort study

Sample size: 30 Patients

Methodology: RT – PCR for detection of fusion genes
or RQ PCR for detection and quantitation of transcript of fusion gene.

Approximate Cost of the Project: Rs: 1,50,000

5.5 Molecular Markers in Chronic Lymphoproliferative Disorders

Introduction: These disorders are characterized by malignant proliferation of mature lymphocytes in the bone marrow and peripheral blood. Chronic lymphocytic leukemia (CLL) and Prolymphocytic leukemia (PLL) are predominantly B cell disorders. T cell disorders only account for 5% of the cases. Most of the malignant disorders occur as a result of chromosomal translocations which lead either to production of fusion genes or bring oncogenes in close proximity to immunoglobulin gene locus and cause increased proliferation.

Chronic lymphocytic leukemia (CLL)
The most common cytogenetic abnormality is trisomy12 either alone by itself or by combination with other cytogenetic abnormalities, other frequently observed structural aberrations are abnormalities of chromosome 13 and chromosome 14.

Burkitt's lymphoma
Three translocations consistently found in Burkitt's lymphoma are; t(8:14)in approximately 80% , t(2:8) in nearly 6% and t(8;22) in 14% of cases. Chromosome 14, 2, 22 encode for immunoglobulin heavy chain, light chain λ, κ gene respectively. Oncogene C MYC is located at chromosome 8 (band q24). As a result of each translocation C MYC is brought in close proximity to one of the Immunoglobulin genes.

Follicular lymphoma

This is the most common B cell malignancy and is associated with t(14:18) in 90 % of cases. This causes deregulation and increased expression of Bcl-2 thus inhibiting apoptosis. Transformation of follicular lymphoma into high grade lymphoma is often accompanied by karyotype evolution.

Mantle Cell Lymphoma

It is characterized by proliferation of small lymphocytes with irregular nuclei derived from normal follicular mantle zone. The translocation commonly involved is t(11:14),it is commonly seen in 70% of the cases.

Anaplastic large cell lymphoma.

The translocation t(2:5) is specific for this lymphoma and result in the fusion of nucleophosmin gene at 5q35to the anaplastic lymphoma kinase gene at 2p23.

Molecular studies are now increasingly employed for detection of chromosomal abnormalities which are found approximately in 80% of these cases. In general it has been observed that those patients with abnormal karyotype have worse prognosis than those with presenting normal cytogenetic. Therefore a study has been planned for detection of Fusion genes resulting from chromosomal translocations.

5.6 Carrier Detection of Haemophilia B by Indirect Linkage Analysis

Introduction: Haemophilia B, also known as Christmas disease, is an X-linked recessive coagulopathy which results from deficiency or an abnormality of procoagulant factor IX (FIX). It is a life long bleeding disorder. As it is an X-linked recessive disorder it affects mainly males. Females are carrier of this disorder who transmits haemophilia B to their sons. Its frequency is about one in 25,000 males at birth. All races and economic groups are affected equally. Hemophilia B can result from several genetic mutations including deletions, point mutations, and frame shift. Point mutation (mis-sense and non-sense) accounts for 95% of the abnormalities.

World Federation of Haemophilia data show that only few people with haemophilia live beyond childhood. The management of haemophilia in developing countries is very difficult because of high cost of treatment. Without proper treatment and care haemophilia has adverse affects on every aspect of life of people suffering from this

disorder. It is estimated that only 25% of the people with haemophilia get proper treatment. Most of 75% of the people who either do not receive treatment at all or are not getting adequate treatment reside in developing countries like Pakistan. Therefore in Pakistan where factor concentrates are hardly available for replacement therapy carrier detection and prenatal diagnosis is the key step for prevention of birth of babies with haemophilia.

Factor IX gene is large in size and complex. The mutations are highly heterogeneous and about one third are de novo so direct mutation detection is very time consuming, costly and difficult.

Material & Methods

Study Design: It is cross-sectional study.

Sample size: 15 families (each consisting of father, mother and brother of the index case).

Methodology: Linkage analysis (also known as indirect gene tracking) is a technique used in the identification of inherited genetic diseases. The technique is based on the fact that special DNA sequences that flank particular genes will travel with the gene when passed from parent to child. These DNA sequences are called 'polymorphic markers' or 'polymorphic repeat sequences'. If a particular polymorphic marker is found only in members of a family with a particular disease then it is likely that a gene located near the marker is associated with the disease.

DNA polymorphic analysis is less costly, quick and also accurate. So in countries where financial resources are limited, carrier detection and prenatal diagnosis by linkage analysis is the method of choice.

The objectives of this study are to determine the informativeness of polymorphic markers in hemophilic families and use these markers in carrier detection in Pakistan

Approximate Cost of the Project: Rs: 100,000

5.7 Prevalence of JAK2 V617F in Patients of Myeloproliferative Disorders

Introduction: Myeloproliferative disorders include are Polycythemia vera (PV), Essential thrombocytosis (ET) and Idiopathic Myelofibrosis (IMF). These are clonal neoplastic diseases characterized by proliferation of one or more hematopoietic lineages.

Recently a mutation of the Janus Kinase 2 (JAK2) gene that leads to the substitution of phenylalanine for valine at position 617 of the JAK2 protein, JAK2 V617F, has been found in 76% to 97% of patients with PV, 29% to 57% of patients with ET and 50% of patients with IMF. This mutation confers constitutive activity on to the JAK2 protein and appears to play an important role in the pathobiology of these conditions. However, not all patients with myeloproliferative disorders have this mutation and it may not be the primary cause of these diseases.

The insights revealed by the foregoing molecular investigations are reshaping our understanding of the pathophysiology, classification, diagnosis, and treatment of MPD. The *JAK2* mutation is the first genetic marker that is directly associated with the pathogenesis of the myeloproliferative disorders, and for this reason it is a powerful tool for analysis of the molecular and cellular basis of these disorders.

Clinical Utility of Direct Diagnostic Test for the V617F JAK2 mutation*:*

- To confirm a diagnosis of PV, ET, and IMF
- To confirm a clonal hematopoietic stem cell disorder
- To confirm MPD in cases with high erythrocyte, leukocyte, or platelet counts
- May obviate the need for slow Endogenous Erythroid Colony (EEC) growth test
- The identification of JAK2 V617F could change the management of MPD patients an paves the way to treatment

The identification of the JAK2 V617F mutation as the cause of MPD is without doubt a first step into the development of specific targeted therapy in this disease. A study will be planned to find out the prevalence of JAK2 V617F in patients of Myeloproliferative Disorders

Material and method:

Study Design: cross_sectional study

Sample Size: Total of 50 patients

Methodology:

PCR

FISH

Estimated Cost: RS**:** 100,000

5.8 Evaluation of Acquired Abnormalities of Platelets in Patients of Chronic Liver Disease.

Introduction: Primary haemostasis is defined as the interaction between platelets and the vessel wall at sites of vascular injury. The chain of events leading to the formation and consolidation of the platelet plug is initiated when circulating platelets adhere to the sub endothelium. This is mediated by the exposure on platelet membranes of specific receptors for the plasma adhesive proteins (mainly von Willebrand factor) followed by the adhesion of platelets to the components of the extracellular matrix and the subsequent aggregation to one another. Normal platelet numbers and function as well as normal plasma von Willebrand factor are therefore essential for primary haemostasis.

Chronic liver disease is characterized by thrombocytopenia due to increased platelet destruction or to increased sequestration in spleen and liver and by thrombocytopathy due to defective thromboxane A2 synthesis, abnormalities of the platelet glycoprotein Ib. The anemia associated with this disease has a negative influence on haemostasis. Most of the patients with cirrhosis have bleeding problems.

Platelet dysfunction can be inversely proportional to whole blood hematocrit in cirrhotic patients.

Materials and methods:

Study Design: It is an analytical cross-sectional study.

Sample size: A total of 50 patients with established chronic liver disease are included in the study

Methodology:

- Platelet aggregation studies will be performed on Chronolog 490-2D platelet aggregometer using ADP, epinephrine, collagen, and ristocetin.
- Complete blood count
- PT & APTT will also be performed.
- Whole blood hematocrit and platelet dysfunction will be determined and their correlation will be discussed.
- A significant number of patients with cirrhosis with low hematocrit may have high degree of platelet dysfunction. A better knowledge of the platelet dysfunction among cirrhotic patients can assist in considering the appropriate treatment.

Estimated Cost of the project: Rs: 100,000

5.9 Determination of Reference Values of Complete Blood Counts of Healthy Population of Different Age Groups in Pakistan

Introduction: Reference values of complete blood count vary according to environmental factors and there is no data regarding reference values of complete blood count on Pakistani population and hence physicians face difficulty in the interpretation of laboratory reports. Ranges of complete blood count will be different from international ranges. In this study complete blood count will be determined and compared with international ranges.

Material and Method

Study design: It will be an analytical cross-sectional study.

Sample Size: Healthy male and female individuals with different age groups and living in Pakistan will be included in this study. Physical examination will be performed. Routine biochemical tests (Fasting blood glucose, renal and hepatic function tests), full blood count and urinary analysis will be performed. Venous blood samples will be drawn from the antecubital vein into 3 ml tubes containing K_2EDTA anticoagulant.

Methodology: Blood samples will be kept at room temperature and tested in one-hour time. Sysmex XS1000i blood counter to determine hemoglobin (Hb), hematocrit (Hct) and red cell indices as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) and red cell distribution width (RDW). Reference values of full blood count parameters will be defined for values of percentiles in 95% confidence limits. Subjects will be healthy males and females of different age groups living in Pakistan and living at the mean altitude of 1050m. Subject's previous diseases, operations, dietary habits and blood donation in the previous six months will be investigated. Acquisition of new reference values from the population living in this region will provide unified standard and more correct ranges of complete blood counts. This will help physicians in the correct interpretation of lab reports.

Estimated Cost of the project : Rs:150,000

5.10 Von Willebrand Disease

Introduction: Von willibrand disease is the most common inherited bleeding disorder. Inheritance is autosomal dominant with varying expression. It is a quantitative or qualitative deficiency of von willibrand factor arising from mutation in the von willibrand factor gene. It remains undiagnosed in many patients with milder form of disease and diagnostic methods for sub types of von willibrand disease are still not established.

Von willibrand factor is produced in endothelial cells and megakaryocytes and stored in Weibel Palade bodies and alpha granules of platelets. It is released from Weibel Palade bodies in the form of ultra large multimers which are the most reactive and adhesive form.

The various types of vWD present with varying degrees of bleeding tendency, usually in the form of easy bruising, mucosal bleeding like nose bleeds and bleeding gums. Excessive blood loss from superficial cuts and abrasions and post traumatic or operational haemorrhage. Women may experience heavy menstrual periods and blood loss during childbirth. Severe internal or joint bleeding is rare (which only occurs in type 3 vWD). Most of the patient have mild bleeding symptoms and thus not aware of having the disease. There are three main types of von willibrand disease.

- *Type1* - due to partial quantitative deficiency. It is the most common and milder form of von willibrand.
- *Type2* - a qualitative disorder with functional abnormality and further sub divided into four sub types.

- 2A is due to decreased platelet adhesion because of absence of ultra large multimers.
- 2B is due to increased affinity for platelet glycoprotein and absence of ultra large multimers.
- 2M is due to decreased platelet adhesion. Ultra large multimers are present.
- 2N is due to decreased affinity for factor VIII.

- *Type 3* - a quantitative disorder due to complete absence of von willibrand factor.

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Material & Methods

Study Design: It is cross-sectional study.

Sample size: At least 50 individuals were included in the study

Methodology: When suspected patient needs to be investigated for quantitative and qualitative deficiencies of vWF. This is achieved by measuring the amount of vWF in a vWF antigen assay and the functionality of vWF with collagen binding assay or ristocetin cofactor activity (RiCof). Normal levels do not exclude all forms of vWD particularly few sub types of type 2 which may only be diagnosed by ristocetin induced platelet agglutination (RIPA) and vWF multimer analysis. Type 2N can only be diagnosed by performing a "factor VIII binding" assay. Other tests performed in any patient with bleeding problems are a complete blood count (especially platelet counts) bleeding time which is replaced by PFA-100, APTT (activated partial thromboplastin time), prothrombin time, thrombin time.

Early and proper diagnosis of sub types of disease is important because only then patients can be treated according to disease type. With the right treatment plan, even people who have type 3 vWD can live normal active lives.

Estimated Cost of the project: Rs:150,000

6. HISTOPATHOLOGY

6.1 Morphological and molecular analysis of muscular dystrophies in Pakistani population.

Introduction: Duchenne muscular dystrophy (DMD) is a fatal muscular disorder, characterised by progressive muscle weakness and wasting. DMD is one of the most common types of muscular dystrophy , with an incidence of one in 3500 newborn boys. Patient is alright at birth with onset of symptoms generally before the age of 5. Affected individuals progressively develop weakness of skeletal muscles, ultimately losing ability to walk and become confined to wheelchair before the age of 12. They usually die in the course of the second or third decade, due to respiratory or heart failure. There is no cure to date for this deadly disease.

Becker muscular dystrophy (BMD) shows a milder phenotype and is less common, with an incidence of 1:20,000 newborn males. BMD is characterised by delayed onset of muscle weakness and clinical symptoms. Many BMD patients remain ambulant later is life and have longer life span than DMD patients.

Material and Methods:

Study Design: The study will be carried out on Pakistani patients coming to various centers dealing with the diseases of the children and some specialised centers dealing with skeletal muscle disorders. In each patient in addition to the clinical parameters basic investigations such as biochemical parameters will be estimated. Serum will be taken to study patterns of deletions in DMD/BMD patients. The biopsy tissues will be studies using various histochemical and immunohistochemical markers.

Sample Size: 80 patients of muscular dystrophies. 25 control patients (normal muscles).

Methodology:

(i) Clinical details of each patient including personal history and family history.

(ii) Biochemical data

(iii) Genetical data of mutations using multiplex ligations dependant probe amplifications (MLPA).

(iv) The result of immunohistochemical markers (6-7 markers).

Estimated Cost of Project : Rs 0.8 millions (approximately).

6.2 Expression of P63 and S-100A6 in The Differentiation of Keratinocytic Tumours of Skin

Introduction: Since late 1990's, S-100A6 and p63 have been demonstrated in various cutaneous tumours. The results suggest that S-100A6 stains mature epithelial cells and p63 stains cells originating from basal stem cells. These two markers give important information regarding the origin of various tumours. Only a few studies have been carried out to see the differential staining pattern of these tumour markers in various subtypes of keratinocytic tumours. The proposed study is intended to see the utility of both these immunohistochemical markers in differential diagnosis of various keratinocytic tumours of skin (WHO Classification). Staining of the tumour with both these markers would be more elaborative as one will stain basal stem cells and the other mature epithelial cells. The results of this study will contribute in the better understanding of the keratinocytic tumours of skin which will help in developing prompt and accurate diagnosis of those tumours that are difficult to differentiate on routine staining.

Materials and Method

Study Design: Analytical Cross-sectional Study

Sample Size: The study will be performed on 100 sample biopsies of keratinocytic tumours.

Methodology

Place of Work: The subjects will be selected from the Department of Dermatology and the Department of Plastic and Reconstructive Surgery, Mayo Hospital, Lahore on the basis of clinical suspicion of keratinocytic tumours. If histology comes out to be suggestive of any keratinocytic tumour, the subjects will be included in the study; otherwise they will be excluded.

Clinical Information: Socio-demographic information (name, age, sex, address, occupation) will be obtained along with relevant clinical information. All the information will be collected on specially designed Proforma recorded.

Laboratory Work: The specimens will be fixed in 10% formalin and brought to the Department of Histopathology, University of Health Sciences, where it will be allocated a laboratory number. Grossing will be done and recorded. Paraffin embedded blocks will be made from each biopsy specimen. Multiple slides will be made from the representative block and stained with H & E and Acid Orcein Giemsa (that shows differential staining for various parts of skin). The slides will be examined for microscopic morphology to establish the diagnosis. All the results will be recorded in the relevant Proforma.

Histochemistry: Two slides from each case of diagnosed keratinocytic tumours will be further processed for immunohistochemical staining of p63 and S-100A6; one for each stain. Immunopositivity will be recorded with respect to staining intensity and pattern.

