Research Agenda
2009 - 2011

University of Health Sciences
Lahore

Index

Biomedical Engineering

Haematology

Histopathology

Human Genetics & Molecular Biology

Immunology

Microbiology

MSc Nursing

Pharmacology

Physiology

Allied Health Sciences

1.1 Cardiovascular Risk Factors in Hemodialysis Patients

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. Study design: Cross-sectional study. Aims/objectives: To assess risk of cardiovascular disease in dialysis patients. Material and methods: 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.

1.2 Zinc and Copper Levels in Young Women Taking Oral Contraceptives

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 (OCR). (2) To compare levels of zinc and copper between women taking OCR and those who are not taking OCR. Material and Methods: Study population will be 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 will be performed by Atomic absorption Spectrophotometer.

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

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 population: 30 children, age range 2-12 years, diagnosed with Acute Lymphoblastic Leukemia will be included in the study. Three samples will be taken. 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

1.4 Serum Trace Metals in Chronic Viral Hepatitis and Hepatocellular Carcinoma

Trace metals are essential nutrients for normal growth and development. Both zinc and copper are involved in several hepatic enzyme systems 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. Hypothesis: 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. Objective: To determine and compare serum levels of zinc and other trace metals in patients with chronic viral hepatitis and hepatocelluar carcinoma Patients with histologically proven chronic liver disease included in the study. 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. Sample Analysis: Analysis of serum zinc and copper with atomic absorption spectrophotometer

1.5 Lipid Abnormalities in Uremia, Dialysis, and Transplantation

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.

Hypothesis: 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.

Objectives:2 Comparison of lipid profile of uremic patients with normal healthy persons without uremia. Material and Methods: Study includes 80 patients, 20 controls without uremia, 25 uremic patients on dialysis, 25 patients without dialysis and 10 patients with kidney transplantation. Highly obese, pregnant and diabetic patients excluded from the study. Laboratory Investigation: Lipid profile will be measured by photometric analysis on Metrolab 2300.

1.6 Prevalence of Hepatitis C in Hemodialysis Patients

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. Study design: Cross-sectional study. Aims and objectives: To measure the prevalence of hepatitis C in patients on hemodialysis in Pakistani population. Material and method: 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.

1.7 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 hormone has been studied. Aims/objectives: Assessment of bone minerals in children treated for acute lymphoblastic leukemia. Study Design: It is a cross sectional study. Material: 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 spectrophotometry. Alkaline phosphatase by standard biochemical methods. PTH by radioimmunoassay.

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

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. Study Design: Cross sectional. Study population: 30 children with nephrotic syndrome and 10 control. Lab investigation: Protein and creatinine quantification in urine will be performed by metro lab 2300.

1.9 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. Study Design: It is a cross sectional study. Aims/Objectives: To assess the immunoglobulin levels in HCV positive patients. Correlation of HCV infection with alteration of serum immunoglobulin. Material and Methods: 50 samples of HCV positive patients will be collected.20 normal control samples will also be collected for comparison. Serum IgA, IgG and IgM will be performed by ELISA.

1.10 Thyroid Dysfunction in Nephrotic Syndrome Patients

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. Study Design: Cross sectional 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. 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.

Anatomy

2.1 Ethambutol Induced Optic Neuritis-Its Prevention by Natural Honey

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.

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. 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. Group C and Group D will be given Natural Honey (Berry Honey) at doses of 15mg\kg\day and 20mg\kg\day orally respectively along with per 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 dissected out and will be stained with Haematoxylin n Eosin stain and will be examined under light microscope to see the toxic effects of ethambutol in Group B and their prevention by Natural Honey at different doses

in Group C & D respectively. The results will be collected and analyzed statistically.

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

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. MSG has specific glutamate receptors which are excitatory and produce excitotoxicity; these are scattered throughout body.

MSG is also being used as bleaching agent for cloths and is likely to be biologically toxic .Despite, most of the reputable international organizations and nutritionists declare it as a safe as taste enhancer. 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.

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.

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

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 pathologist 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.

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.The results will be collected and analyzed statistically.

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

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 accumulate 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 days 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.

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.

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

Local Anesthetics have been widely used in wound infiltration, nerve block, epidural, and intrathecal anesthesia. The presently used local anesthetics popularly used in obstetrics cross the placenta readily. Teratogenic effects of general anesthetics have been studied in past variously but the local anesthetics hither to had not been given proper attention. In an in vitro study teratogenic effects of Lidocaine on neural tube have been observed. Another recent study revealed marked teratogenicity of cocaine in albino mice. Bupivacaine is one 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; 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.

Thirty two female mice (6-8week old) weighing 25-30 gm will be used; these comprised twenty four females and eight males. Three female and one male will be housed together for mating. When pregnancy will be confirmed, twenty four pregnant mice will be divided into three groups, having eight mice each. The control group A received daily intraperitoneal injections of normal saline from day 7 to 14 during 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 and microscopic abnormalities.The data will be collected and analyzed using SPSS 16.0

2.6 Perinatal Histogenesis of Pancreatic Islet α and β-cells

pancreas consists of exocrine and endocrine components, the later is composed of aggregate of cells called islets of langerhans. beta and alpha cells are predominant and secrete 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.

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.

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.

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 kruskal-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.

Biochemistry

3.1 Basic Development Need Program (BDNP)

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.

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

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. 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.

3.3 Relationship between Obesity, Lipid Profile and Osteoarthritis

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.
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.

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

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).

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

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. Osteoprosis 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.

The study will include following parameter

1. Bone mineral density by using DXA
2. Serum level of Calcium and Vitamin D
3. Urinary deoxypyridinoline, a biochemical marker for bone resorption
4. 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.