Estimated Cost of Project

Sr. No.	Reagents / Stains / Slides	Company	Quantity	Estimated Price in Rs.
1.	Acid Orcein Giemsa	Local/Imported	250ml	8,500/-
2.	DAB Universal Detection Kit	Labvision USA	200 Tests	28,500/-
3.	p63 Antibody (RB-9424-R7)	Labvision USA	7 ml	20,000/-
4.	S100A6 Antibody (RB-1805-R7)	Labvision USA	7 ml	25,000/-
5.	Super-frosted Plus slides	USA/Germany/UK	200	4,500/-
Total				**86,500/-**

6.3 Morphological study of renal lesions in systemic lupus Erythematosis

Introduction: Systemic lupus erythematosus (SLE) is a chronic, inflammatory, autoimmune disease affecting multiple organ systems including the kidney, skin, joints, heart, lungs, central nervous system and the serous membranes. Immune mediated nephritis is a common complication of SLE evident in around 50% of the patients, being more common in certain ethnic groups and in children.

Autoimmunity plays a major role in the pathogenesis of lupus nephritis causing deposition of immune complexes in the kidney that can subsequently be observed on biopsy specimens and can clinically present as asymptomatic haematuria / proteinuria, nephrotic syndrome, or nephritic syndrome .This may be accompanied by varying degrees of hypertension and renal dysfunction.

A revision of the classification of lupus nephritis sponsored by the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) was put forward in 2002.

Our view is that this system of classification is more standardized and provides important information about the class, severity, activity and chronicity of the renal disease that cannot be accurately predicted on the basis of clinical manifestations alone. Based on these findings it is important to diagnose the pattern of immunological type of lupus nephritis as it can help in choosing the efficient therapy and diagnosis.

Materials and Methods

Study Design: Analytical & Cross sectional study

Sample Size: 30 samples of lupus nephritis

Methodology: The subjects will be selected from the Department of nephrology Sheikh Zayed Hospital and Services Hospital, Lahore.

Socio-demographic information (name, age, sex, address) will be obtained along with relevant clinical information. All the information will be collected on specially designed Proforma-A (attached) and recorded.

The specimens will be fixed in 10% formol saline and brought to the Department of Histopathology, University of Health Sciences, where it will be allocated a laboratory number. Grossing will be done and recorded. Paraffin embedded blocks will be made from each biopsy specimen. Multiple slides will be prepared from the representative block and stained with H & E. Special staining with PAS, Gomori & Trichrome stains will be performed. The slides will be examined for microscopic morphology to establish the diagnosis. All the results will be recorded in the relevant Proforma-B (attached).

Estimated Cost of the Project: 75,000/-Rs.

6.4 Morphological Study of IgA and Henoch-Schönlein Nephropathies

Introduction: Primary or idiopathic IgA nephropathy is recognized as the most common form of primary glomerulonephritis, although the prevelance of IgA nephropathy shows considerable variations among geographical regions and different renal biopsy practice. Henoch Shonlein Nephritis is the systemic form of IgA nephropathy. It is mainly predominantly involving the children.The mainstay of diagnosing IgA nephropathy remains immunoflouresence microscopy. It shows predominantly IgA deposits and C_3 deposits in glomeruli involving the mesengium with or without staining of peripheral capillary walls. Induction of inducible nitric oxide syntheses (iNOS) occurs as part of initial response to immune injury in proliferating forms. The hypothesis of this study is to determine the presence of IgA deposits on immunofluorescence and iNOS in diagnosing proliferative pattern. The study shall consist of 36 cases, comprising 12 cases each of Henoch Shonlein Nephritis, idiopathic IgA nephropathy and of chronic glomerulonephritis. Renal biopsy samples will be taken from Sheikh Zayed Hospital, Services Institute for Medical Sciences and Institute of Child Health and Children Hospital, Lahore. Paraffin embedded sections will be stained with H&E, PAS, Gomori Methanamine Silver and Trichrome. Sections from fresh and unfixed biopsy will be frozen in cryostat and direct immunofluorescence will be done on each case. One slide from each case of diagnosed IgA nephropathy will be further processed for immunhistochemical staining of iNOS.

Materials and methods

Study Design: Analytical Cross-sectional Study

Sample Size: The study shall consist of 36 samples, of which 12 will be the cases fulfilling the criteria of idiopathic IgAN, 12 will be of chronic GN and 12 will be from patients of HSN. The number of cases of IgAN and HSN are based on the assumption that only this number of cases(as per ward records) will be available during this period.

Methodology

Place of Work: The subjects will be selected from the Department of Nephrology Sheikh Zaid Hospital,Services Hospital and Children Hospital, Lahore.

Clinical Information: Socio-demographic information (name, age, sex, address) will be obtained along with relevant clinical information. All the information will be collected on specially designed Proforma-A (attached) and recorded.

Laboratory Work: The specimens will be fixed in 10% formalin and brought to the Department of Histopathology, University of Health Sciences, where it will be allocated a laboratory number. Grossing will be done and recorded. Paraffin embedded blocks will be made from each biopsy specimen. Multiple slides will be made from the representative block and stained with H & E. Special staining with PAS, Gomori Methanamine Silver & Trichrome stains will be done. The slides will be examined for microscopic morphology to establish the diagnosis. All the results will be recorded in the relevant Proforma-B (attached).

Immunoflourescence: Sections from fresh and unfixed biopsies will be frozen in cryostat. Cryostat sections will be cut at 5um and incubated with antibodies specific for IgA, IgG, IgM, C3. Direct immunofluorescence microscopy will be done on each case.

The intensity of fluorescence will be graded on the following scale:

Absent	-	0	(No staining)
Weak	-	1	(5% or less)
Moderate	-	2	(5-50%)
Strong		-	3 (more than 50%)

The location of the deposits will be reported separately.

Immunohistochemistry: One slide from each case of diagnosed IgAN will be further processed for immunohistochemical staining of iNOS. Immunopositivity will be recorded with respect to location and distribution pattern.

Estimated cost of project: 75,000 Rs

6.5 Morphological study of HCV Associated Nephropathy

Introduction: Hepatitis C virus (HCV) is an enveloped RNA virus in the flaviviridae family and accounts for vast majority of viral hepatitis. Extrahepatic manifestations of HCV include mixed cryoglobulinaemia, lymphoproliferative disorders, and kidney disease. HCV is strongly associated with mixed cryoglobulinaemia. It is found that there is high prevelance of anti HCV antibodies in the serum and cryoprecipitates along with serum HCV RNA. Concomitant cryoglobulinaemia and HCV infection is associated with membranoproliferative glomerulonephritis (MPGN).

Patients present with impaired renal function, haematuria and proteinuria. Renal biopsy examination reveals membranoproliferative glomerulonephritis type I, membranous glomerulonephritis IgA nephropathy, amyloid nephropathy post-infectious glomerulonephritis, focal segmental glomarulosclerosis, fibrillary and immunotactoid glomerulonephritis. Immunofluorescence shows IgG, IgM, C3 & C1q deposits in the glomerular capillary walls immunohistochemistry detects antigens. Immunohistochemistry shows linear or granular deposits with HCV NS3 antibody. The hypothesis of this study is that expression of anti HCV-NS3 has diagnostic value in HCV-associated nephropathy. The aim of our study is to see the different morphological patterns of HCV-associated nephropathy and associate it with expression of HCV-NS3. The study shall consist of 30 cases of renal biopsy samples. These samples will be taken from Sheikh Zayed Hospital and Services Institute for Medical Sciences , Lahore.

Paraffin embedded sections will be stained with H&E, PAS, Gomori Methanamine Silver and Trichrome. Sections from fresh and unfixed biopsy will be frozen in cryostat and direct immunofluorescence will be done on each case. One slide from each case of diagnosed HCV nephropathy will be further processed for immunhistochemical staining of HCV NS3.

Materials and methods

Study Design: Analytical Cross-sectional Study

Sample Size: The study will be performed on 30 biopsies from patients of nephropathy associated with HCV. The number of cases was calculated after going through the retrospective data of the Nephrology departments of Sheikh Zayed Hospital and SIMS Hospital, Lahore.

Methodology

Place of Work: The subjects will be selected from the Department of Nephrology, Sheikh Zayed Hospital and SIMS,Hospital, Lahore.

Clinical Information: Socio-demographic information (name, age, sex, address, occupation) will be obtained along with relevant clinical information. All the information will be collected on specially designed Proforma-A (attached) and recorded.

Laboratory Work: The specimens will be fixed in 10% formol saline and brought to the Department of Histopathology, University of Health Sciences, where it will be allocated a laboratory number. After processing of the tissue, paraffin embedded blocks will be made from each biopsy specimen. Multiple slides will be made from the representative block and stained with H & E. Special staining with PAS, Gomori & Trichrome stains will be done. The slides will be examined for microscopic morphology to establish the diagnosis. All the results will be recorded in the relevant Proforma-B (attached).

Immunoflourescence: Sections from fresh and unfixed biopsy will be frozen in cryostat. Cryostat sections will be cut at 5um and incubated with antibodies specific for IgG, IgM, C3, C1q. Direct immunofluorescence microscopy will be done on each case.

The intensity of fluorescence will be graded according to the following scale:

Absent	-	0	(No staining)
Weak	-	1	(5% or less)
Moderate	-	2	(5-50%)
Strong	-	3	(more than 50%)

The location of the deposits will be reported separately.

Immunohistochemistry: One slide from each case of diagnosed HCV-associated nephropathy will be further processed for immunohistochemical staining of HCV-NS3. Immunopositivity will be recorded with respect to location and distribution pattern.

Estimated cost of project: 75,000 Rs

6.6 A Study of Alterations in Mucin Expression and Cellular Changes as Prognostic Indicators in Colorectal Carcinoma

Introduction: Colorectal carcinoma happens to be the fourth most common malignant tumour in females, and in males only behind Bronchial Carcinoma. The incidence of the disease has generally increased over the recent decades in both developed and developing countries. It develops by a multistep process that can be influenced by hereditary, genetics, environmental or acquired factors.

The assessment of cell proliferation in colorectal tissue provides information with both prognostic and therapeutic implications. High counts of total white cells and individual high counts of eosinophils, mast cells, plasma cells, and neutrophils had significant prognostic value in predicting overall prolonged survival. Changes in mucin composition have also been reported in Colorectal Carcinomas. The expression of sulphomucin is high in normal mucosae and much lower in colorectal carcinoma, whereas increased amount of sialomucin in the transitional zone when stained with HID-AB, has been associated with a relatively poorer prognosis in colorectal carcinoma.

The hypothesis of this work is that an increased number of inflammatory cells and an altered expression of mucin in the transitional zone of the colorectal carcinoma act as prognostic indicators.

Materials and methods

Study Design: Analytical cross sectional study.

Sample Size: The study shall consist of 50 samples fulfilling the inclusion criteria The number of cases of CRC are based on the assumption that only this number of cases will be available during this period.

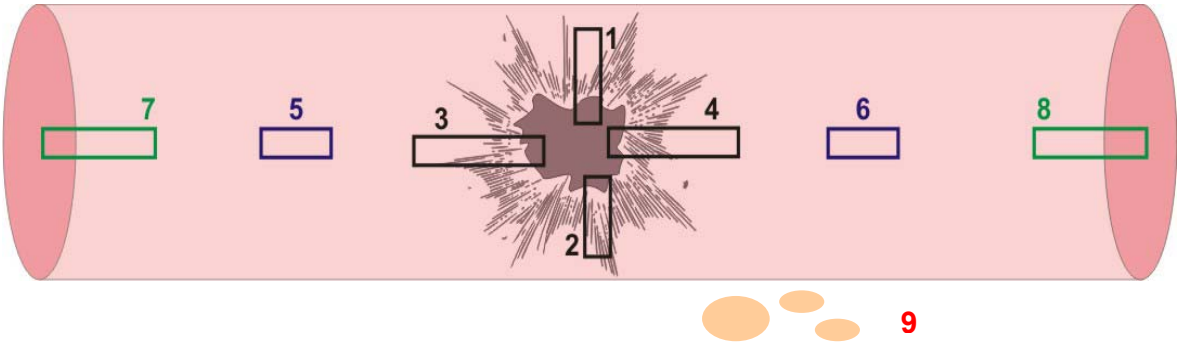
Methodology:

Place of Work: The study shall be conducted in the Department of Morbid Anatomy and Histopathology at University of Health Sciences, Lahore.

Specimens: Fifty subjects will be selected from Mayo, Jinnah and Lahore General hospitals, using the inclusion / exclusion criteria of colorectal carcinoma.

Laboratory processing: All specimens will be fixed in 10 % formalin. Grossing will be done and recorded. Paraffin embedded blocks will be made from each biopsy specimen and one from main tumour mass, three from transition zones and two from excision margins. The blocks will be examined for microscopic morphology. Slides from each block will be stained with H & E, Giemsa, Toluidine blue, Mucicarmine and HID-AB for morphological examinations**.** There will be a panel of three pathologists to assess the morphology independently. In case of difference of opinion, two similar results out of the three will be considered final. I will be a part of this process from the collection of cases till final recording of the results. All the results will be recorded in the relevant proforma (attached) and compiled.

Gross Examination & Blocks Of Tissue:



1,2,3,4 : Sections from tumour region including apparent transitional zone

5,6 : Sections from Paratumour region

7,8 : Sections from Proximal & Distal ends

9 : Sections from Lymphnode (If any)

Estimated cost of project

Sr. No	Chemical/Reagents	Quantity	Cost (Estimated)
1	Tolouidine blue Stain	50 grams	2600
2	Giemsa stain	500 ml	5000
3	Ferric chloride	1 pack	1650
4	N,N-dimethyl-meta-phenylenediamine dihydrochloride	25 Grams	23500
5	N,N-dimethyl-para-	25 Grams	25000

	phenylenediamine dihydrochloride		
6	Mucicarmine Staining kit	01 kit	12000
7	Alcian blue	01 kit	6000
Total Pak. Rs:			**75750**

6.7 Morphological Patterns of Premalignant Conditions in Association with Carcinoma Breast

Introduction: Breast cancer is the most common female malignancy world-wide with the highest incidence in US and other affluent countries. Over the last 20 to 30 years a trend of increasing incidence has been observed in less resource countries, especially Pakistan in which one in every nine women is suffering from carcinoma breast. Among other known risk factors, pre-malignant breast lesions have a great importance because these act as precursor lesions.

Best characterized pre-malignant breast lesions are usual ductal hyperplasia, atypical ductal hyperplasia, atypical lobular hyperplasia, ductal carcinoma in situ and lobular carcinoma in situ. It is suggested that the breast cancer evolves in a linear progression through sequential stages of hyperplastic benign breast lesions with and without cellular atypia to carcinoma in situ, and then to invasive carcinoma.

The aim of study is to look for various patterns of pre-malignant breast lesions in association with cancer breast, so as to improve our assessment of risks that will eventually help therapeutically in order to prevent progression into invasive cancer.

Materials and Methods:

Study Design: Cross sectional analytical study.

Sample Size: 50 mastectomy specimens

Methodology: 50 subjects will be selected from Mayo hospital, using the inclusion / exclusion criteria, undergoing relative surgeries on clinically diagnosed cases of Breast carcinomas.

Socio-demographic information (name, age, sex, address, family history) will be obtained along with relevant clinical information. All the information will be collected on specially designed Proforma.

All specimens will be fixed in 10 % formalin. Grossing will be done and recorded. Paraffin embedded blocks will be made from each specimen. The slides will be stained with H & E and then examined for microscopic morphology. There will be a panel of three pathologists to assess the morphology independently. In case of difference of opinion, two similar results out of the three will be considered final. Not more than five cases will be reported per day. I will be part of this process from collection of cases till final recording of the results. All the results will be recorded in the relevant Proforma.

Estimated Cost of the Project: Total cost of project (Approximately): 60,000/-

7. HUMAN GENETICS & MOLECULAR BIOLOGY

7.1 The Heritability and Phenotypic Pattern of Mandibular Prognathism in Pakistan.

Introduction: Mandibular prognathism (MP) is a craniofacial anomaly which gives a heterogeneous presentation. Mandibular prognathism is a polygenetic familial disease, which is inherited as an autosomal dominant trait with incomplete penetrance. Affected people usually ignore MP until abnormal mandibular rotation and growth create functional problems. As it shows incomplete penetrance and poly genetic so different genes when contribute in different fashion gives a presentation from mild to severe. It is common in Asian population like Japanese, Korean and less in European population. Even in Pakistan MP is quit common but its frequency in our multiethnic population is not known. This study will be helpful to identify the frequency of MP in Pakistani population. Frequency of this trait is still unknown in many populations due to lack of interest and research tools in that field. In Pakistani population no study has been done on the subject. The findings from this population study would be applicable to familial cases to aid in future genetic analyses, such as genotyping and linkage studies.