3.6 Prevalence of Environmental Asthma Due to Dust Storm in Multan

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.

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.

Biomedical Engineering

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

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.

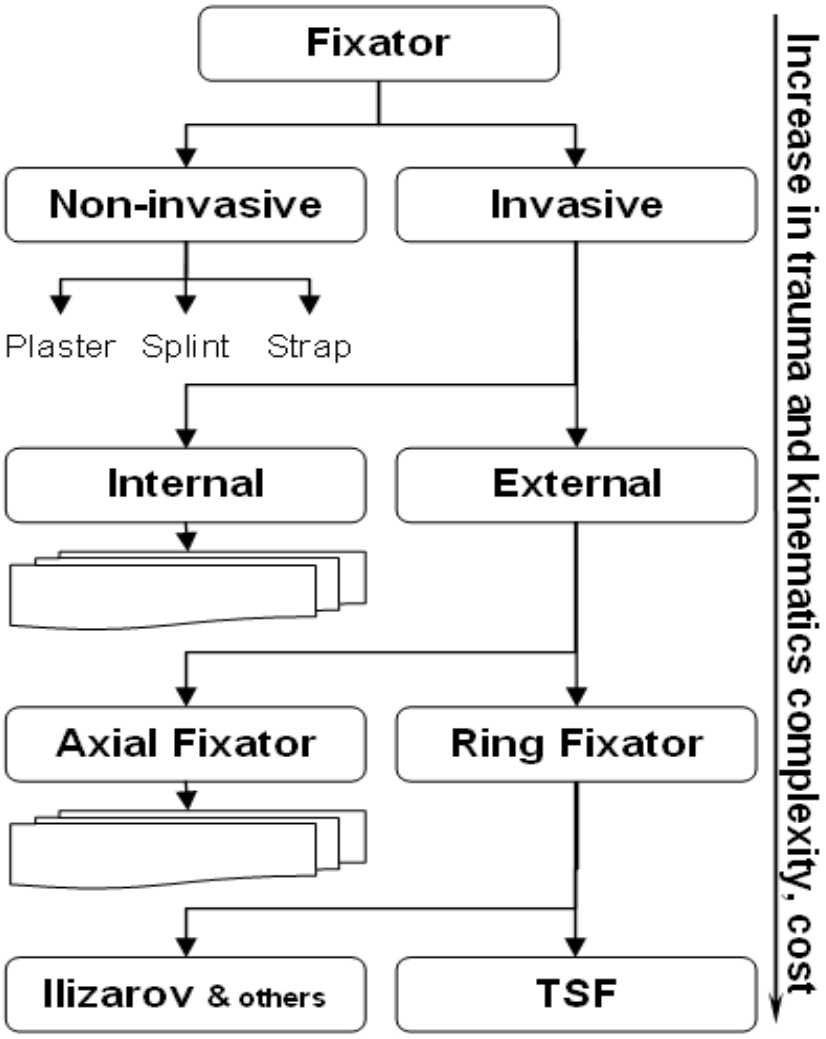

Figure 1. Types of orthopedic fracture fixation

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.

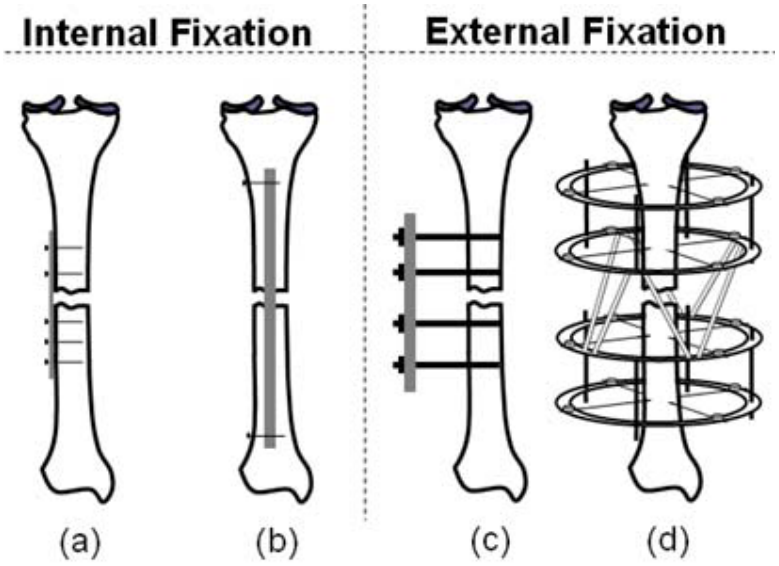

Figure 2. Examples of invasive fracture fixation methods. a) plate and screws, b) intramedullary nail fixed with screws inside the bone, c) unilateral bar connected to bone via half-pins, d) TSF (Taylor Spatial Frame) ring fixator with two accessory rings and connection to bone via 8 fine wires.

4.2 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.

Equipment Required (for titles 1 & 2):

Following is the detail of the basic equipment required for such type of lab.

- 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

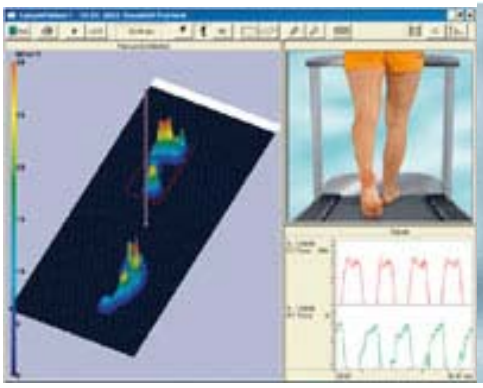

Figure: Gait Analysis

Figure: Floor-gait-plate

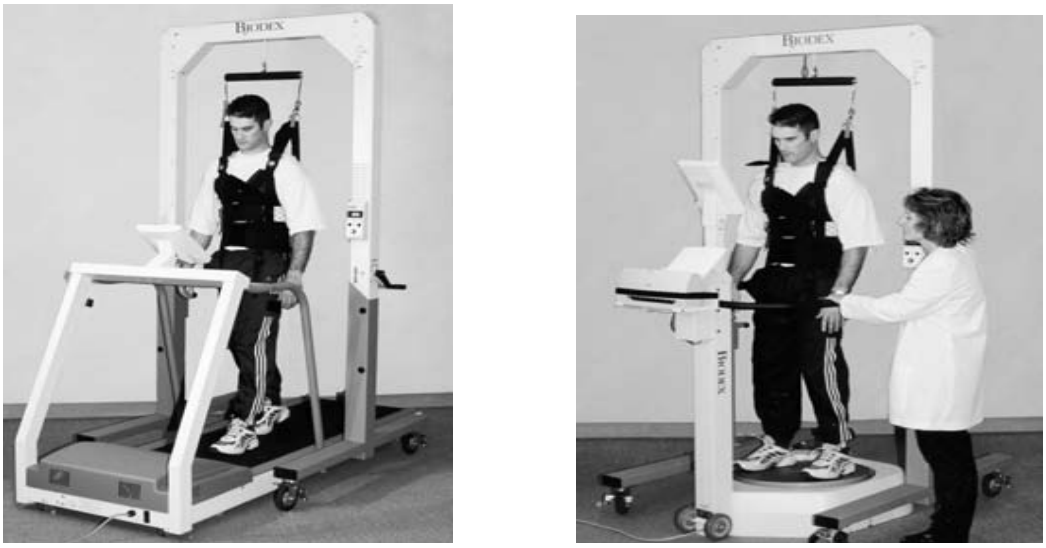

Figure: Standard un-weighing system for treadmill and balance evaluation system

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

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 are selected and are checked for their real time interaction with human cells 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.

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.

Equipment Required :

Following are the basic equipments required for this project on our end.

- CO_2 Incubators.
- Cell Culturing Lab.
- Laser Scanning Microscopes
- Latest computers along with appropriate softwares.

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

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.

Equipments Required :

Following are the basic equipments required for this project.

- CO_2 Incubators.
- Cell Culturing Lab.
- Laser Scanning Microscopes
- Latest computers along with appropriate softwares.

4.5 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.

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.

Equipments 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.

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. This is essential software for this research project and estimated cost of this software is around 1.7 million.

4.6 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".

This project is actually related to project 1 of Gait Analysis Lab. 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 and after finalization of a design, prototypes can be made to implant in the human bodies and then study their performance with the help of Gait Analysis Lab.

Equipments Required:

Computer simulation lab is needed. Same lab can be used as for project 5 but with separate specialized stress analysis softwares.

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

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.

Students can perform various research projects on this equipment related to characterization of various biomaterials depending upon their specific implementation.

Equipments Required:

This specialized equipment is specifically engineered to perform the following functions for the testing of various biomaterials

Figure: 858 Mini Bionix Test System

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 expension
- Response characteristic

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

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.

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 theatre 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.

Haematology

5.1 Prevalence of Alloantibodies in Multipara Females and Multitransfused Patients

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. It will be an Analytical Cross sectional study. Samples will be obtained from Multipara females**,** patients of Thalasemia and of chronic renal failure.Antibody screening will be carried out by using 'Three cell panel' and antibody detection will be done by 'Eleven cell panel'.

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

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.
It will be an analytical cross-sectional study. 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. 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.

5.3 Childhood Anaemia

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.

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

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)

5.5 Molecular Markers in Chronic Lymphoproliferative Disorders

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

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.

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

5.7 Myeloproliferative Disorders

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.

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

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.

- Whole blood hematocrit and platelet dysfunction will be determined and their correlation will be discussed.

- 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.

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.

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

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.

It will be an analytical cross-sectional study. 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. 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.

5.10 Von Willebrand Disease

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.

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.

Histopathology

6.1 Muscular Dystrophies: Their Correlation with Various Proteins & their Genetic Correlation.

This study project has been prepared from the point of view of finding a correlation of various types of muscular dystrophies, such as DMD, BMD and LGMD. In this project we will be using various types of markers to explain different aspects of diseases as well as their genetic correlation. These markers include dystrophins, (ROD, CN types) β- spectrin, α-sarcoglycane, β-sarcoglycane, dysferlin, caveolin-3, laminin α2, desmin and integrin. In addition, we will be initially using some specialised stains as well, so that we are in a position to differentiate congenital muscular dystrophies as well.

This project when completed is likely to give us a lot of information regarding how to differentiate muscular dystrophies? What is their correlation with each other and what are the genetical backgrounds? This will also explain the prognosis longevity of life in these children. Lastly the role of female careers and a higher percentage of female victims in muscular dystrophies as observed in one of our pilot studies finished recently.

6.2 Morphological Study of IgA and Henoch SchÖnlein Nephropathies.

Primary or idiopathic IgA nephropathy is recognized as the most common form of primary glomerulonephritis, while Henoch Schonlein nephropathy is the systemic form of IgA nephropathy, predominantly seen in children. The proposed study will focus on morphological patterns of IgA nephropathy and their relationship with clinical findings by applying special stains, immunoflourescence and immunohistochemistry by using iNOS.