Material & Methods:

Study Design: Analytical cross sectional study.

Sample Size: The study will consist of 50 probands and their extended families.

Methodology: Fifty subjects will be selected from the institute of de Montmorency college of Dentistry, using the exclusion and inclusion criteria. A consent form will be signed by the proband and their relative to participate in the study (Annexure A). Socio-demographic information (name, age, sex, address, family history) will obtain along with relevant clinical and radiological information. All the information will be collected on specially designed Proforma.

The facial pictures will be taken. Lateral cephalomatric analysis will be done by tracing on standard acetate paper (17.5 x 17.5 cm and 0.07 mm thickness) with a 0.3 mm graphite mechanical pencil, transparent ruler to the nearest 0.5 mm, protractor to the nearest 0.1º, template and adhesive tape. All tracings will be done on a light illuminator. The cranial and facial structures will be outlined and the ANB, SNA and

SNB angles will be measured. Not more than 2 cases will be reported per day. All the results shall be recorded in the approved Proforma.

National Importance: Information about Mandibular prognathism, which causes multiple aesthetic and functional complications, is not available in the literature. This study will pave the way in controlling this heritable disorder through genetic counseling and modify the treatment modalities.

Estimated Cost of the Project: 0.125 million

7.2 Genetic and Pathophysiological Role of Coupling Factor 6 in Coronary Artery Disease among High Risk Profile Families

Introduction: Coronary artery disease (CAD) refers to coronary insufficiency caused by atherosclerosis leading to myocardial ischemia and infarction. Despite much investigation into cause, mechanism of disease progression and risk factors its incidence is relentlessly on the rise. As compared with other illnesses CAD causes greater loss of life and economic burden. It is reported that by 2020 it might attain a magnitude of epidemic proportions. The events occurring during pathogenesis of CAD include endothelial dysfunction, inflammatory changes and atheroma formation. Normally vascular endothelium itself plays a role in regulation of blood flow by virtue of its glycocalyx component and prostacyclin formation. Prostacyclin in turn is responsible for vasodilatation and inhibition of platelet aggregation by antagonizing thromboxane A2, thrombosis and plaque formation. Multiple risk factors are associated with the pathogenesis of disease. In addition to conventional risk factors, recently a substance coupling factor 6 has shown an association with coronary artery disease. It is a vasoactive peptide, derived from ATP synthase, released from endothelial cell surface and mitochondrial membrane, causing vasoconstriction. It also inhibits cytosolic phospholipase and availability of arachidonic acid thus suppress the formation of prostacyclin. It also inhibits NO synthase and attenuates NO (vasodilator) synthesis. Recent studies have shown increased plasma level of CF6 in CAD. As a positive family history of CAD is one of the conventional risk factors of the disease, the present study is planned to investigate and explore the role of CF6 in patients with CAD (diagnosed on angiogram findings) and in their families, both horizontally(siblings) and vertically (in parents and offspring). At molecular level CF6

gene polymorphism studies have been planned to find the role of inheritance and genes in pathogenesis of CAD. The observations will be used to chart the pedigrees and find out the relationship of consanguineous marriages with the disease and further elaborate the role of family history (inheritance).A total of 200 subjects will be studied.

Objective: To evaluate the efficacy of CF6 as an early marker of coronary artery disease and its relationship with CF6 gene polymorphism.

Data Collection Techniques: Levels of CF6 will be estimated in plasma by RIA and CF6 gene polymorphism by using PCR technique and gene sequencing.

Data Analysis: The data will be analyzed through the statistical package for the social sciences 18.0 (SPSS).

National Importance

1. This study would signify the role of coupling factor 6 in predicting future cardiovascular events due to coronary artery disease and assessing the present status and its role in CAD risk inheritance.
2. It would help in decreasing the morbidity and mortality rate of coronary artery disease.
3. It would also help us in understanding the pathophysiology and pathogenetics of CAD.
4. It will also achieve the national goal of control and prevention of CAD through genetic counseling.

Budget: PKR 1.62 million

7.3 New Locus Involved In Autosomal Recessive Congenital Cataract in Affected Families from District Peshawar.

Introduction: A cataract is a clouding of a part of the eye known as the crystalline lens. The lens works with the transparent cornea, to focus light on the retina at the back of the eye. The lens consists mostly of water and protein. When the protein clumps up, it clouds the lens. When the lens becomes cloudy, or cataractous, light cannot pass to the retina properly, and vision is blurred and decreased. The word

“Cataract” comes from the Greek word “Waterfall” and was named for clouded opaque lens. Cataracts remain a leading cause of blindness worldwide accounting for 42% of all blindness. Lens with cataract is removed and an artificial intraocular lens (IOL) is placed inside the eye. Approximately one third of these cases have a familial origin. Hereditary cataract can be autosomal dominant, autosomal recessive or X-linked. 75% of the cases of congenital cataract are of autosomal dominant type. Less commonly, the inheritance may be autosomal recessive. Congenital cataract can be syndromic or non-syndromic. There are large variety of chromosomal and dysmorphic syndromes, in which the child will have a high risk of having congenital cataract. Different syndromes associated with congenital cataract are Down’s syndrome, Cataract-Dental Syndrome and cerebro-oculofacial skeletal syndrome.

Materials and methods

Sample size: The number of subjects to be enrolled has no logical upper limit, but will be at least 50 families affected with hereditary cataract.

Study design: After identification of the families’ ophthalmological examinations of patients will be carry out. After this blood samples from parents, affected and normal siblings will be collected. DNA is extracted from blood samples and amplification is performed by microsatellite markers by PCR. Screening of families for linkage will be performed to identify the known recessive cataract loci. Unlinked families will be subjected to genomewide scan. The region of homozygosity will be identified and narrowing it down with the help of linkage data for the families.

Proposed budget: The cost on whole project will be about 1.248 million.

7.4 “Ecology and Genetics of Susceptibility of HCV RNA Virus Gene in Various Isonym Groups In Pakistan”.

Introduction: Hepatitis C virus (HCV) is an RNA virus that is associated with chronic infection in the majority of people infected. Chronic infection with HCV is the cause of significant morbidity and mortality worldwide and associated with a large spectrum of liver disease including cirrhosis and hepatocellular carcinoma. HCV is a blood-borne virus that is and always was the major cause of "transfusion hepatitis," which can develop in patients who are given blood or most blood products except for gamma-

globulin. Like all other under developing countries Pakistan has high prevalence of HCV. Thus there is a direct need to investigate the ecology and genetics of hepatitis C virus human susceptibility in Pakistan, where we have cultural consanguinity and susceptibility of human population.

The objectives of this study area are:

1) To determine and isolate the HCV RNA virus susceptibility gene in human population.
2) To determine variation of genetic susceptibility of HCV RNA virus in different isonym groups.

Subjects and Methods: Quantification of serum HCV-RNA and HCV genotyping will be studied in 50 probands, patients with chronic hepatitis C undergoing interferon treatment. Pretreatment serum HCV-RNA levels were quantified using competitive RT-PCR and compared to a quantitative RT-PCR assay based on co-amplification of HCV-RNA with a synthetic RNA standard. HCV genotyping was performed using a line probe reversed hybridization assay or direct solid-phase sequencing

Sample size: The number of probands will be 50, from whom vertical (i.e. parents and progeny) horizontal (i.e., sibs) relatives will be tested for susceptibility to HCV RNA virus. It means that from 10 to 15 relatives will be tested: in total from 500 to 750 individuals.

Methodology

Research Plan:

First Year:

1). Establishment of Molecular Biology Laboratory.

2). Purchase of supplies e.g. Glass ware and chemicals and etc.

3) Preparation of questionnaire as proband and his/her family history.

4) Field testing of questionnaire.

5) Collection of the blood samples from different ethnic groups in Pakistan.

6) Analysis of the samples as

a) For nucleic acid analysis.

b) Extraction of HCV RNA on a large scale so that they can be used till end of the project.

c) Preservation of Extracted RNA for a long term.

Second Year:

1) Amplification of HCV RNA.
2) Quantification of HCV RNA
3) Sequencing of HCV RNA samples.
4) Study of polymorphism of HCV RNA samples.
5) Genotyping studies of HCV RNA samples.
6) Analysis of sequencing of RNA (with the help of software).
7) Multiplex Amplification.

Third Year:

1) Computerization of the data.
2) Phylogenetic Analysis.
3) Statistical analysis.
4) Thesis writing.
5) Submission of final thesis.

National Importance: Information gleaned from this study about the genetics of HCV, which has become a national scourge, will be helpful in correct gene identification and treatment. This genetic study will also be of tremendous importance to delineate what percentage of population is susceptible to Hepatitis infection, which will help in sequencing new genes in our local community of Hepatitis C patients. This will lead the way in identification of susceptible individuals and the control of hepatitis C.

Estimated project cost: Rs. 1.58 million

7.5 Eco-genetics of non-syndromic recessive mental retardation in isolated tribes of Buner Pakistan

Introduction: Buner is a rural area with only a few urban settlements like Pir Baba and Swari towns. The district lies between 34º-11' to 34º-43' north latitude and 72º13' to 72º-45' east longitudes. The total area of the district is 1,865 sq. km. It is administratively divided into six Tehsils and twenty seven Union Councils. The total population of Buner district is 506,048. The number of males and females is 253,035 and 253,013, respectively; i.e. sex ratio is almost 100 %. The main tribes are: Yousafzai, Mandar, Syed, Gujar, Hindu and Sikh. Generally, the marriages are held

within tribe. Limited health facilities are available in Buner. Disabled persons constitute 2.9 % of the total population. Among them almost 55 % are males and 45 % females. Of disabled persons, 4.2 % are mentally retarded. Unfortunately, mental retardation is well known in populations where cousin marriages are common, as in rural areas of Pakistan, which puts a heavy psychological as well as financial burden not only on the affected families but also on society as a whole. Mental retardation (MR), a developmental disability, affecting 1 to 3 % of the general population is common in the United States, according to the Morbidity and Mortality Weekly Reports. However, the prevalence of MR has been reported as 7.6 per 1,000 people between the ages of 6 and 64 years.

The American Psychiatric Association defines Mental retardation as the combination of: (1) Tested IQ at or under 70, (2) Problems with learning and adaptation and (3) Symptoms that begins before 18 years of age.

Objectives:

The objectives of the present work are as follows:

Prevalence of Mental Retardation in isolated tribes of Buner Pakistan

Impact of consanguinity and environmental factors on Mental retardation.

Identification of a novel genes/loci causing non-syndromic recessive mental retardation (NSRMR), which will lead to the cure of the disease.

Subjects and Methods:

Study design: Random sampling from all the 6 Tehsils, 27 Union Councils and 56,227 households.

Sample size: About 2.0% (10,120) of the total population.

Methodology: It is planed to visit every 50^{th} house in each Union Council of each Tehsil. Thus 1124 houses will be surveyed for the number of inhabitants and their health status about whether or not anybody suffering from mental retardation. The data will be recorded on specially designed questionnaire. Families with two or more mentally retarded individuals will be selected. Family history, pedigree and blood/buccal swab samples will be collected personally by visiting the families. DNA will be isolated from the blood/buccal swab samples by standard methods. Microsatellite markers, spaced at 10-cM intervals, from ABI PRISM linkage-mapping

set version 2.5 will amplified by multiplex PCR, using standard protocols. Amplified markers will be electrophoresed on an ABI 3100 DNA capillary sequencer and will be analyzed with GENESCAN and GENOTYPER software. All family members will be screened and Haplotypes will be constructed to either include or exclude the linkage regions. The families that do not show linkage with known loci will be separated and subjected to genome scan to find out new loci or genes causing non syndromic recessive mental retardation.

National importance: This study will give us information about the environmental and genetic effect on the development of mental retardation which will help in the control and cure of the disease on national level.

Estimated project cost: Rs. 1.46 million

7.6 Identification of Deafness Loci in Isolated Ethnic Tribes of Dir Pakistan.

Introduction: Among the diverse sensory defects in human, deafness constitutes the major portion. Deafness is defined as partial or complete hearing loss that leads to impaired speech, language and effective communication skills. More or less 1 in 1000 infants is affected by severe or profound deafness at birth or during early childhood, i.e., the prelingual period. More than 60% of the cases of profound early-onset deafness are caused by genetic factors, which in most of the cases are due to single gene mutation. Though, the etiology of profound childhood deafness is also ascribed to some environmental factors. Of the total hereditary hearing loss, 30% is syndromic, while 70% of genetically determined cases are non-syndromic. According to Van Camp and Smith, the analyses of Pakistani consanguineous family pedigrees, besides sharing most of these mutations, have revealed 14 loci and 9 genes for the first time. Recessively inherited diseases are common in populations where cousin marriages are frequent, as in Pakistan. As a consequence of the unique socio-cultural practices in the population of Pakistan ca 60% of marriages are consanguineous, of which more than 80% are between first cousins. It is estimated that, the prevalence of profound bilateral hearing loss is 1.6 per 1000 in Pakistan and 70% of hearing loss arises in

consanguineous families. Hence local population provides a valuable genetic resource for mapping deafness loci.

In the background of the above information, it is proposed to study consanguineous families with multiple affected individuals to elucidate the molecular basis of deafness. Such families' yield high LOD score (Log of Odd Ratios) and hence are very suitable for identification of new loci and deafness genes. Ultimately identification of new loci / genes involved in hearing impairment will provide us a better understanding of sound transduction.

Enrollment of Affected Families: Families are identified through student profiles in the deaf schools. Pedigree construction and detailed history are taken to map out the mode of inheritance, after visiting the families. History will be taken meticulously from each family to curtail the presence of any abnormalities and environmental causes for deafness. Families would be questioned regarding skin pigmentation differences in the eye color, hair pigmentation, problems relating to balance, vision, night blindness, thyroid, kidneys, heart, diabetes, antibiotic usage, injury, infectious diseases, like meningitis etc. Hearing ability of affected will be accessed by audiometry, where possible. The above clinical information will help to establishing nature of deafness as either syndromic or non-syndromic.

Collection of Blood Samples: 10 ml blood samples of all affected individuals, their normal siblings, parents and grand parents are to be collected, by venipuncture. EDTA is used as an anticoagulant and blood will be stored at $4^{o}C$ for not more than a week before DNA extraction. 4 Buccal swabs are taken from those individuals who are reluctant of giving blood.

Plan of work and Methodology:

Enrollment of affected families

Collection of blood samples

DNA extraction

Exclusion of known *DFNB* loci

1.1 Genome scan

1.1 Refinement of candidate regions

Sequencing of the candidate genes

Sample Size: According to 1998 census the population of Dir is 1,368,000. So the expected total deaf population in this area is 136. However, because of high consanguinity the affected individual may exceeded from the expected number.Our sample will consist of 50 Probands and their family members, both in vertical and horizontal lineage.

National importance: This genetic study about deafness will help in developing genetic counseling strategies, paternal diagnostic procedures. Thus lessening the socio economic burden of the affected families as well as of the country.

Estimated Cost: Rs. 1.27 Million

7.7 Epidemiology and Prevalence of Breast Cancer in the District of Bahawalnagar, Punjab, Pakistan.

Introduction: Bahawalnagar is located between 20°-51° to 30°-22° north latitudes and 72°-17° to 73°-58° east longitudes. It covers an area of 8,878 sq km. Average annual rainfall is 119.4 mm. The total population of Bahawalnagar is 2,061,447. It has 5 Tehsils, 118 union councils and 1,444 villages. Average house hold size is 6.7 and the sex ratio is 107.4. The total number of females is 994036 which represent 48.2% of the total population. The number of females 15 years and above is 568,592 which represent 57.2% of total females. Total numbers of houses in the district are 307653. Health facilities in the district consists of 1 District Head Quarter Hospital, 4 Tehsil Head Quarter Hospitals, 10 Rural Health Centers,101 Basic Health Units, 34 Sub Health Units, 10 Civil Dispensaries, 3 T.B Dispensaries/ Clinics, 47 Rural Dispensaries and 7 M.C.H Centers. Generally people visit their nearest health facility from where they are referred to DHQ for further diagnosis and treatment.

Breast cancer, worldwide, is the second most common type of cancer after lung cancer and fifth most common cause of cancer death. In 2004, breast cancer causes

519,000 deaths worldwide. Breast cancer is about 100 times as frequent among women as among men, but survival rates are equal in both sexes.

Objectives: Unfortunately the prevalence of cancer particularly breast cancer is not available in Pakistan. To fill this lacuna it is important to do some work on population genetics / community medicine / eco-genetics and genetics epidemiology.

Subjects and Methods:
Study design: Random sampling from all the 5 tehsils, 118 union councils and 1,444 villages.
Sample size: 1.10% of the total population of the district.

Methodology: It is planed to visit every 25th house of the locality and will be surveyed for the number of inhabitants and their health status about whether or not anybody suffering from cancer particularly breast cancer. The data will be recorded on specially designed questionnaire.

National importance: About breast cancer, the second most common cause of cancer and the fifth biggest cause of death due to cancer, nothing is known about its prevalence in our population. This epidemiological community based study will help in providing us data for its management and control. #

Estimated project cost: Rs 0.521 million.

7.8 Ecology and Cytogenetic Studies in Couples Experiencing Recurrent Spontaneous Miscarriages.

Introduction: A miscarriage is one of the most frequent complications of human pregnancy. It is the expulsion or extraction from its mother of an embryo or fetus weighing 500 gms or less, in clinical terms it is usually the gestational age of 20 weeks or less. Spontaneous miscarriage affects 15 % of all the clinical recognized pregnancies. Recurrent spontaneous miscarriage (RSM) is the loss of three consecutives miscarriages at or before 20 weeks of gestation. The frequency of RSM in couples attempting to have a child is between 1-5 %. Parental chromosomal

aberrations represent 2-8 % of the causes of RSM. These parental chromosomal aberrations include balanced translocations either reciprocal or robertsonian and inversions. It is recommended by the American College of Obstetrics & Gynaecology as well as Royal College of Obstetrics & Gynaecology to karyotype both parents for chromosomal aberrations as a part of evaluating the causes of recurrent pregnancy losses.

The present studies aims to evaluate the prevalence and pattern of chromosomal defects in the local couples experiencing recurrent spontaneous miscarriages also the pattern of consanguinity in these couples. This would help in identification of one of the etiological factor of RSM, which in turn would be helpful in offering genetic counseling and prenatal diagnosis to the affected couples.

Material & Methods:

Study Design: Analytical cross sectional study.

Sample Size: The study will consist of 50 couples experiencing RSM.

Methodology: Couples experiencing recurrent spontaneous miscarriage will be identified from the Gynaecology units of the local public sector hospitals. Complete obstetric, gynecological, medical and family history of the couples will be taken and noted down in the pre-designed proforma. Informed consent will be taken from the couples to participate in the study. Blood will be drawn from both the partners in sodium heparin vacutainers. Peripheral white blood cells will be cultured for the analysis of chromosomes by G-banding technique.

National Importance: Cultural consigunity creating national problem of high problem of genetic maladies including infertility and recurrent miscarriages. Study will show us to guide the people for early diagnosis and genetic counseling.

Estimated Cost of the Project: Total Estimated Cost (PKR): 0.253 millon

7.9 Cytogenetic Aberrations Found in Children with Acute Lymphoblastic Leukemia (ALL).

Introduction: ALL is the most common form of Leukemia in children. Its incidence is highest at 3-7 years of age. Cytogenetic analysis is helpful in depicting different patterns of chromosomal abnormalities in infants, children & adults, which partly explains the different prognoses of these groups. ALL cases are stratified according to the number of chromosomes in the tumor cells (ploidy) and by specific molecular abnormalities. Hyperdiploidy cells have >50 chromosomes & generally have good prognosis where as hypodipliody cases carry poor prognosis. The most common specific abnormality of the childhood ALL is the t (12:21) (p13; q22) TEL-AML1 translocation. The frequency of Philadelphia chromosome translocation t (9; 22) increases with age & carries poor prognosis. Translocation of chromosome 11q23 involve MLL gene. Fluorescence in Situ Hybridization (FISH) analysis is more sensitive genetic test in which some cases with normal by conventional cytogenetic testing are found to have fusion genes & other genetic abnormalities. These molecular genetic changes also carry prognostic significance whether or not corresponding chromosomal change is present.

Aims & Objectives of the proposed Research are to identify chromosomal aberrations in the local patients of ALL & correlate the aberrations with severity of disease & treatment outcome.

Material & Methods:

Study Design: Analytical cross sectional study.

Sample Size: The study will consist of 50 children diagnosed with ALL.

Methodology: Bone marrow sample will be taken from the children already diagnosed with ALL, coming to children's Hospital Lahore. Samples will be cultured using the RPMT 1640 media for 24-72 hours. After harvesting the cultures, slides will be prepared & stained with Geimsa to ascertain & identify the chromosomal defects.

Estimated Cost of the Project: Total Estimated Cost (PKR): One Lac & Fifty Thousand only.

8. IMMUNOLOGY

8.1 Anti - CCP Antibodies: A Better Diagnostic Tool for RA

Introduction: The anti-CCP belong to a group of autoantibodies able to react with several citrullinated peptides on multiple proteins (flaggrin, vimentin, fibrin, alpha enolase), and for this reason, indicated as anti-citrullinated protein/peptide antibodies (ACPA). Citrullination is the post-translational modification of protein-bound arginine into the non-standard amino acid citruuline.Enzymatic conversion of arginine to citrulline is catalyzed by peptidylarginine (PAD) enzyme. In different cohorts of RA patients, various researchers have reported specificity of anti-CCP antibody testing between 95-98%.

Material & Methods:

Study design: Cross sectional

Sample size: 50-samples in each group of sero-positive and sero-negative RA patients

Methodology: A group of RA patients will be collected and they will be divided in to two i.e. sero-positive and sero-negative patients. Both of the groups will be screened for the presence of anti-CCP antibodies in their serum.

Aims:

a. Determine the sensitivity and specificity of anti-cylic citrullinated peptides antibodies in RA patients using ACR criteria as the gold standard

b. Compare the sensitivity and specificity of anti-cylic citrullinated peptides antibodies with rheumatoid factor (RF)

c. Find out the more reliable diagnostic marker for rheumatoid arthritis

Estimated Cost: Rs= 80000/=

8.2 Th1 and Th2 Cytokine Profile in Case of Unexplained Infertility

Introduction: Like other countries, infertility is one of the serious concerns of Pakistan as well. There are various causes attributed to infertility but quite a number of cases

remain undiagnosed. Literature discusses the role of cytokines in such unexplained infertilities. Cytokines are the proteins which are secreted in the body under various circumstances. The cytokines are broadly divided in to two groups' i. e. Th1 and Th2. Each of these groups contains a set of cytokines. It is well documented that different cytokines play important roles during various stages of the disease. Therefore, intentions are to select two cytokines from each group and find out their levels in the unexplained infertile females.

Material & Methods:

Study design: Cross sectional

Sample size: 80 patients with no definite reason for their infertility

Methodology: A group of female patients will be selected from Obstetric and Gynecology clinics of different hospitals of Lahore. These patients will be screened for the levels of cytokines of Th1 and Th2 groups.

Aim: Determine the levels of INF-gamma, IL-6 (Th1) and IL-10, IL-12 (Th2) in the serum of patients of primary infertility

Estimated cost: Rs= 90000/=

8.3 Study Immune Mechanisms Involved in the Pathological Manifestations of Type-I and Type-II Diabetes Mellitus

Introduction: Diabetes mellitus specially (type 2) is one of the leading causes of morbidity and mortality in the world. It affects about more than 170 million people each year worldwide. Diabetes is associated with many complications such as retinopathy, nephropathy, cardiovascular disease, etc. It is suggested that early steps in the pathogenesis of diabetic complications could be due to a breach in tolerance. During early stages antipericyte and anti endothelial cell antibodies have been found. Similarly, with the progression of disease, levels of various cytokines such as TNF-α, IL-8, soluble IL-2 receptor increases. There is also abnormal expression of HLA-DR and HLA-DQ antigens, deposits of immunoglobulins, T and B lymphocytes, activated complement components and monocytes at various sites in the body and retina is one of them. All these findings suggest that diabetes may be an immune mediated

disease. The purpose of the study is to divided diabetic patients in to different groups depending upon their clinical manifestations and find out the immune dysfunction in them.

Material & Methods:

Study design: Cross sectional

Sample size: 80 included 30 patients with type-1 and 30 with type-ll diabetes mellitus and 20 healthy controls.

Methodology: The study population will be divided in two groups according to the disease manifestations. Blood of these subjects will be analyzed with Calibur 4-color analyzer (BD) for the percentage of T regulatory cells, determination of levels of cytokines by ELISA method and their comparison with healthy controls.

Aims:

a. Select diabetic patients with various clinical menifestations

b. Find out the proportion of T-regulatory cells and cytokines in these patients

Estimated Cost: 85000/-

8.4 Prevalence of Antibodies against Helicobacter Pylori among Healthy Individuals

Introduction: The rate of infection with helicobacter pylori is not uncommon in our society and most of the cases remain undetected. The clinicians treat these cases as they are strongly suggestive of this disorder and interestingly a good number of patients do get better, this way. Like other diseases there is no data available on the prevalence of this infection as well. The idea is to get base line information on the rate of infection in healthy individuals with this organism in our own community. Statistically significant population will be selected from the healthy volunteers to obtain this information.

Material & Methods:

Study design: Cross sectional

Sample size: 80 healthy volunteers

Methodology: A group of healthy subjects will be selected from local community. Blood of these subjects will be screened for the prevalence of antibodies against *Helicobacter pylori* by ELISA technique.

Aim: Find out the prevalence of antibodies against *H. pylori* in healthy adult population

Estimated Cost: 80000/-

8.5 Association of Immune Status in Preterm Babies and Occurrence of Sepsis

Introduction: Since the immune system of the new born babies is still not developed, therefore there is a very strong correlation between the preterm babies and the occurrence of infection in them. In case of premature new born babies, this rate of infection becomes quite high. To get the clear picture of our society, this study has been planned. The blood of premature babies will be collected to find out the level of immunoglobulin in their serum and these babies will be followed for a period of six to find out the rate of infection among them At the same time a group a term babies will also be included in the study to be used as a control group.

Material & Methods:

Study design: Longitudinal study

Sample size: 80 subjects including 40 preterm babies and 40 term babies

Methodology: The study population will be divided in two groups i.e. 40 preterm and 40 term babies. Serum immunoglobulin levels will be determined by ELISA technique. The same subjects will be followed for a period of six months and frequency of sepsis in these subjects will be determined with the levels of various immunoglobulins.

Aims:

a. Determine the serum immunoglobulin levels in preterm babies
b. Repeat the serum immunoglobulin levels of the same babies at six month interval

Estimated Price: 75000/-

8.6 Comparison of Immunological Markers in the Cord Blood of Preterm and Term Babies

Introduction: Newborns are more prone to the bacterial and viral infections as compared to young adults. The likelihood of infection is increased if the new is premature. It is suggested to study the level of immunoglobulin, T , B and NK cells in the pre term and term babies. Cord blood will be drawn from babies to check their parameters.

Material & Methods:

Study design: Cross sectional

Sample size: 80 subjects including 40 preterm babies and 40 term babies.

Methodology: The study population will be divided in two groups' 40 preterm babies and 40 term babies. Blood samples will be analyzed on Calibur 4-color analyzer (BD) for the determination of T, B and NK cells. Serum immunoglobulin levels will be determined with ELISA technique.

Aim: Determine serum immunoglobulin, T and B cells in the cord blood of preterm and term babies

Estimated Price: 95000/-

8.7 The Effect of Honey Ingestion on Total IgE and Symptoms Score in Allergic Rhinitis Patients

Introduction: Honey is a natural product, and it is found in abundance in Pakistan. There are many varieties of natural honey available in the market. Honey had been documented as a good anti-inflammatory product and some of its kinds have been recommended by FDA to be used as medicine.Considering various roles of honey, the following two projects are suggested. Allergic rhinitis is a very common disorder which is related to pollens and it shows seasonal variation. Blood samples will be collected from a group of allergic rhinitis patients and their serum IgE level will be performed.

These patients will be advised a particular brand of honey to be used in addition to their regular medicines, these patients will be followed for a period of three months. After three months again their blood sample will be collected to find out the serum IgE levels.

Material & Methods:

Study design: Cross sectional

Sample size: 80 subjects including 60 allergic patients and 20 healthy controls.

Methodology: ELISA will be performed to determine the IgE levels for both groups.

Aims:

a. Determine the effect of honey in the treatment of allergic rhinitis

b. Determine the magnitude of total IgE reduction after honey treatment

c. Determine the magnitude of allergic rhinitis symptoms improvement with honey treatment

Estimated Price: 70000/-

8.8 Effects of Adjuvant Honey Therapy on Disease Activity, Lymphocyte Function and Lymphocyte Subsets in SLE Patients

Introduction: Systemic lupus erythmatosus (SLE) is a common autoimmune disorder. Its occurrence is nine times more common in females as compare to male population. Although exact etiology of this disorder is not know, but in the literature there are multiple reasons for that. One thing is for sure that in this disorder there are a number of site in the body where the process of inflammation is going on. A group of SLE patients will be selected and their blood samples will be collected to find out the base line levels of various cells. These patients will be advised a particular brand of honey to be used in addition to their regular medicines, these patients will be followed for a period of six months. After this period again their blood sample will be collected to find out the changes in the parameters which were done at the beginning of the treatment.

Material & Methods:

Study design: Longitudinal study

Sample size: 60 SLE patients will be divided in to two groups. Half of the patients will be put on the honey along with their regular medicine. Second half of the patients will receive their regular medicines only. These patients will be followed for a period of six months and they will be repeated for the tests again.

Methodology: Blood samples of both groups will be analyzed to find out the base line levels of various cells on flowcytometer.

Aims:

a. Compare the effects of adjuvant honey versus conventional therapy alone on T lymphocytes in SLE patients

b. Compare the effects of adjuvant honey therapy versus conventional therapy alone on B lymphocytes in SLE patients

c. Compare the effects of adjuvant honey therapy versus conventional therapy alone on SLE Disease Activity Index score in SLE patients

Estimated Price: 95000/-

8.9 Frequency of CD4+CD8+ T Cells in the Blood of Tuberculosis Patients and Normal Healthy Subjects

Introduction: Tuberculosis (TB) is a common and often fatal infectious disease caused by mycobacteria, mainly *Mycobacterium tuberculosis*. It is the leading cause of death associated with infectious diseases globally. The correlation between TB and human immunodeficiency virus (HIV) has contributed to a significant increase in the worldwide incidence of tuberculosis. The interaction of T cells with infected macrophages is central to the protective immunity against *M. tuberculosis* and depends on the interplay of cytokines produced by each cell. IFN-γ has got an important role in the regulatory and effector phases of the immune response to *M. tuberculosis*. T lymphocytes are broadly divided in to T helper (CD4) and T suppressor (CD8) cells. Both of these cells are important for the normal functioning of the body.

The proportion of CD4 and CD8 cells is disturbed in different diseases and HIV infection is an example of it. This study has been designed to highlight the role of various T cells in tuberculosis and for this purpose a group of normal healthy individuals will also be included in the study.

Material & Methods:

Study design: Cross sectional

Sample size: 40 subjects including 40 tuberculosis patients and 40 normal healthy subjects.

Methodology: A group of tuberculosis patients will be selected from Gulab Davi Hospital Lahore. Blood cells of these subjects will be analyzed with Calibur 4-color analyzer (BD). The percentages of CD4 and CD8 T cells will be determined in their blood and it will be compared with health subjects.

Aim:

a. Find out the proportion of CD4 and CD8 T cells in the blood of tuberculosis patients

b. Determine the significance of different subsets of T cell population in tuberculosis patients by comparing it with the healthy subjects

Estimated Price: 100000/-

8.10 Establishment of Normal Ranges of CD3+, CD4+, CD8+, B19+ and CD56+ Lymphocytes in Selected Pakistani Population

Introduction: In the literature it is documented that there is geographical variation in the normal values of different parameters. Therefore, Pakistani population may have different levels of $CD3^+$, $CD4^+$,$CD8^+$, $B19^+$ and $CD56^+$ lymphocytes than the European or American populations due to many factors such as diet, environment, difference in there hormonal levels, habitual differences, differences in there life style etc. By

finding the normal values of our population will help to clear the confusion of making a proper diagnosis because since to date we depend upon the normal values of European countries. Further, the physician will be in a better position to advise proper treatment.