6.3 Study of SLE Related Renal Lesions.

SLE is an autoimmune disorder affecting multiple systems in body nephritis is a common complication of SLE evident in more than 50% of the cases. Renal biopsy of lupus nephritis and its classification according to the ISN/RPS system

provides an important information about class, severity, activity and chronicity of the renal disease. That can't not be accurately predicted on the basis of clinical manifestation alone.

6.4 Morphological Study of Hepatitis C Virus Associated Nephropathy

HCV is associated with mixed cryoglobulinaemia. Concomitant cryoglobulinaemia.and HCV infection is associated with membranoproliferative glomerulonephritis type I, membranous GN, IgA nephropathy, amyloid nephropathy, post infectious GN, focal segmental glomeruloscelrosis, fibrillary and immunotactoid GN. Immunofluoroscence show IgG, IgM, C3 and C1q. deposits and immmunohistochemistry shows linear or granular deposits with HCV NS3 antibody.

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

Colorectal carcinoma happens to be the fourth most common malignant tumour in females, and in males only behind Bronchial Carcinoma. The assessment of cell proliferation in colorectal tissue and changes in mucin composition provides information with both prognostic and therapeutic implications. Objectives of the proposed study are to determine the morphological types & their histological grading of colorectal carcinoma and mucin type, cell types and their counts in the transitional zone of colorectal carcinoma. An increased number of inflammatory cells and an altered expression of mucin in the transitional zone of the colorectal carcinoma act as prognostic indicators.

6.6 Study of premalignant conditions of breast in relation to carcinoma breast.

Breast cancer is the most common female malignancy worldwide, with the increasing incidents in low resource countries, specially in Pakistan, in which one in every 9 females is suffering from carcinoma breast. Among well known risk factors premalignant breast lesions are precursors of cancer breast. Our aim of study is to look for these premalignant breast lesions in mastectomy specimen, for accurate assessment of risk which may help us to prevent progression of disease into invasive cancer.

6.7 Morphological Study of Renal Biopsies in Diabetics.

Diabetic nephropathy in occurs in 30% patients of diabetes. Nephropathy in diabetes can follow three patterns; either it will be diabetic nephropathy, non-diabetic renal disease in diabetes or non diabetic renal disease superimposed on diabetic nephropathy. Renal biopsies taken from patients with rapid progression of diabetes in the absence of retinopathy, heavy protinuria, haemturia or with the deranged renal functions will be studied morphologically.

6.8 Expression of p63 and S-l00a6 in the Differentiation of Keratinocytic Tumours of Skin.

Immunohistochemical characterization of skin tumours, in adjunct with routine histopathology, has resulted in a level of distinction between diagnoses which have not been previously possible. In the same context, recently few studies have been carried out to see the expression of p63 and S100A6 in various cutaneous tumours and their correlation to skin carcinogenesis. The p63 gene plays an essential role in epithelial development and the proliferation of limb and craniofacial structures, and is involved in the maintenance of a basal/stem cell population in stratified epithelia. S100A6 has been found in fibroblastic and epithelial cell lines, cells with rapid proliferative activity and cells undergoing differentiation. Its expression in melanomas and other melanocytic lesions have

been well documented in various studies. Regardless of the data available concerning p63 & S100A6 expression in several types of human neoplasms, no studies have involved both these markers to compare the respective staining patterns in the differential diagnosis of cutaneous tumours, especially keratinocytic tumours. The proposed study is intended to study the expression of p63 and S100A6 in different keratinocytic tumours of skin & to associate the expression of these immunohistochemical markers with the diagnosis of different types of keratinocytic tumours of skin. This will add an efficient method to diagnose rather undifferentiated/poorly differentiated epidermal carcinomas.

Human Genetics & Molecular Biology

7.1 Diagnostic Role of Conventional Cytogenetics and Fluorescence In Situ Hybridization (FISH) In Hematological Malignancies

Cytogenetic analysis is an important aid in the classification of hematological disorders. Most types of leukemia display either numerical chromosomal abnormalities or structural rearrangements, mainly translocations. The established relationship between malignancy and chromosomal changes has made cytogenetic studies a significant part of a number of hematological disorders. Increasingly recognized, nonrandom chromosomal abnormalities are especially useful in diagnosing the leukemia subtypes and predicting treatment outcomes. A major progress in human cytogenetics is the development of a molecular technique of Fluorescence In Situ Hybridization (FISH). This technique has provided the cytogeneticist with considerably increased ability to identify chromosome segment, to correlate chromosome structure with gene location, to reveal cryptic abnormalities that are undetectable using standard banding techniques and to analyze and describe complex rearrangements.

Aims and objectives of the proposed research are to identify chromosomal aberrations in local patients of leukemia, to correlate these aberrations with the severity of disease and treatment outcomes and to compare sensitivity of conventional cytogenetics with FISH technique. This would in turn facilitate establishing FISH diagnostic services in our laboratory.

7.2 21-Hydroxylase Genotyping in Pakistani Patients with Congenital Adrenal Hyperplasia

Congenital Adrenal Hyperplasia (CAH) due to deficiency of the enzyme 21-hydroxylase (21-OH) is one of the most common autosomal recessive inherited disorders. It affects about 1 in 5,000 births and is the most common cause of genital ambiguity at birth. CAH results from a deficiency in one or another of the enzymes of cortisol biosynthesis. In about 95% of cases, 21-hydroxylation is impaired in the zona fasciculata of the adrenal cortex so that 17-hydroxyprogesterone (17-OHP) is not converted to 11-deoxycortisol. Because of defective cortisol synthesis, ACTH levels increase, resulting in overproduction

and accumulation of cortisol precursors, particularly 17-OHP, proximal to the block. This causes excessive production of androgens, resulting in virilization.