Material & Methods:

Study design: Cross sectional

Sample size: 100 healthy volunteers.

Methodology: Blood sample of these subjects will be analyzed on Calibur 4-color analyzer (BD) to find out the levels of various cells to establish a normal range of these cells for local population and compare this data with other countries.

Aims:

a. Find out the level of various cells in our population

b. Compare these levels with the known values from other countries

Estimated Price: 100000/-

8.11 Acute and Long Term Alterations in Immunological Factors and Cytokines in Rheumatic Fever – Rheumatic Heart Disease

Introduction: Children with acute rheumatic fever and carditis showed an increase in serum IgG, IgA and antistreptococcal antibodies during the acute stage. Lymphocyte transformation responses to phytohaemagglutinin and streptococcal antigens were reduced but this was due to a serum suppressor effect. After recovering from acute rheumatic fever a lymphocytosis and an increased lymphocyte blastogenic response to streptococcal antigen were found. T-cells, T-helper cells and T-suppressor cells showed some changes in acute rheumatic fever. In rheumatic fever, there is a predominant involvement of vascular endocardium and hence the blood supply to the

endothelium and myocardium will decrease affecting the heart muscles severely. The changes in the levels of different cytokines like IL-12 may be helpful in the early diagnosis of the suspected patients of the rheumatic arthritis.

Material & Methods:

Study design: Longitudinal study

Sample size: 30 subjects with rheumatic fever and they will be followed for three months

Methodology: Specific sets of lymphocytes such as T, B, and T regulatory cells will be determined by flowcytometer. ELISA will be performed to determine the level of cytokines. Both lymphocyte population and cytokines will be determined at the time of diagnosis and after three months interval.

Aims:

a. Investigate the role of immunological factors (specific lymphocyte subsets for NKC, regulatory lymphocytes, T and B cells) in the development of RF and its complication

b. Investigate the role of specific cytokines: IL-2, IL-17, and IL-23 in the development of RF and its complication

Estimated Price: 85000/-

8.12 Comparison of Rate of Apoptosis in the Lymphocytes of SLE Patients with the Normal Healthy Controls

Introduction: Systemic lupus erythmetosus (SLE) is a common autoimmune disorder. It is documented that there are Patients with SLE suffer from various immunological abnormalities. Apoptosis plays a major role in immune regulation and there is dysfunction of apoptosis in patients with SLE. Defect in apoptotic process may contribute to abnormal T and B cell function, maturation of antibody responses. Study

will be carried out to determine the rate of apoptosis in patients with SLE and then it will be compared with healthy individuals.

Material & Methods:

Study design: Cross sectional

Sample size: 60 subjects including 40 SLE patients and 20 healthy controls.

Methodology: Blood samples of both groups will be analysed on flowcytometer to determine the rate of apoptosis.

Aims:

a. Find out SLE patients at various stages of disease

b. Compare the rate apoptosis of lymphocytes from these SLE patients with the normal controls

c. Relate this comparison to the clinical and immunological data

Estimated Price: 70000/-

8.13 Frequency of CD4+CD25+ T Regulatory Cells in Males versus Females

Introduction: Autoimmune diseases are more prevalent in females than in males. The precise reason for this finding is not known but it appears to be partly hormone dependent. Because immune cells express estrogen and androgen receptors, sex hormones may act directly on Ag-specific T cells to alter their functions. The level of CD4+CD25+ T regulatory cells decreases in the SLE patients as compared to the normal population. There is a defect in Treg development,maintenance or function. It has been associated with several human autoimmune diseases including Systemic Lupus Erythematosus (SLE). Some studies suggest that sex steroids induce immune deviation from a Th1 to a Th2 phenotype; a subset of CD4 + cells called CD4 + CD25 + regulatory T (Treg) cells that expresses Forkhead box P3 (Fox P3) can control autoimmune responses and estrogen modulates the expression of Fox P3 mRNA. Therefore, it is planed to carry out a study to find out the level of these cells in our population.

Material & Methods:

Study design: Observational, Cross sectional

Sample size: 90 Health subjects.

Methodology: The study population will be divided in two groups of age matched males and females. Blood cells of these subjects will be analyzed with Calibur 4-color analyzer (BD). The percentages of T_{reg} in these groups will be determined and compared.

Aims:

a. Find out the level of T-regulatory cells in our population

b. Correlate the findings of this study with the predisposition of females towards autoimmune disorders

Estimated Price: 100000/-

9. MICROBIOLOGY

9.1 Prevalence and Genotyping of MDR and XDR-Mycobacterium Tuberculosis in Lahore region.

Introduction: Tuberculosis remains a major cause of morbidity and mortality causing two million deaths worldwide. Pakistan with population of 141 million has the seventh highest tuberculosis rate despite the widespread BCG vaccination.
The objective of the study is to conduct a multicenter study to estimate the overall disease burden and prevalence of the MDR and XDR tuberculosis in Lahore District.

Materials and Methods
Study Design: Observational Cross Sectional

Sample Size: 2000 sputum samples

Methodology: We will evaluate all isolates of *M. tuberculosis*, in which drug susceptibility testing will be performed at our institution using BACTEC MGIT 960, for multi and extensive drug resistance. The genotyping of MDR and the XDR-TB isolates will be analyzed by spoligotyping using commercially available kit according to the manufacturer's protocols. The spoligotypes will be scanned and analyzed using Bionumerics software, version 2.0 (Applied Maths, Kortijk, Belgium).

Estimated Cost: - 4,000,000

9.2 Molecular Epidemiology of Metallo-Beta-Lactamase Producing Pseudomonas aeruginosa

Introduction: *Pseudomonas aeruginosa* is an important bacterial pathogen most frequently responsible for nosocomial infections, especially in immuno-compromised patients. Carbapenems, are potent agents for the treatment of infections due to multidrug resistant *Pseudomonads.* Carbapenemases belong to two major molecular families, distinguished by the hydrolytic mechanism at the active site. Carbapenemases are members of the molecular class A, B, and D. Class A and D enzymes have a serine-based hydrolytic mechanism (i.e. Serine Carbapenemase), while class B enzymes are metallo-β-lactamases that contain zinc in the active site.

Catalytic activity of MBL depends on zinc ions, and is lost if it is chelated with EDTA. The metallo-β-lactamases belong to the IMP, VIM, SPM, GIM, and SIM families.

Materials and Methods

Study Design: Observational Cross Sectional

Sample Size: 5000 *Pseudomonas aueroginsa* isolates

Methodology: Screening of MBL will be done by phenotypic method of disk potentiation test and Molecular characterization will be done using RT-PCR and PFGE (pulse field gel electrophoresis).

Estimated Cost: - 50, 000, 00/

9.3 Molecular Characterization of Extended Spectrum Beta Lactamases (ESBL) in Pakistan

Introduction: ESBLs are beta-lactamases that hydrolyze extended-spectrum cephalosporins with an oxyimino side chain. These cephalosporins include cefotaxime, ceftriaxone, and ceftazidime, as well as the oxyimino-monobactam aztreonam. There is no data available in Pakistan regarding molecular characteristics of ESBLs. These belong to Ambler class A, C and D.

Materials and Methods

Study Design: Observational Cross Sectional

Sample Size: 8, 000 Enterobacteriaceae isolates

Methodology: Screening of ESBL will be done by phenotypic method of combine disk and double disk diffusion method and Molecular characterization will be done using RT-PCR and PFGE (pulse field gel electrophoresis). The objective of the study will be to determine whether it is chromosomal or plasmid mediated as well as comparison of genotypes with internationally existing enzymes.

Estimated Cost: - 60, 000, 00

*9.4 **In vitro Antibiotic resistance and** Genotyping of Helicobacter pylori.*

Introduction: The increasing incidence of *H. pylori* infection, its strong association with MALT and growing resistance against the known drug regimens requires the prevalence and susceptibility pattern to various drugs undertaken.

Materials and Methods

Study Design: Observational Cross Sectional

Sample Size: 1000 gastric biopsy specimens

Methodology:

Collection of Biopsy Samples

Each biopsy specimen will be processed as per standard techniques for culturing, DNA isolation and sensitivity testing. Genotyping of the isolates will be performed using molecular techniques.

Estimated Cost: 500,000

9.5 Efficacy of Honey against methicillin resistant Staphylococcus aureus systemic infection in mouse model

Introduction: Methicillin resistant S*taphylococcus aureus* (MRSA) is a major human pathogen responsible for a number of diseases. It can cause serious clinical infections ranging from hospital acquired pneumonia to sepsis and even death. It has become increasingly prevalent worldwide since it was first reported in a British hospital.

Honey, a thick, sweet liquid made by the bee from the nectar of flowers, is one of the oldest known medicines. It is increasingly becoming part of modern medicine and has been approved by FDA for treatment of chronic skin infections, wounds and burns. Honey possesses a strong antibacterial potential that is primarily due its acidic pH, osmolarity, H_2O_2 and non-peroxide activity. Although honey is proved to be extremely successful in topical infections, its role in systemic infections like bacteraemia remains

to be determined. The present study is designed to evaluate the antibacterial potential of honey in bacteraemia induced by MRSA

Materials & Methods:
Study Design: Experimental Study

Animals: *Balb*/c mice between 8 weeks to 12 weeks of age, weighing 20-25gms will be purchased from National Institute of Health Islamabad and will randomly be divided into 4 groups:
1) Normal control (10 mice)
2) Experimental group (40 mice)
This group will be further divided into 4 subgroups each comprising 10 mice each.
3) Positive control (10 mice)
4) Negative control (10 mice)
Animals will be obtained with full health reports and will be housed under specific pathogen free conditions with controlled temperature, humidity, and light condition

Methodology: Culture of MRSA in a volume of 200µl mice will be given intravenously in Balb/C mice. Honey injections will be given through intravenous/intraperitoneal route. Blood samples will be drawn for evidence of bacteraemia. Various organs like kidney, heart, liver and spleen will also be taken out to determine effect of treatment with honey on their bacterial load.
The results will be analyzed using computer software program SPSS. Data will be analyzed with appropriate tests.

Estimated cost of the project: (200,000).

9.6 Detection and Identification of Enterohemorrhagic E. coli (EHEC) Strains of Serotype Ol57:H7 by Polymerase Chain Reaction (PCR) in drinking Water.

Introduction: The enterohemorrhagic *E. coli* (EHEC) strains of serotype Ol57:H7 causes hemorrhagic colitis, which may develop into life-threatening hemolytic uremic syndrome. We will use Polymerase chain reaction (PCR) in order to assess the presence Ol57:H7. PCR is a powerful tool to multiply a target molecule to detectable quantities. In the multiplex PCR method, two or more primer sets are used to

simultaneously amplify multiple target sequences. Many researchers developed multiplex PCR for the detection of the LT (heat-labile toxin), SLT-I (Shiga-like toxin) and SLT-II producing *E. coli.* Antibody- or DNA- based assays for identifying SLTs or bacteria-carrying SLT genes not discriminate O I57:H7 isolates from the numerous other serotypes that also produce SLTs enterotoxins.

Materials and Methods:

Study design: Cross sectional study

Sample size: Sample size will be: 200

Methodology: The samples will be processed by Membrane Filtration technique and the filters then will be placed on the Chrome agar and MacConkey agar simultaneously which has the ability to detect coliform and *E. coli* at the same time as they produce two different enzymes β-galactosidase and β-glucoronidase respectively. Coliform form pink coloured colonies and E. coli will form blue purple coloured colonies. These blue coloured colonies will further identified as follows;
The colonies will be sub-cultured on MacConkey agar and identified by API 20E. Serology will be done to identify EHEC OI57:H7. Then PCR will be applied in order to identify Shiga Labile Toxin (SLT) genes of EHEC.

Estimated cost of the project: 500,000

9.7 Prevalance and genotyping of Dengue Haemorrhagic fever.

Introduction: Dengue is a mosquito-borne infection that in recent decades has become a global public health concern. Dengue is found in tropical and sub-tropical regions around the world. Dengue haemorrhagic fever (DHF), a potentially lethal complication, was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. Dengue (DF) and dengue hemorrhagic fever (DHF) are caused by one of four closely related, but antigenically distinct, virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), of the genus *Flavivirus.* Antibodies produced by infection with one of the serotype do not protect from other serotypes.

Materials and Methods

Study Design: Observational Cross Sectional

Sample Size: 1000 clinically suspected cases of dengue fever

Methodology: The blood sample of clinically suspected cases of dengue fever will be collected. The dengue fever case will be confirmed in microbiology laboratory along with hematological picture. The serotypes of the virus will be determined. Molecular characterization of the serotype will be done to verify the existence of internationally known serotype or any newer one.

Estimated Cost: - 40, 000, 00/= Rupees

9.8 Management and control of nosocomial infections

Introduction: Nosocomial infections are infections which are a result of treatment in a hospital or a healthcare service unit, but secondary to the patient's original condition. Infections are considered nosocomial if they first appear 48 hours or more after hospital admission or within 30 days after discharge.

The most common nosocomial infections are of the urinary tract, surgical site and various pneumonias. Hospital acquired infections are an emerging problem in our tertiary care hospitals. Hospital acquired bugs are resistant to multiple antibiotics rendering all the first and second line drugs ineffective clinically which narrows the treatment options and thus leading towards a high rate of mortality. Hospital microbiology & molecular laboratories can help to detect resistant strains and thus can prevent the occurrence of outbreaks. The objective of the project will be to establish a hospital infection control committee and to conduct a project on hospital microbiology and devise different methods to reduce this alarming rate of nosocomial infections in the tertiary care hospitals.

Material and methods:

Study design: It is a case-control study/ observational type of study.

Sample size: 1000 specimens will be processed for this project. Specimens will be collected from hospital environment, staff's hands, in use disinfectants, patient therapy

equipments and patients themselves. Patient samples may be blood, urine, pus, wound swabs, ear/nose swabs and sputum.

Methodology: All clinical specimens will be identified by morphological appearance, cultural characteristics and Analytical profile index, 20 Enterobacteriaceae (API 20E) and 20 non Enterobacteriaceae (API 20NE). Antimicrobial susceptibility will be determined using disk diffusion method according to CLSI 2009. Molecular typing of the resistant strains will be done via PFGE.

Estimated Cost of the project: 2,000,000

10. MSC NURSING

10.1 Workplace Stress in Nursing in Tertiary Care Hospitals in Lahore

Introduction: Stress perception is highly subjective, so the complexity of nursing practice may result in variation between nurses in their identification of sources of stress especially when the workplace and roles of nurses are changing. Nursing provides a wide range of potential workplace stressors as it is a profession that requires a high level of skill, team work in a variety of situations, provision of 24-hour delivery of care and input of what is often referred to as emotional labor.

The aims of this study are to identify different type of workplace stressors for nurses working in tertiary care settings to address the sources of stress in healthcare and in particular to reverse the shortfall in nurse recruitment and retention and to introduce a participative style of management.

This study will be done to answer the following research questions:

- Is there commonality of sources of workplace stress for nurses?
- Are sources of workplace stress for nurses changing?
- Will recent organizational interventions introduced to reduce the sources of stress for nurses be effective.

Material & Methods

Study Design : Qualitative longitudinal exploratory study

Sample Size : Fifty participants from six tertiary care hospitals in Lahore

Methodology: Questionnaires will be devised and distributed among the participants at two points in time.

Estimated Cost of the Project: cost (PKR): 27,000

10.2 Family Experiences of Home Caring For Patients with Tuberculosis

Introduction: Tuberculosis is a problem of global significance, estimated to cause about 8 million new cases of disease and about 3 million deaths each year, more than half of which are in Asia. Most of the patients with tuberculosis need admission to the hospital because when they are diagnosed they already have developed extensive disease. As long as these patients remain in the hospital their condition improves, but when they are under home care their condition deteriorates. Due to the nature of communal life, it is a common practice to care for one's loved one at home. With the current burden of tuberculosis, although the community members are keen to care for their loved ones, people are more sympathetic to other chronic conditions, such as cancer.

Aims and Objectives:

1. Exploring and describing the experiences of family members who care for the patient with tuberculosis at home.
2. Developing guidelines for family members in caring for patients with tuberculosis at home.

Material &Methods

Study Design: Qualitative, phenomenological, explorative study design

Sample Size: In-depth phenomenological interview will be conducted of fifty families who care for the patient with tuberculosis.

Methodology: A qualitative, phenomenological, explorative, descriptive, and contextual design was chosen as the most appropriate for this study. This design allows for personal feelings to be shared without the restrictions and pressure of being objective. The population in this study will be included family members who are caring for patients with tuberculosis at home in the rural

communities of. A criterion for selection is based on the frequent return of patients back to the hospital immediately after discharge.

Estimated Cost of the Project: 35, 000

10.3 Nurses' Knowledge and Attitude for Assessing and Managing for Depression and Anxiety in Pakistan with Breast Cancer Undergoing Mastectomy.

Introduction: Depression and anxiety disorders are common psychiatric disorders in Pakistan, and their prevalence is high in patients with carcinoma of the breast. Depression and anxiety can affect the mortality and morbidity of these patients; thus it is important to screen for symptoms of anxiety and depression, especially in cancer patients in developing countries

Objective of this study is to examine the prevalence and risk factors for depression and anxiety in patients of breast cancer undergoing mastectomy.

Material & Methods

Study Design : Quantitative exploratory study

Sample Size : Twenty five diagnosed cases of breast cancer who have not received any treatment. Twenty diagnosed cases of breast cancer who have undergone treatment

Methodology: All patients admitted to oncology unit of a tertiary care hospital in Lahore for a planned mastectomy over a period of twelve weeks will be evaluated with clinical interview using Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria, Hospital Anxiety and Depression Scale (HADS), and Quality of Life (QoL) scale

Estimated Cost of the Project: cost (PKR): 30,000

10.4 Graduate Courses Nursing Student's Perception of the Hospital Learning Environment during Clinical Placements

Introduction: Nursing is an action profession and nurses learn by doing, so imparting the fundamental clinical skills must be a key component of courses leading to registration. The last five to seven years have seen widespread changes to nurse education in Pakistan but the clinical field remains an invaluable resource in preparing students for the reality of their professional role supporting the integration of theory and practice and linking the 'knowing what' with the 'knowing how'. The clinical-learning environment represents an essential element of nurse education that needs to be measured and warrants further investigations.

Objective of the study is to develop an innovative tool to assess hospital-learning environments. This study will examine under graduate student nurses perception of the hospital-learning environment during clinical placements together with the key characteristics of the students' preferred learning environment utilizing an established tools

Material & Methods

Study Design: A quasi-experimental, pre-post test design will be used for the study

Sample Size: The population will be consisted of thirty final year Bachelor of Nursing students from the three nursing colleges at Lahore. The students must be enrolled in the relevant clinical unit and to undertake a four week clinical placement

Methodology: All patients admitted to oncology unit of a tertiary care hospital in Lahore for a planned mastectomy over a period of twelve weeks will be evaluated with clinical interview using Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria, Hospital Anxiety and Depression Scale (HADS), and Quality of Life (QoL) scale

Estimated Cost of the Project: cost (PKR): 30,000

10.5 Nursing Assessment and Intervention for Fluid Restriction in Patient Undergoing Hemodialysis

Introduction: Noncompliance is a common problem in hemodialysis (HD) patients. Patients are asked to comply with medical advice that may disturb their normal routine. In addition to the dialysis sessions two to three times a week for many years, patients have to take many medications and adhere to diet and strict fluid intake restrictions.

Noncompliance is found in all aspects, but adhering to the fuid restriction is the most difficult aspect for most patients.One of the tasks of nephrology nurses is counseling the patients and helping them cope with their regimens, especially the fluid restriction. It would be very helpful for nurses to know how many HD patients suffer from thirst and how to prevent or treat thirst.

Objective of the study is to identify why thirst is a difficult concept to measure and study and to describe the relationship between thirst and interdialytic weight gain in hemodialysis patients.

Material&Methods

Study Design: Exploratory descriptive design

Methodology: The potential patient subjects will be identified, who will be then screened by using inclusion criteria. The interviewer will explain the study verbally to these eligible patients, and a written description of the study will be given to the patients and their caregivers. All of the nurses in the units will be asked to participate in the study after the purpose and procedures of the study have been explained to them. Those nurses who volunteer to participate will sign a written consent and self-report on the questionnaire. Both patient and nurse subjects will rate the perceived importance of each of the barriers listed in the questionnaire.

Estimated Cost of the Project: Total estimated cost (PKR): 27, 000

10.6 Communication Barriers Perceived by Patients and Nurses in Health Care Delivery

Introduction: Effective communication between patients and nurses is an important factor for patient satisfaction, treatment and patient compliance. Good communication may boost patient recovery, whereas poor communication can be distressing for both nurse and patient. Effective communication between nurse and patient is a critical factor in the patient's perceived quality care.

Hypothesis of this study is that there is an association between nurse patient communication and health outcome.

Aim of the study is to identify the barriers to effective communication in nursing (nurse, patient environment related) and to find out new communication strategies.

Material & Methods

Study Design: Non-descriptive exploratory design

Methodology: The potential patient subjects will be identified, who will be then screened by using inclusion criteria. The interviewer will explain the study verbally to these eligible patients, and a written description of the study will be given to the patients and their caregivers. All of the nurses in the units will be asked to participate in the study after the purpose and procedures of the study have been explained to them. Those nurses who volunteer to participate will sign a written consent and self-report on the questionnaire. Both patient and nurse subjects will rate the perceived importance of each of the barriers listed in the questionnaire.

Estimated Cost of the Project: cost (PKR): 27, 000

11. PHARMACOLOGY

11.1 Anti-Hyperlipidemic Effect of Berry Honey in Diet Induced Hyperlipidemic Rats

Introduction: Cardiovascular diseases are major health challenges caused by many factors that result into hyperlipidemia which can be primary or secondary to other conditions leading to abnormal lipid profile. To regulate normal lipid profile a lot of clinical trials as diet, surgery and drugs established. In spite of all that, we should focus towards traditional medicine treatment such as honey. Unfortunately, research documenting the effectiveness of honey for clinical conditions is scarce. On the other hand, medications for these conditions abound, rendering honey a less popular choice. Research into these areas is preliminary but potentially promising. This traditional treatment will be helpful to lift the blinds off to give its due recognition. So, we designed experimental study to investigate the antihyperlipidemic effect of Pakistani berry honey in patients with hyperlipidemia.

Study Design: The study will be carried out on 32 subjects. Eight rats with normal lipid profile fed on normal diet as control, eight rats with high cholesterol diet induced hyperlipidemia, eight hyperlipidemic rats with Simvastatin and eight hyperlipidemic rats with berry honey treatment will be included. The results of parameters will be analyzed to determine Symptoms and signs mainly qualitative and presented as descriptive statistics of frequencies and proportions. The outcome measures will include lipid profile as total cholesterol, LDL-C, HDL-C and triglycerides qualitatively and will be presented as proportions.
The study will provide an alternative method of treating hyperlipidemia and help in proper prognosis of hyperlipidemia as well as help in future identification and separation of antihyperlipidemic constituents of honey.

Estimated cost of the project: Rs 45,000-50,000

11.2 Effect of Date Palm Pollen (DPP) on the Spermatogenesis of Prepubertal Rats

Introduction: Delayed puberty may result in low self-esteem in boys, and in difficulty separating from parents due to apparent immaturity. Adolescents affected by delayed puberty may be treated as less mature than real age by adults and peers hence face difficulties in getting work due to apparent immaturity. Considerable evidence exists for the efficacy and safety of short courses of low-dose testosterone therapy for appropriately selected individuals. There is not yet sufficient evidence for the routine use of other therapies.

Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats. The present study is therefore designed to see the effect of Date Palm Pollen (*Phoenix dactylifera* L.) on the spermatogenesis of prepubertal rats with hopes to add a valuable contribution in advancement to the therapies for delayed puberty in males.

Study Design: In this study, 4-day old, twenty-four male albino rats will be divided into 3 groups, I, II, and III. Group I will serve as control, Group II and III will receive 120mg/kg and 240mg/kg of DPP suspension respectively, dissolved in 1 ml of distilled water, daily for 35 days starting at 4^{th} day of age. At the end of experimental period all the rats will be weighed and then sacrificed; testosterone levels will be measured in blood samples taken. Testes will be weighed, sectioned and then stained with haematoxylin and eosin. The histological findings will be recorded and interpreted. The seminal vesicles and ventral prostate will be removed for wet weight determination without removing the secretions from them. The liver will also be removed and weighed so that the weight of sex organs could be compared with an organ that is not dependent on sex hormones for growth.

If the postulated effects of *Phoenix dactylifera* L. pollen are validated by the present study, it may be used to promote spermatogenesis and treat the patients of delayed puberty with impunity.

Estimated cost of the project: Rs 40,000-45,000

11.3 Comparison of the Effects of Zingiber Officinale (Ginger) and Prednisolone on the Characterized Rat Model of Allergic Airway Inflammation

Introduction: Asthma is one of the most common chronic inflammatory diseases, affecting about 300 million people worldwide, and a total that is expected to rise by an additional 100 million mainly in children over the next 15-20 years. Asthma accounts for about one out of every 250 deaths worldwide and has profound health-care costs in terms of emergency room visits and hospitalizations. Glucocorticosteroids are the main stay of treatment in asthma despite of their disastrous adverse effects. In Pakistan most commonly used glucocorticoid for asthma treatment is the prednisolone. Ginger as a natural herb is renowned for a long time for its anti-inflammatory activity. So the present study is designed to compare the anti-inflammatory effect of prednisolone with the ginger on the rat model of allergic airway inflammation.

Study Design: Thirty two rats will be divided into four groups i.e. group I, II, III and IV. Group I will reserve as control, while II, III and IV will be immunized and challenged with ovalbumin to induce airway inflammation. Group III and IV will receive prednisolone at dose of 10mg/kg body weight and ginger 500mg/kg body weight through intra-peritoneal route for seven consecutive days. After euthanization, the anti-inflammatory effect of both will be compared by measuring delayed type hyper sensitivity, observing the changes in lung histology, presence of inflammatory cells in the blood, lungs and Broncho Alveolar Lavage Fluid (BALF). The mRNA expression of pro-inflammatory cytokines IL-4, IL-5 and IL-10 will be compared by RT-PCR.
If the study could prove that *Zingiber officinale* has anti-inflammatory effect on allergic airway inflammation comparable with prednisolone, we might avoid the side effects of prednisolone by replacing it with *Zingiber officinale.*

Estimated cost of the project: Rs 50,000-55,000

11.4 Reno-Protective Effect of Aqueous Extract of Carum Carvi (Kala Zeera) on Streptozotocin Induced Diabetic Nephropathy in Rats.

Introduction: Diabetic nephropathy is one of the major complications of diabetes. It occurs in 30-40% of diabetic patients. The early stages of diabetic nephropathy are characterized by an elevation of urinary albumin excretion, decline of glomerular

filtration rate and renal hypertrophy. The urinary albumin excretion progresses to overt proteinuria and finally results in end stage renal failure in diabetic patients. Caraway plant (*Carum carvi* L.), locally known as kala zeera, is a medicinal plant which is known to posses antibacterial, antiulcerogenic, antiproliferative, antioxidant and antihyperglycemic properties. Phytochemical investigation of caraway seeds revealed the presence of carvone, limonene, carveol, dihydrocarveol, thymol, beta caryophyllene and flavonoids. The reno-protective effect of *Carum carvi* is presumed to be due to its constituents, which are known to be responsible for lowering blood cholesterol and glucose concentration. Other reno-protective property of *Carum carvi* may be because of its antioxidant property, thereby reducing oxidative stress induced by diabetes.

Study Design: The study will be carried out on 32 male Wistar rats weighing. In this study, rats will be divided into four groups, each having 8 animals. Group-A will serve as normal control, group-B (diabetic control) while groups C and D will be experimental groups. Diabetes will be induced by single intraperitoneal injection of streptozotocin at 60 mg/kg body weight. Rats having fasting blood glucose over 280 mg/dl will be included in this study. Groups C and D diabetic rats (experimental groups) will receive aqueous extract of 30 mg/kg body weight and 60 mg /kg body weight of *Carum carvi* seeds, respectively. Blood samples will be taken at 0 day (when diabetes is confirmed) and after 60^{th} day. After that animals will be sacrificed under chloroform vapors and kidney will be taken out for microscopic examinations. In addition, following parameters will be studied: body weight, urine volume, total urinary protein, urinary albumin level, serum urea, serum creatinine, and Serum glucose.

If we could prove that *Carum carvi* seeds have reno-protective effect we could prevent the kidney damage and ultimately renal failure which occurs after diabetes.

Estimated cost of the project: Rs 40,000-45,000

11.5 Transdermal Drug Delivery System of Glipizid in Rats

Introduction: Type II diabetes is treated by oral hypoglycemic drugs. Sulfonylureas are the most commonly prescribed oral hypoglycemics, and among this class glibenclamide and glipizide are used routinely. The major action of sulfonylureas is to increase insulin release from the pancreas. All oral hypoglycemics have an inherent risk for severe hypoglycemia, which may be fatal in some cases. Various alternatives are being tested these days to overcome this problem. Transdermal drug delivery system is one of them.

This project is aimed to study the feasibility of transdermal drug delivery system of glipizide in diabetic rats with an objective of subsequent application of this technique to the human type II diabetics, to improve the compliance of the patients. Therefore keeping in view the expected advantages and feasibility of transdermal drug delivery system this study will be carried out to compare the oral versus transdermal administration of glipizide in the diabetes-induced rats.

Study Design:

Male Wistar rats will be divided into 2 groups each comprising of 24 rats. Group A will serve as control while group B as Diabetes-induced rats. Each group will be further subdivided into three subgroups each comprising of 8 animals:

$A_{1:}$ Control

$A_{2:}$ Oral glipizide treated

$A_{3:}$ Transdermal glipizide treated

$B_{1:}$ Control Diabetic

$B_{2:}$ Oral glipizide treated

$B_{3:}$ Transdermal glipizide treated

To induce Type-II diabetes, rats will be fed high-fat diet (58 % of calories as fat) for 2 weeks. After 2 weeks of dietary manipulation rats will be injected intraperitoneally with low dose of streptozotocin (35 mg kg^{-1}). Blood glucose levels will be measured after 24 hrs and animals with blood glucose levels > 250 mg/dl will be selected. Transdermal patches will be prepared according to the mercury substrate method. Animals in subgroups A_3 and B_3 will be treated with the transdermal patches. To subgroups A_2 and $B_{2,}$ Glipizide will be administered orally at a dose of 5 mg/kg in 2 % acacia suspension after overnight fasting. Blood samples will be drawn from the dorsal pedal vein at the interval of 0, 2, 4, 8,12,24,48 and 72 hours and blood glucose levels will be measured. For the untreated subgroups A_1 and B_1, after an overnight fasting, the glucose levels will be estimated at the similar intervals.

Estimated cost of the project: Rs 35,000-40,000

11.6 Aqueous Extract of Azadirachta Indica (Neem) has Hepato-Protective Effect on Alcohol Induced Hepato-Toxicity

Introduction: Alcohol abuse is major cause for liver disease in western countries. In USA there are estimated 25,000 deaths per year from cirrhosis, half of which are related with alcohol. Chronic liver disease is the tenth leading cause of death in USA. Alcohol and hepatitis C virus are synergistic in hastening the development of cirrhosis. Alcohol consumption is associated with alcoholic hepatitis, fatty infiltration of liver, accelerated progression of liver disease, higher frequency of liver cirrhosis, higher incidence of hepatocellular carcinoma and death.

Azadirachta indica popularly known as Neem is a medicinal plant that grows throughout greater parts of India, Pakistan and Burma. AI is known to posses' anti-inflammatory, anti-pyretic, anti-microbial, and anti diabetic properties. More than 135 compounds have been isolated; these are divided into two major classes, Isoprenoids and Non isoprenoids.

Study Design: In the study 4 groups of rats will be taken, each having 12 animals. Group 1 will serve as control, In group 2 alcohol toxicity will be induced, group 3 will receive 500mg/kg body weight of *Azadirachta Indica* leaf extract and group 4 shall receive 1g/kg body weight of *Azadirachta Indica* leaf extract. Blood samples will be taken for biochemical analysis and following tests will be done.

1. Serum Alanine Amino Transferase (ALT.)
2. Serum Aspartate Amino Transferase (AST)
3. Serum Creatinine Kinase (CK)
4. Serum Bilirubin

Liver will be examined both macroscopically and microscopically.