The synthesis of 21-OH is controlled by the active CYP21A2 gene located at a distance of 30 kb from a highly homologous pseudogene designated CYP21A1P on short arm of chromosome 6 (6p21.3). In most population, CYP21A2 mutations in the form of deletions comprise about 20% of classic 21-hydorxylase deficiency. Besides deletions, point mutations resulting in nonsense and frameshift mutations have also been reported.

Purposed research aims to identify frequency and pattern of 21-OH gene mutations in local patients of Congenital Adrenal hyperplasia. This information would be useful in establishing carrier screening and prenatal diagnosis services in our population.

7.3 Search for Schizophrenia Susceptibility Genetic Loci

Schizophrenia is a complex and a common neurophsyciatric disorder with a life time prevalence of about 1% of population worldwide. The illness is characterized by psychotic disturbance, positive symptoms such as delusion, hallucinations and negative symptoms such as social withdrawal and poor motivation. Although the exact etiology of the disorder is not understood, results obtained from family, twin and adoption studies support a major role for genetic factors in pathogenesis of schizophrenia. However the disorder does not display simple mendelian inheritance in most families but the genetic transmission comparable with a multi-factorial background. Those who have a third degree relative with schizophrenia are twice as likely to develop schizophrenia as those in the general population. Those with a second degree relative have a several-fold higher incidence of schizophrenia than the general population, and first degree relatives have an incidence of schizophrenia an order of magnitude higher than the general population. The current belief is that there are a number of genes that contribute to susceptibility or pathology of schizophrenia, but none exhibit full responsibility for the disease.

The aims of the proposed study are to identify the familial pattern of schizophrenia in local population and to identify association of different genes with this disorder. Finding the specific gene or genes that lead to schizophrenia has large social implications. The discovery of these genes is the first step towards a more detailed biochemical and mechanistic understanding of schizophrenia and possible gene therapy.

7.4 Inheritance Pattern and Genetic Basis of Idiopathic Epilepsy

Epilepsy is a broad term which refers to a condition in which an individual is susceptible to repeated, unprovoked seizures. During seizures, large numbers of neurons in the brain are activated involuntarily and synchronously. Epilepsy can have a significant impact on quality of life, including social interactions and relationships, discrimination and prejudice, as well as the side effects of anti-epileptic medications.

Epilepsy is a heterogeneous entity with great variation in aetiology and clinical features. It has been suggested that there are certain genetic factors which contribute to a general susceptibility to seizures and which have a variable expression depending on additional, more specific genetic factors. Idiopathic epilepsies are believed to arise from a combination of genetic and environmental factors. The genetics of epilepsy are currently an area of intense research activity and progress.

The aim of the proposed research is to ascertain the inheritance pattern of idiopathic epilepsy and to identify loci and genes involved in the pathogenesis of this disorder. This knowledge will improve diagnosis, prognosis, management and prevention of epilepsy.

7.5 Genetics of Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder characterized by hyperandrogenemia, hyperinsulinemia, insulin resistance, and

chronic anovulation. It is the most common endocrine disorder in women of reproductive age. Pathophysiology and molecular basis of this disease is still unknown. The high prevalence of affected individuals and the wide range of phenotypic expression can be explained by the interaction of a number of key genes with environmental factors. Although evidence of familial segregation and clustering of the disease in first-degree relatives of women diagnosed with PCOS has been presented, no particular pattern of inheritance has emerged. Currently, PCOS is considered a polygenic trait that might result from the interaction of susceptible and protective genomic variants and environmental factors. Molecular defects in gonadotrophins and their receptors, in enzymes involved in steroidogenesis, as well as those underlying insulin action and secretion pathways, have been under continuous and intense investigation with variable results.The aim of the proposed research is to ascertain the familial clustering of PCOS and to identify loci and genes involved in the pathogenesis of this disorder.

7.6 Candidate Loci for Type-1 Diabetes in Pakistan

Type-1 diabetes (T1D), once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin. It is a common autoimmune disorder that arises from the action of multiple genetic and environmental risk factors. It is characterized by T-cell mediated selective destruction of the insulin producing beta-cells of the pancreatic islets. Type 1 diabetes is usually diagnosed in children and young adults.

Multiplex families studies, using genetic markers allowed the identification of various genes, including HLA, insulin, SUMO-4, IL10, IL19, IL20, GLIS3, CD69 and IL27and CTLA-4 all being linked with different degrees to disease risk. The difference in susceptibility among T1D patients suggest the development of the disease as resulting from the interaction between genetic and environmental factors. The aim of the proposed research is to identify the genetic loci for the susceptibility to type-1 diabetes mellitus in our population.

7.7 Clinical and Molecular Manifestations of Inherited Neurological Disorders

Neurological diseases are common disorders resulting in various degrees of disability and loss of productive life. Hundreds of different disorders affect the brain and nervous system, complicating efforts to prevent and treat them. A large number of these disorders are genetic in origin. Almost half of all human genes are expressed in the brain and third of all known genetic defects affect the nervous system. A number of genes have been identified that can cause or contribute to neurological disease. Among these are genes associated with Alzheimer's disease, Parkinson's disease, and genes known to cause Huntington's disease, Friedreich's ataxia, Batten disease, neurofibromatosis, spinal muscular atrophy, and several forms of epilepsy. While many of the less common neurological disorders are due to defects in single genes, it is now becoming clear that combinations of multiple genes interact to influence disease susceptibility, progression, and severity in the more common disorders. A better understanding of these genetic influences is essential for preventing and treating these disorders.The aims of the proposed study are to determine the prevalence of inherited neurological disorders in our population, identify their clinical manifestations and to discover molecular defects responsible for these disorders.