Documentation of this study will be a step in search of a natural source for reverting hepatic changes brought about by chronic intake of alcohol. Aqueous Neem extract can be commercially packed in the form of capsules, tablets and as syrup.

Estimated cost of the project: Rs 45,000-50,000

11.7 Antihyperlipidemic Effects of Olea europea (Olive oil) in Comparison with Atorvastatin

Introduction: Coronary heart disease, or atherosclerotic, is the number one killer disease in the United States and worldwide. In Pakistan, this is the third biggest killer

disease and is on the surge. Most patients with coronary artery disease have some identifiable risk factor. Hypercholesterolemia and other lipid abnormalities are important modifiable risk factors for atherosclerosis causing coronary artery disease. Risk increases progressively with higher levels of low-density lipoprotein (LDL) cholesterol and declines with higher levels of high-density lipoprotein (HDL) cholesterol. To regulate normal lipid profile, lots of drugs are already in the market. Because of the concerns about the side effects of conventional medicines, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. The miraculous properties of olive oil are mentioned in The Holy Quran and The Bible. Unfortunately, research documenting the effectiveness of olive oil for clinical conditions is scarce. The present study is designed to compare the antihyperlipidemic effect of olive oil and Atorvastatin in hyperlipidemia induced rats.

Study Design: The study will be carried out on 32 rats. Eight rats with normal lipid profile on normal diet as control, eight rats with high cholesterol diet induced hyperlipidemia, eight hyperlipidemic rats with Atorvastatin and eight hyperlipidemic rats with olive oil treatment will be included. The lipid profile of all the groups will be compared after euthanization. Lipid profile will include total cholesterol, LDL-C, HDL-C and triglycerides levels in serum.

The study benefits include: To avoid the side effects induced by Atorvastatin and help in future identification and separation of antihyperlipidemic constituents of olive oil.

Estimated cost of the project: Rs 45,000-50,000

11.8 Molecular Mechanism Involved in the Inhibition of T-cell Proliferation by Black Seeds in Allergic Asthma

Introduction: Recent era has witnessed an increased prevalence of allergy and asthma. This requires more attention and effective therapies since the current therapeutic approaches have high side effects. Search for novel treatments has significantly advanced in recent years due to increased prevalence of allergy and asthma. This attention has led to the exploration of alternative medicines with particular interest in plant products.

The black seeds, from the *Ranunculaceae* family, have been traditionally used by various cultures throughout the world as a natural remedy for several ailments. In this

study, we aim to evaluate the role of black seed oil as an immunomodulator in a rat model and find out the mechanism by which black seed oil can inhibit T-cell proliferation.

Study Design: The 24 Wistar rats will be divided into three different groups. One group given phosphate buffered saline intraperitoneally, and challenged intranasally with PBS, other sensitized intraperitoneally and challenged intranasally with Ova-albumin. The last group will be sensitized intraperitoneally and challenged intranasally with Ova-albumin and treated with black seed oil intraperitoneally. Broncho-alveolar Lavage Fluid (BALF), serum, lung and the spleen tissue will be analyzed for the presence of allergic inflammation by checking the infiltration of inflammatory cells and nitric oxide production in BALF; histopathological lesions in lung tissue; levels of IgE and IgG1 production in the serum by ELISA; pro-inflammatory cytokine IL-4, IL-5 and IL-13 mRNA expression levels in lungs; OVA-specific T-cell proliferation in spleen measured by ELISA;. In spleens the expression of CD80, CD86, CD28 and CTLA4 will be measured by FACS.

The rats sensitized and challenged with Ova-albumin will develop allergic airway inflammation with a Th2 type of immune response. The group treated with black seed oil will exhibit a significant reduction in all the markers of allergic inflammation including the T-cell proliferation. To find out the exact mechanism by which T-cell proliferation was inhibited spleen cells suspension will be performed FACS to check the expression of CD28, CD80, CD86 and CTLA4. BSO might suppress the expression of CD80 or CD86 on APC's or CD28 on T-cells. It may also increase the expression of CTLA4 which gives negative feed back to decrease the T-cell activation and proliferation.

Following are the expected results:

1- Black seed oil ameliorates allergic airway inflammation by inhibiting all the markers allergic airway inflammation including inflammatory cell invasion in lungs, lungs lesions, nitric oxide level in serum, IgE and ova-specific IgG, Th2 cytokines expression.

2- The anti-inflammatory effect of BSO is due to the inhibition of activation and proliferation of T- lymphocytes.

3- BSO can block the receptors of CD80 or CD86 expressed on APC's, hence downregulating their expression.

4- BSO can inhibit the expression of CD28 molecule on T-cells

5- BSO may upregulate the expression of CTLA4 which might result in attenuation of T-cell activation and proliferation hence attenuating Th2 type inflammation.

Estimated cost of the project: Rs 80,000-100,000

11.9 Comparison of The Bioavailability and Bioequivalence of a Single Dose of "A" Tablet (Test Formulation, Generic Drug) With That of a Single Dose of "B" Tablet (Reference Formulation, Brand Drug) Under Fasting Conditions

Introduction: The rationalization of health care expenditures is a high priority for the governments of many countries in the industrialized world, and the introduction onto the market of generic drugs produces notable savings. When drug patents expire-6 to 10 years after registration in the European Union (EU)-generic drugs having the same formula as the brand-name drug can he marketed at a much lower price, as their manufacturers have not had to hear the costs of the original registration studies. In 2002, the Italian Ministry of Health saved an estimated 25 million as a result of the introduction of generic drugs. However, although generic formulations are always less expensive than the corresponding brand name drugs, they are not always as safe or effective. Concerns over potential therapeutic inequivalence have prompted many clinicians to question current regulatory requirements for both establishing bioequivalence, as well as product substitution. Use of generic drugs, which are bioequivalent to brand-name drugs, can help contain prescription drug spending. However, there is concern among patients and physicians that brand-name drugs may be clinically superior to generic drugs.

Study Design:

Using a two-treatment, two-period, two-sequence, randomized crossover design, test and reference formulations will be administered as individual single doses to 24 healthy male rabbits under non-fed conditions, with 4 days washout period between dosing. 17 blood samples will be drawn from each subject over a 12-hour period. Pharmacokinetic parameters, Cmax, AUC0-t, AUC0-infinity and Cmax/AUC0-infinity will be calculated from the plasma concentration-time data of each individual and during each period by applying non-compartmental analysis. Analysis of variance will be carried out using logarithmically transformed and non-transformed values of the

stated pharmacokinetic parameters. Data for test and reference formulations will be analyzed statistically to test for bioequivalence of the two formulations.

If we could prove that the test formulation "A" (Generic Drug) is bioequivalent to reference formulation (Brand Drug) and both formulations are well tolerated, then test formulation can be considered a pharmaceutically and therapeutically equivalent alternative to Branded drug which is sold at higher cost.

Estimated cost of the project: Rs 30,000-35,000

11.10 The Anti-Tubercular Effect of Allium Cepalinn (Onion) in Tuberculosis Induced Albino Rats.

Introduction: According to the World Health Organization, nearly 2 billion people—one third of the world's population—have been exposed to the tuberculosis pathogen. Annually, 8 million people become ill with tuberculosis, and 2 million people die from the disease worldwide. In 2004, around 14.6 million people had active TB disease with 9 million new cases. Tuberculosis is the world's greatest infectious killer of women of reproductive age. Multi-drug resistant strains of tuberculosis pose a serious threat in many third- and first-world countries. The aim of this case report is to describe a potential new method for treating those with primary pulmonary tuberculosis using phytochemicals.

Study Design: For this purpose we choose to prove the ant-tubercular effect of *Allium Cepalinn* in the rats. Briefly rats will be inoculated with suspension of Mycobacterium tuberculosis, bovine strain. In the other group alongwith inoculation the rats will be given the aqueous extract of *Allium Cepalinn*. On the 10^{th}, 20^{th}, 30^{th}, 40^{th} and 50^{th} days after inoculation the animals will be autopsied. The relative weight of the spleen and lungs will be determined. The internal organs will be examined with the naked eye and the lungs, liver, and spleen histologically. Lungs, spleens, and lymph nodes will be stained with hematoxylin and eosin (H&E) to evaluate histopathology, and modified acid-fast tissue stain to look for the presence of acid-fast bacilli (AFB). Representative samples of fixed lung tissue will also be evaluated with immunohistochemical stains

specific for either B or T lymphocytes. Lung and spleen tissue samples will be used for culture of MTB. Colonies will be counted. The mRNA expression levels of cytokines IL-2, IL-10 and TGF-B will be measured by RT-PCR in all groups.
If we could prove that aqueous extract of onion declined the disease score, it would be a better choice of treating tuberculosis patients having drug resistance.

Estimated cost of the project: Rs 45,000-50,000

11.11 Effect of Tribulus Terrestris on Intra Testicular Testosterone (ITT)

Introduction: Intratesticular testosterone (ITT) is thought to play a key role in the control of spermatogenesis in males but is rarely measured. The purposes of this study will be (1) To examine the relationship between intratesticular fluid and serum testosterone (T) at baseline and during treatment with *Tribulus terrestris* extract a known compound to enhance spermatogenesis in males and (2) To measure intratesticular fluid androgenic bioactivity.

Study Design: 20 albino rats will be divided in 2 groups, Group I (control) and Group II (Experimental). *Tribulus terrestris* will be administered to experimental group daily for 20 days. Testicular fluid will be obtained by percutaneous aspiration at baseline and on 20th day of the treatment. The rats in both groups will then be sacrificed and the testes will be removed and sectioned along the midline and immersed in Bouin's fixative for 24 hours. The tissue will embedded in paraffin and paraffin blocks will be prepared. These will be stained with H&E and PAS and examined with a light microscope at different magnifications. Spermatogenesis will be assessed by a method which depends upon scoring 'cross sectional' profiles of seminiferous tubules. Right and left testicular fluid samples will be pooled for ITT measurement. Serum T, LH, and FSH will be measured by immunoflourometric assay.
We hope this study will be a step forward to treat the male infertility.

Estimated cost of the project: Rs 40,000-45,000

12. PHYSIOLOGY

12.1 Predictive Role of Pulmonary Function Test in Asymptomatic Rheumatoid Lung Disease Patients

Introduction: Rheumatoid arthritis is a chronic systemic inflammatory disease, predominantly affecting joints. It affects the physical activities of the patients and has poor morbidity and mortality. In rheumatoid arthritis patients extra-articular manifestations are up to 50 percent. Among the extra-articular manifestations lung involvement is up to 40 percent. Rheumatoid lung disease is one of the major causes of mortality among rheumatoid arthritis patients. There is a great lack of information regarding rheumatoid arthritis as a chronic relapsing, remitting disease and its management among local patients and in general population. As lungs are the most commonly affected among rheumatoid arthritis patients, the present study is designed to analyze the pre clinical decline in pulmonary functions, in those rheumatoid arthritis patients who have not yet developed symptoms of rheumatoid lung disease.

Materials and Methods: The study will be conducted in Physiology Department, University of Health Sciences, Lahore. Rheumatoid arthritis patients will be taken from Medical and Rheumatology out door patient departments / wards of tertiary care hospitals of Lahore. The study will include 80 known rheumatoid arthritis patients aged between 30-60 years, having no pulmonary disease / symptoms and 40 age, sex and BMI matched healthy control subjects, having no pulmonary symptoms.

Methodology: After taking complete history, physical and systemic examination, spirometry will be done. 3ml of blood will be taken from each patient; the serum taken after centrifugation will be analyzed for C-reactive protein and RA factor titers. Disease severity will be assessed by the score calculated after filling the Stanford Health Assessment Questionnaire Disability Index (HAQ-DI) from each patient. Pulmonary functions of rheumatoid arthritis patients will be analyzed and they will be correlated with the disease duration, severity, C-reactive protein levels and RA factor titers.

Estimated Cost of the Study: Rs. 25, 000/-

12.2 Comparative Study of Serum Rheumatoid Factor, Anti-Cyclic Citrullinated Peptide Antibody and Anti-Mutated Citrullinated Vimentin Antibody for Diagnosing Rheumatoid Arthritis

Introduction: Rheumatoid Arthritis is a systemic autoimmune disease of unknown etiology characterized by chronic joint inflammation that often leads to joint destruction. It is a lifelong progressive disease with worldwide estimated prevalence of 0.5-3%, 2-3 times more in women than in men, most frequent during fourth and fifth decades of life. There is no specific diagnostic tool for RA and early diagnosis mainly depends on clinical symptoms which are usually mild and nonspecific. Moreover, patients usually do not fulfill the American College of Rheumatology (ACR) criteria and remain undiagnosed. Rheumatoid factor has low specificity as it can be detected in sera of patients with other autoimmune and infectious disorders as well as in the normal, healthy elderly individuals and might be absent in Rheumatoid Arthritis sufferers. The present study is being designed to fulfill the critical need for a specific diagnostic marker for rheumatoid arthritis. In this study the sensitivity and specificity of rheumatoid factor (IgM isoform), anti-CCP and anti-MCV antibody shall be compared to evaluate the validity of these markers in diagnosis of the disease.

Materials & methods:

Study design: Analytical, cross sectional study

Sample size: A total of 80 subjects will be included in the study, these will comprise of three groups

Group1: thirty known patients of rheumatoid (fulfilling the ACR criteria)

Group 2: twenty-five patients with other rheumatic diseases

Group 3: twenty-five normal healthy individuals (age and sex matched)

Methodology: The subjects will be selected from public sector hospitals and institutions. After obtaining a written informed consent, thorough examination will be performed. The venous blood samples will be taken and secured in vacutainers. Serum will be extracted by centrifugation and stored at -20˚C till analysis. Rheumatoid factor, anti-CCP and anti-MCV antibody titers will be determined by ELISA method. The data obtained will be analyzed by using SPSS version 16.0.

Estimated cost of the project: Rs.75, 000

12.3 Insulin Resistance In Relation to Body Iron Stores In off springs of Type 2 Diabetics

Introduction: Type 2 diabetes mellitus (T2DM) is a predominant public health concern world wide, accounting for 90% of the cases of diabetes globally. Most of the patients with T2DM are middle aged, not elderly and 80% of the cases are found in low and middle income countries. Currently, T2DM is thought to occur in genetically predisposed individuals who are exposed to various environmental factors that precipitate the onset of clinical disease. Pathogenesis of T2DM involves insulin resistance, defective insulin secretion and increased glucose production by the liver. Recent studies have revealed that insulin resistance is positively correlated with body iron stores. Insulin being an anabolic hormone stimulates the uptake of various nutrients by the cells, including the iron by different mechanisms. Increased iron stores further aggravate the insulin resistance by catalyzing many redox reactions in the cells. Insulin resistance, compensated by hyperinsulinemia is present several years before the onset of T2DM in genetically predisposed individuals.

Methodology: The present study is a cross-sectional study. It is designed to see the relationship between insulin resistance and various parameters of iron stores including serum iron, serum TIBC, transferrin saturation and serum ferritin in non diabetic offsprings of type 2 diabetics.

Materials and Methods: This study will include eighty offsprings of type 2 diabetics. Subjects will be selected by convenient sampling from registered cases of T2DM in different Diabetic Centers of Lahore. Written informed consent will be taken. Thorough examination will be done; blood samples will be drawn and secured in vacutainers. Blood tests will be conducted following standard procedures. Data will be analyzed by SPSS-16.

Cost of the Study: The estimated cost of the project is Rs 50,000/-.

12.4 Comparative Study of Non Invasive Parameters in Predicting and Grading Esophageal Varices.

Introduction: The chronic ongoing injury to the liver due to multiple reasons, leads to extensive scaring of liver tissue with ultimate impairment in its function. The world wide incidence of liver cirrhosis is 5-10%, however in Pakistan 15 million people are

suffering from it. Viral Hepatitis is the commonest cause in our country, as compared to alcohol in the west. Cirrhosis causes irreversible damage and commonly accompanied by portal hypertension leading to development of esophageal varices. According to recent recommendation every diagnosed liver cirrhotic patient should undergo endoscopy for the presence of esophageal varices. Endoscopy is an invasive and expensive procedure; therefore the non invasive predictors of esophageal varices are needed to be known. There are different controversies regarding the reliability of different non invasive parameters as predictors of esophageal varices. This study will provide information regarding the reliability of non invasive parameters as predictors of esophageal varices. The results of this study will help the clinicians to predict cost effectively those patients who are at high risk of bleeding and need strict follow up, hospitalization, prophylaxis and emergency interventions. This will help to prevent bleeding and in turn a number of complications and will also help to reduce the anxiety and pain of patient which he/she suffer during endoscopy.