7.8 Adult pseudohypertrophic muscular dystrophy

Minimal diagnostic criteria: Positive family history in at least one additional male subject; elevated serum enzymes, weakness especially in proximal muscle groups.

Etiology: X-linked recessive

Pathogenesis: Unknown expects that the clinical and pathologic features are associated with progressive deterioration of the striated musculature of the body due to some unknown cause. The increase in elevation of the serum

enzyme levels is a secondary event due to muscle fibre necrosis or to a defect in the sarcolemmal membrane of striated muscle fibres.

7.9 Albinism, Hemorrhagic Diathesis and Pigmented Reticuloendothelial

Minimal diagnostic criteria**:** Universal albinism with a prolonged bleeding time and pigmented reticuloendothelial cells in the bone marrow.

Etiology: Autosomal recessive

Pathogenesis: Unknown for the pigment found in reticuloendothelial cells and in walls of small blood vessels. A deficiency of tyrosinase is a known defect in albinism.

7.10 Anophthalmia

Minimal diagnostic criteria: Anophthalmia is clinically suspected and is diagnostically confirmed microscopically.

Etiology: Autosomal recessive. In conjunction with other defects it can be transmitted as an autosomal dominant, x-linked recessive, or found sporadically with a chromosomal aberration.

Pathogenesis: Elements of the eye are missing; minute traces of mesodermal elements may be present. Lack of differentiation of the optic plate following the development of the rudimentary forebrain which results in failure of formation of the optic vesicle. One theory postulates pressure upon the head of the embryo by thickened amnion with suppression of growth of the optic vesicles.

7.11 Autosomal Recessive Pseudohypertrophic Muscular Dystrophy

Minimal diagnostic criteria: Proximal weakness; hypertrophy, especially of the calf muscles; occurrence in both male and female children; moderate elevation of serum cpk and muscle biopsy consistent with myopathy or dystrophy

Etiology: Autosomal recessive

Pathogenesis: unknown. Progressive deterioration of striated muscle fibres, predominantly in the early years in the proximal muscle groups. The condition is slowly progressive.

7.12 Carpal-Tarsal Osteolysis And Chronic Progressive Glomerulopathy

Minimal diagnostic criteria: Osteolysis of carpal and tarsal bones associated with moderate-to-marked involvement of adjacent tubular bones, proteinuria and microscopic haematuria.

Etiology: Autosomal dominant

Pathogenesis: ?

7.13 Chin Fissure (Marker)

Minimal diagnostic criteria: Constant depression of the soft tissue part of the chin.

Etiology: Autosomal dominant with high degree of penetrance and variable expressivity for all types.

Pathogenesis: Fissures of the chin may occur by partial fusion of the mental muscles with the overlying skin. Local absence of subcutaneous fat and

muscular tissue in the area of the gnathion may cause an adhesion of the dermis to the periosteum by means of collagen bundles or even a ligament. These developmental variations leading to chin furrows may be determined around the end of the first trimester.

7.14 Cleft Lip or Palate and Lip Pits or Mounds

Minimal diagnostic criteria: Pits of the lower lip associated with cleft lip or palate, or pits in the proband and cleft occurrence in pedigree, or cleft in the proband and occurrence of pits in pedigree.

Etiology: pleiotropic autosomal dominant transmission with 80% penetrance. expressivity is variable: pits (69.6%) and cleft (36%) of affected persons and there is significant association between the types of cleft in parents and their children. possibly the development of cleft in this syndrome is influenced by modifying genes or by different mutant alleles with a predilection for the different types of cleft.

Pathogenesis: Small invaginations appearing on the embryonal mandibular process, “lateral sulci” could be identified in normal embryos 7.5 to 12.5 mm long. Aberrant mutant prevents the normal obliteration of these “lateral sucli” which results in labial pits. The same mutant gene prevents closure of primary or secondary palate.

7.15 Deafness, Stippled Epiphyses and Goitre with High Pbi

Minimal diagnostic criteria: Goitre and thyroid over activity associated with high levels of free (protein unbound) circulating thyroid hormone in the absence of hyperthyroidism.

Etiology: Behaves as a rare autosomal recessive.

Pathogenesis: It appears to be a congenital metabolic defect associated with variable degrees of tissue response to the action of thyroid hormone, which has been partially of the presence of an inhibitory substance or variable degree of tissue penetration of hormone cannot be excluded.

7.16 Distal Muscular Dystrophy

Minimal diagnostic criteria Weakness in the distal limb muscles. Electromyogram consistent with a myopathy. Muscle biopsy consistent with a myopathy or muscular dystrophy.

Etiology: Autosomal dominant

Pathogenesis: Unknown. There is a specific and slowly progressive deterioration of muscle fibres, primarily in the peripheral muscles.

7.17 Ear Lobe Pit (Marker)

Minimal diagnostic criteria: Pit in ear lobe

Etiology: Autosomal dominant with variable expressivity and incomplete penetrance. In about half the cases the pit is in only one lobe and in a few cases the gene may be carried without showing pits in either lobe.

Pathogenesis: ?

7.18 Ectopic Placement of Pinna

Minimal diagnostic criteria: Normally contoured pinna found in a displaced position without relationship to the external auditory canal, eardrum and middle ear which are in normal position or normally contoured pinna with associated canal, drum and middle ear all displaced from their usual position.

Etiology: ?

Pathogenesis: Unknown. Possible intrauterine trauma that led to transplantation of pinna precursor to another area of skull or amniotic fibrous adhesions that pulled developing pinna into abnormal position

7.19 Lactate Dehydrogenase

Minimal diagnostic criteria: Relatively simple eletrophoretic technics are available for determining the isozyme composition of tissues and body fluids. In normal serum, LDH isozymes are present in the following proportions: LDH-2 > LDH-1 > LDH-3 >LDH-4 >LDH-5. A variety of diseases exhibit unique changes in serum isozyme patterns.LDH-1 is markedly increased in myocardial infarction and LDH-5 in infectious hepatitis.

Analysis of tissue isozyme patterns may also have diagnostic application .In chickens and humans a form of muscular dystrophy is associated with a failure of development of the adult isozyme pattern. Whether this abnormality is a primary or secondary event is unknown. These findings suggest that isozymic analyses may be helpful in determining at what period of development a metabolic abnormality occurs.

Etiology: Autosomal dominant transmission of isozymes. Discovery of polypeptide A and B variant in human and animal tissues, together with appropriate genetic studies, showed that the synthesis of A and B polypeptides is controlled by two separate non allelic genes. Observations on pigeon testes have shown that the synthesis of the LDH-X subunit (C) is controlled by a third genetic locus in the pigeon. The total complement of LDH isozymes can be explained on the basis of the activity of genes at three loci, A, B and C, each being responsible for the synthesis of a corresponding polypeptide. The X locus, in contrast to the other loci, is not activated until pubescence in the male and remains inactive in the female.

Pathogenesis: ?

7.20 Limb-Girdle Muscular Dystrophy

Minimal diagnostic criteria: Muscular weakness of one or both groups of girdle muscles in a symmetric fashion. Electromyographic evidence of myopathic response. Possibly elevation of serum enzymes, especially cpk (creatine phosphokinase). The enzyme elevations in this disease may be significant, but oftentimes are minimal by contrast with the rises in serum enzymes in childhood pseudohypertrophic muscular dystrophy (x-linked duchenne type). A positive muscle biopsy indicating evidence of a myopathy.

Etiology: Unknown. Usually inherited as an autosomal recessive character, but in rare instances as an autosomal dominant trait. A few cases may be sporadic.

Pathogenesis: Unknown. The mechanisms of weakness involve structural breakdown of the striated musculature in the affected muscles.

7.21 Mucopolysaccharidosis I

Minimal diagnostic criteria: Presence of constant clinical signs, excess mucopolysaccharidurіa and either 1) specific “curing” characteristics of fibroblasts or 2) abnormal sulphate incorporation and degradation by cultured fibroblasts. metachromatic staining of fibroblasts and leukocyte inclusions are non specific findings.

Etiology: Autosomal recessive

Pathogenesis: The Hurler features develop because of progressive deposition of acid mucopolysaccharide in various tissues. Deficient function of an enzyme responsible for degradation of AMPS is presumed but not proven to underlie this condition. Skeletal abnormalities result from combination of soft tissue MPS deposition and disruption of normal cartilage-bone transition.

7.22 Mucopolysaccharidosis II

Minimal diagnostic criteria: Male with the above described clinical findings, mucopolysacchariduria and specific “curing” characteristics of cultured fibroblasts.

Etiology: X-linked recessive

Pathogenesis: Accumulation of acid mucopolysaccarides in tissue underlies most of the observed clinical features. The enzymatic block in mucopolysaccarides metabolism has not been found.

7.23 Mucopolysaccharidosis III

Minimal diagnostic criteria: Severe mental deterioration, mild somatic defects, and the urinary excretion of heparitin sulphate alone are findings sufficient for the diagnosis of mps iii. metachromatic staining is non specific. Confirmatory “correction” studies and sulphate incorporation studies on fibroblasts are desirable.

Etiology: Autosomal recessive

Pathogenesis: An enzymatic error in degradation of acid mucopolysaccharide is suspected but not proven. The cause of sever mental decline, whether due to heparitin sulphate toxicity, to deficiency of essential metabolic products, or to other factor is unknown.

7.24 Mucopolysaccharidosis IV

Minimal diagnostic criteria: Pathognomonic roentgenologic finding plus clinical features and keratosulfaturia.

Etiology: Autosomal recessive

Pathogenesis: Unknown. keratosulfaturia suggests that a defect in sulphate metabolism may be basic to the development of the soft tissue abnormalities and the disruption of cartilage-bone transition.

7.25 Mucopolysaccharidosis V

Minimal diagnostic criteria: Corneal clouding, mild or absent intellectual impairment, variable but generally mild somatic features plus mucopolysaccharriduria plus specific “curing” characteristics in fibroblast culture.

Etiology: Autosomal recessive

Pathogenesis: Presumed to be a failure of enzymatic mechanism of mucopolysaccharide degradation leading to deposition of AMPS in soft tissues and interruption of normal bone development.

7.26 Mucopolysaccharidosis VI

Minimal diagnostic criteria: Sever hurler-like somatic features with normal intelligence, urinary dermatan sulphate excess, specific “curing” characteristics of fibroblasts.

Etiology: Autosomal recessive

Pathogenesis: Presumed to be a defect in degradation of acid mucopolysaccharide, allowing accumulation of AMPS in soft tissues and disruption of bone development.

7.27 Oculocutaneous Albinism and Deafness

Minimal diagnostic criteria: Sensorineural deafness, translucency of the irides, nystagmus, hypopigmentation of fundus oculi, hypoplasia or aplasia of fovea, diffuse unpatterned absence of melanin in the skin, hair and eyes. Probable tyrosinase-negative albinism.

Etiology: Autosomal recessive (in two related consanguineous jewish-moroccan sibships).

Pathogenesis: ?