Materials and Methods:

Sample Size: - 196 diagnosed patients of liver cirrhosis.

Study Design: - Analytical cross sectional study.

Methodology: 196 diagnosed patients of liver cirrhosis irrespective of etiology and categorised on the basis of severity of disease. Endoscopy for presence and grading of varices will be performed. Ultrasonography for relevant measurements and blood samples for analysis of ammonia, platelet count and albumin will be obtained. These parameters will be compared with presence and grading of esophageal varices on endoscopy.

Estimated Cost of the Study: Rs. 60,000/-

12.5 VDR Polymorphisms (Fok1, Bsm1) and Breast Cancer Risk in Pakistani Population

Introduction: Breast cancer is one of the most common cancers world wide. In our part of the world it tends to occur at a much earlier age. The conventional risk factors, identified for breast cancer including late age at first birth, lesser duration of breast feeding and use of hormonal contraceptives, are not common in our females.

However, the strong association between family history of breast cancer and the subsequent increased risk of breast cancer advocates an important role that genes may play in the etiology of breast carcinoma. Recently vitamin D receptor (*VDR*) gene has come up as a potential candidate in modulating the risk for breast cancer. Various polymorphisms in the *VDR* gene (*Fok*1 and *Bsm*1) have been related to alter the risk for breast cancer. But most of these studies have been carried out in a Caucasian population few in an Asian population and none in Pakistan. Present study is aimed at determining *VDR* polymorphisms *Fok*1 and *Bsm*1, in the *VDR* gene and their relationship to breast cancer in Pakistani population.

Materials and Methods: It will be a case-control study, recruiting 100 diagnosed patients of breast cancer and 100 controls after informed written consent, from the Shaukat Khanum Memorial Hospital and Research Centre. The subjects will complete a questionnaire regarding risk factors for breast cancer and demographic features of the subjects.

Methodology: Blood samples will be collected in EDTA coated vacutainer tubes. DNA would be extracted and amplified by PCR. Polymorphisms would be evaluated by Restriction Fragment Length Polymorphism.

Estimated Cost of the Project: Rs. 52,000/-

12.6 Association of Serum Uric Acid with Renal Function Decline in Normoalbuminuric and Microalbuminuric Type 1 Diabetics

Introduction: Diabetes mellitus is a chronic metabolic disease which affects many organ systems of the body including kidneys. The gradual renal function loss which ensues over a period of years leads to what is called diabetic nephropathy. The nephropathy is described in terms of amount of proteins lost in the urine per day, and is accordingly divided into microalbuminurea (30-300mg/24hr) and macroalbuminurea (more than 300mg/24hr). The progressive renal function decline is associated with many biochemical and metabolic changes in the body. Serum uric acid is one of the biochemical parameter which increases invariably in end stage renal disease. The association of serum uric acid with progression of renal function decline in

macroalbuminurea has been described in many studies but little work has been done to evaluate the association of serum uric acid in those patients who fall in microalbuminuric stage or in those who still lose normal amount of proteins (normoalbuminurea). This study is aimed at finding this association in type 1 diabetics of local population.

Materials & Methods: It will be a descriptive cross-sectional study which will be conducted in University of Health Sciences, Lahore. Only those diagnosed patients of type-I diabetes will be included in the study that will fulfill the inclusion and exclusion criteria. A total of 90 volunteer patients will be recruited from the tertiary care hospitals of Lahore.

Methodology: After getting consent from the patients, their history will be recorded and their examination findings will be documented. The blood samples will be taken from the patients and brought back to the university to run the tests. The renal function of the patients and the stage of diabetic nephropathy will be assessed by measuring glomerular filtration rate and the amount of proteins in the urine respectively. Finally the levels of uric acid will be determined in the serum and its association will be established with the renal function decline and stage of diabetic nephropathy.

Estimated cost of project: Rs. 28000/-

12.7 Expression of Circadian Clock and Cell Cycle Genes in Chronic Lymphocytic Leukemia".

Introduction: Circadian rhythms are daily oscillations of multiple biological processes driven by endogenous clocks. The circadian timing system comprises peripheral oscillators located in most tissues of the body and a central rhythm generator located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is a master circadian clock tissue, generating self-sustained circadian oscillators which determine the pace and amplitude of the expression of the circadian clock genes in peripheral tissues through neuronal and hormonal signals, these peripheral clocks in turn control the output of circadian physiology and behavior.

The cellular mechanism of circadian rhythmicity involves the regulation of three *Period* genes (*Per 1–3*) and two *Cryptochrome* genes (*Cry1* and *2*) by a dimer of the proteins

CLOCK and BMAL1. Through the CLOCK/BMAL1 complex, transcriptional circadian regulation extends beyond clock genes to include various clock-controlled genes (CCGs) including cell cycle genes such as *Wee1*, *c-myc* and *Cyclin D1*. The genetic or functional disruption of the molecular circadian clock may result in genomic instability and accelerated cellular proliferation, two conditions that favor carcinogenesis. Thus, aberrant expression of circadian clock genes could have important consequences on the transactivation of downstream targets that control the cell cycle and on the ability of cells to undergo apoptosis, potentially promoting carcinogenesis.

Different lines of evidence in mice and humans suggest that cancer may be a circadian-related disorder and aberrant expression of clock genes has also been observed in some leukemia (Gery S, 2005; Yang M, 2006). However, little is known about possible molecular mechanisms underlying this clock-cancer connection in Chronic Lymphocytic Leukemia (CLL) which represents the common leukemia of the adults. Based on the reported involvement of the circadian clock in the development of several cancers including some leukemia, the current study has been designed to explore the possible roles of clock genes in the development of human CLL.

Materials & Methods

Study Design: This is cross sectional analytical study which is based on random sampling.

Sample Size: The study will involve 60 adults; including 30 CLL patients and equal number of age and sex matched healthy individuals comprising the control group.

Methodology: The expression levels of five clock genes (*per1, per2, per3, clock* and *Bmal1*) and three clock-controlled cell cycle genes (*Cyclin D1, c-myc and wee1*) will be determined by qRTPCR. The methodology will involve blood samples collection, RNA isolation, cDNA synthesis and qPCR. Statistical analysis will be done for association between different variables using software package SPSS 16. It is expected that this study will provide supportive data for clock-cancer connection and role of clock genes in the etiology of CLL. Moreover, understanding the molecular links between the cell and the circadian cycles may lead to new therapeutic approaches to cancer as well as other challenging diseases.

Estimated Cost of the Project: The project involves highly sophisticated and advanced techniques of Molecular Biology ant its estimated cost is around Rs. 500000/-

12.8 Growth, Obesity and Pubertal Development Pattern in School Girls of the Punjab

Introduction: Child's health and nutrition are best indicated by the somatic growth which in turn is evaluated by serial recording of height, weight, waist circumference, skinfold thickness and body mass index. Body growth is a process sustained throughout from fetal development to early adulthood. The greatest growth occurs during infancy with a decline in growth during childhood. A plateau like phase emerges in mid-childhood followed by a decline before the onset of pubertal growth spurt. Body growth is thus assessed in relation to the reference values for these anthropometric measurements in order to monitor long-term health in children and adolescents. In humans, the pubertal growth spurt is due to augmentation in GH secretion, which in turn is stimulated by elevated sex steroids

An alarming increase in prevalence of pediatric obesity and the associated metabolic dysfunction is also known to affect onset and progression of puberty in girls. Menstrual bleeding in perimenarcheal girls is dependent upon reproductive hormones which in turn are effected by leptin released from body fat. The estimates for overweight and obesity in the girls can thus provide valuable information regarding the prevalence of obesity and its effect on menarche in the girls of Punjab. Our pediatricians use American norms for evaluating somatic and pubertal growth due to the lack of national data on anthropometric indices and thus standard percentile charts. However, the effect of ethnicity as well as environmental and nutritional status on growth limit the use of these growth charts in our pediatric population. Reference anthropometric values of our population shall provide pediatricians a tool that would avoid the use of charts which are outdated. The BMI cut-off points for underweight, overweight and obese in children has to be ethnic-specific and thus need to be evaluated for national data. It is expected that this study will contribute in providing the pediatricians latest growth norms to evaluate the growth status in the Punjabi girls and will help in recording the incidence of obesity along with its correlation with menarche.

Materials and Methods: The study shall comprise 10,000 school girls aged at 8-16 years and categorized as Group I (8-10 yrs), Group II (11-13 yrs), Group III (14-16 yrs).

Methodology: Measurement of height, body weight, waist and hip circumference, skinfold thickness will be carried out in all subjects. Approximately 5 ml of blood will be drawn by venepuncture for the analysis of serum FSH and serum growth hormone in randomly selected subjects. Statistical analyses will be carried out for association between different variables using software package SPSS 16.

Estimated Cost of the Project: The estimated cost of the project will be 610,000 rupees.

12.9 Molecular Markers of Susceptibility to Prion Diseases in Local Humans and Domesticated Bovids

Introduction: Prion diseases or Transmissible Spongiform Encephalopathies are neurodegenerative conditions usually characterized by the accumulation of conformational isomers of normal host-encoded prion protein in the central nervous system. Clinical symptoms include myoclonus, dementia, and behavioral changes. Prion diseases are the only disorders that can occur in all three forms viz. sporadic, familial, and infectious. A single gene *PRNP* is responsible for the development of these diseases and a number of DNA variants have been found in this gene to be associated with their different types like Cruetzfeldt-Jacob disease (CJD) in humans and BSE or mad cow disease. Recently, BSE infection has resulted in a variant CJD form that can cause death at an earlier age (29 years) in comparison with common CJD (65 years). This has raised public health concerns all over the world. There is a lack of information about the presence of prion diseases in Pakistan. Although no prion case has been reported in this region, more than 120 probable cases of CJD have been registered in India, which indicate that there may also be some CJD cases in Pakistan.

Materials and Methods: The present study therefore aims at finding the risk of occurrence of BSE and vCJD at molecular level in Pakistan. In this study, a total of four DNA sequence variations in the prion protein gene (*PRNP*) ─ 12bp and 23bp

insertions/deletions (indels), and E211K variant in bovine *PRNP* and a common M129V polymorphism in human *PRNP* will be targeted in 1116 DNA samples using various reagent concentrations and polymorphism targeting techniques, such as amplification refractory mutation detection system (ARMS), restriction fragment length polymorphisms (RFLP), multiplex PCR, and DNA sequencing. Forced and multiple primer pairs will be employed to enhance the reliability and simplicity of data analyses. The selected polymorphisms predispose to either BSE or CJD. These polymorphisms will also be studied in other bovid species like sheep and goats to gain information about evolution and selection pressure exerted on *PRNP* locus by prion diseases.

Outcomes: The data will be analyzed with genetic analysis softwares (i.e. PopGene) and basic statistical modules (i.e. SPSS) and will be compared with the data reported for other countries. These analyses will provide information about the risk of occurrence of prion diseases in Pakistan and their evolution.

Estimated Cost of the Project: Self funded.

13. RESEARCH PROJECTS OF AFFILIATED INSTITUTES

Synopsis Title	Institute	Course
1. Comparison of multidetector computed tomographic angiography vs conventional coronary angiography for assessment of coronary artery bypass grafts.	PGMI	M.D. Cardiology
2. Validation of TIMI risk score in patients presenting with Unstable Angina/Non-ST elevation Myocardial Infarction.	PGMI	M.D. Cardiology
3. In-hospital outcome of percutaneous transvenous mitral commissurotomy in patients with severe pulmonary hypertension.	PGMI	M.D. Cardiology
4. Efficacy and comparison of radial versus femoral approach for percutaneous coronary intervention	PGMI	M.D. Cardiology
5. Prognostic value of heart rate variability after acute myocardial infraction	PGMI	M.D. Cardiology
6. Diagnostic accuracy of multi detector computed angiography Vs Conventional coronary angiography for detection of coronary artery disease	PGMI	M.D. Cardiology
7. Comparison of topical (Lindane and Permethrine) and oral (Ivermectin) treatment of scabies	PGMI	M.D. Dermatology
8. Frequency and severity of diabetic retinopathy among type-I and type-II diabetes mellitus at the time of diagnosis	PGMI	M.D. Internal Medicine
9. Prevention of lamotrigine induced orofacial cleft in mice fetuses with folic acid supplementation	PGMI	M.Phil Anatomy
10. Histological study of soleus muscle of rats in response to nandrolone decanoate and exercise	PGMI	M.Phil Anatomy
11. Relationship between serum Interleukin-6, C-reactive protein levels and Insulin Resistance Score in asymptomatic obese children and adolescents.	PGMI	M.Phil Chemical Pathology

12.	Associations of serum adiponectin, insulin and lipoproteins in patients with type 2 diabetes mellitus and non-diabetic subjects	PGMI	M.Phil Chemical Pathology
13.	Assessment of nutritional status of primary school children of Quetta City	IPH	M.Phil Community Medicine
14.	Effect of lifestyle changes on control of borderline hypertension	IPH	M.Phil Community Medicine
15.	Evaluation of maternal health services provided by City District Government, Lahore	IPH	M.Phil Community Medicine
16.	Appraisal of general practitioners in the management of acute watery diarrhoea for children under 5 years of age	IPH	M.Phil Community Medicine
17.	Assessment of hepatitis-B vaccination services under National Immunization Programme	IPH	M.Phil Community Medicine
18.	Immunoglobulin gene rearrangement in detection of minimal residual disease in acute lymphoblastic leukaemia	AFIP	M.Phil Haematology
19.	Comparison of different treatment modalities of chelation therapy in beta thalassemia major patients	PGMI	M.Phil Haematology
20.	Determination of optimal shelf life of cord blood by measurement of storage related changes and its comparison with adult blood	PGMI	M.Phil Haematology
21.	HFE gene mutation in patients with increased bone marrow iron	AFIP	M.Phil Haematology
22.	Prevalence of Alpha Thalassaemia in Northern Pakistan	AFIP	M.Phil Haematology
23.	Identification of the laboratory markers of disease activity for Pakistanis suffering from rheumatoid arthritis	AFIP	M.Phil Immunology
24.	Vaginal carriage rate of Group B Streptococcus in pregnant women and its transmission to the newborn neonates.	RMC	M.Phil Microbiology
25.	Sero prevalence of dengue fever in Rawalpindi	RMC	M.Phil Microbiology

26.	Efficacy of Zinc sulfate as an antibacterial agent against enteric pathogens	AFIP	M.Phil Microbiology
27.	Correlation of AgNOR pattern with HER2/neu receptor status in carcionoma of the breast	PGMI	M.Phil Morbid Anatomy & Histopathology
28.	To study the effects of Interferon-α on thyroid functions during treatment of Chronic Hepatitis C	PGMI	M.Phil Pharmacology
29.	Comparative lipid lowering effect of extra virgin olive oil and atorvastatin in dyslipidemia in type 2 Diabetes Melllitus	PGMI	M.Phil Pharmacology
30.	Relationship between Serum Total Homocysteine Level and Lipid Profile in different age group patients with coronary artery disease	PGMI	M.Phil Physiology
31.	Intraocular pressure in normal, hypertensive, diabetic and diabeto-hypertensive age-matched subjects	PGMI	M.Phil Physiology
32.	Off-pump Vs. On-pump coronary artery bypass grafting: Comparison of graft patency angiographically at 6 months	PGMI	M.S. Cardiac Srugery
33.	Off-Pump Vs On-Pump coronary artery bypass grafting: Randomized Study	PGMI	M.S. Cardiac Srugery
34.	Comparative study of sublay and onlay mesh repair for paraumbilical hernias	PGMI	M.S. General Surgery
35.	Visual improvement after transsphenoidal excision of pituitary macroadenomas	PGMI	M.S. Neurosurgery
36.	Outcome and complications in clipping of anterior circulation aneurysms.	PGMI	M.S. Neurosurgery
37.	Role of anterior cervical decompression and fixation with bone graft placement in lower cervical spine trauma cases	PGMI	M.S. Neurosurgery
38.	Fracture shaft of femur in children of 6 to 12 years of age treated with titanium elastic nail	PGMI	M.S. Orthopaedics
39.	Incidence of leak in interrupted single layer extra-mucosal suture intestinal anastomosis in paediatric age group	PGMI	M.S. Paediatric Surgery