7.28 Oculocerebrorenal Syndrome

Minimal diagnostic criteria: Bilateral cataracts at birth, mental retardation and hypotonia in a male child are the clinical hallmarks. laboratory findings, which may vary in intensity, stem from the evidence of renal tubular dysfunction, including generalized renal hyperaminoaciduria; “tubular” proteinuria (soluble with heat after initial precipitation with 20% sulfosalicylic acid) comprising β globulins: low-t_m glucosuria; high renal clearance of inorganic phosphate with hypophoshatemia; renal tubular acidosis with impaired bicarbonate conservation and with evidence for a particular defect in h^+ secretion and ammonia production; titratable organic aciduria not totally accounted for by the α-aminoaciduria, but as yet unidentified as to composition. The tubular dysfunction increased in severity with age, beginning in early infancy; its manifestation are believed to be absent at birth onwards. Rickets is considered to be secondary to the hypophosphatemia. Progressive glomerular and interstitial fibrosis have been reported in the syndrome.

Etiology: X-linked recessive

Pathogenesis: It is presumed that all features of trait are related to an undefined derangement of energy metabolism. Trait is expressed prenatally since the patient is born with some manifestation e.g. cataracts.

7.29 Oculopharyngeal Muscular Dystrophy

Minimal diagnostic criteria: Evidence of ptosis and pharyngeal muscle weakness along with a positive family history of this condition, or a muscle biopsy disclosing myopathic or dystrophic features.

Etiology: Autosomal dominant

Pathogenesis: ? There is progressive deterioration of the muscle fibres.

7.30 Oculoauriculovertebral Dysplasia

Minimal diagnostic criteria: There is no accepted definition of what constitutes the oculoauriculovertebral dysplasia syndrome. It is a variation of the first and second arch syndromes. The distinguishing features of this syndrome include unilateral facialk hypoplasia, dermoids of the eyes, coloboma of the upper eyelid and vertebral abnormalities.

Etiology: It is possibly secondly to a vascular abnormality during embryogenesis. Although there are two possible cases of the syndrome occurring in sibs, it does not appear to be an inherited disorder. Chromosomal analyses have been normal.

Pathogenesis: It is postulated that a vascular insufficiency or abnormality disrupts the normal embryologic development of first and second arches.

7.31 Ocular Colobomas

Minimal diagnostic criteria: Depends on total ophthalmologic evaluation.

Etiology: Multifactorial. Iris coloboma may be autosomal dominant. Embryonic fissure derived ocular colobomas uncertain. Expressivity is quite variable and

penetrance said to be 20 to 30%. Environmental factor can not be ruled out as in colobomatous cysts where transmission has been documented.

Pathogenesis: Accentuation of inversion of the inner layer of the optic cup anywhere along the fetal fissure results in non closure of varied degree and position yielding the diverse colobomas described.

7.32 Pectus Carinatum

Minimal diagnostic criteria: Prominence of sternum with lateral depression of ribs.

Etiology: ?

Pathogenesis: Several theories have been proposed but none proven. brodkin believes that the various sternal deformities are the result of failure of the development of muscle in the ventral segment of the diaphragm and that these portions of the muscle exert a pull on the attached chest wall as a result of the unopposed action of muscles on the other side. Others believe this deformity is due to an overgrowth of the costal cartilages,

7.33 Polydactyly

Minimal diagnostic criteria: An extra digital triradius is found at the base of the extra digit. When it is a pedunculated post minimus that was surgically removed or fell out spontaneously, the extra triradius in the dermatoglyphics may be the only evidence of postaxial polydactyly.

Etiology: Polydactyly of a triphalangeal thumb, polydactyly of index finger and postaxial polydactyly are autosomal dominant traits with variable penetrance and expressivity. Thumb polydactyly is frequently unilateral and the cases are sporadic with no evidence of inheritance.

Pathogenesis: ?

7.34 Syndactyly

Minimal diagnostic criteria: In syndactyly type I (zygodactyly), there is usually webbing between the third fourth fingers, either complete reaching to the nails, or partial, and occasionally associated with fusion of the distal phalanges of these fingers. Other fingers are sometimes also involved but the third and fourth fingers are the most commonly affected. In the feet, there is usually webbing between the second and third toes, either complete or partial.

In syndactyly type II (synpolydactyly), there is usually syndactyly of the third and fourth fingers associated with polydactyly of all components or of part of the fourth finger in the web. In the feet, there is polydactyly of the fifth toe.

In syndactyly type III (ring and little finger syndactyly), syndactyly between the ring and the little fingers is usually complete and bilateral. The fifth finger is short with absent or rudimentary middle phalanx. Feet are usually not affected in this type. This type of syndactyly is the hand malformation in oculodentodigital dysplasia.

In syndactyly type IV (haas type) there is complete cutaneous fusion of the fingers giving the hands a cup-like appearance.

In syndactyly type V, there is an associated metacarpal and metatarsal fusion. the metacarpals and metatarsals most commonly fused are the third and fourth or the fourth and fifth. Soft tissue syndactyly usually affects the third and fourth fingers and the second and third toes. syndactyly is usually more extensive and complete.

Etiology: Autosomal dominant for each type

Pathogenesis**: ?**

7.35 Xeroderma Pigmentosum

Minimal diagnostic criteria: Photosensitivity and photophobia, early development of skin cancers.

Etiology: Autosomal recessive

Pathogenesis: Failure to repair DNA damage after ultraviolet exposure. This demonstrated in cultured fibroblasts and lymphocytes and in vivo epidermis. endonuclease probably missing to remove damaged DNA. endonuclease deficiency may lead to carcinogenesis. Only 10-20% repair of DNA in xeroderma pigmentosum.

Immunology

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

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%.

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

8.2 Th1 and Th2 Cytokine Profile in Case of Unexplained Infertility

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.

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

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

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.

Aims:

a. Select diabetic patients with various clinical menifestations
b. Find out the proportion of T-regulatory cells and cytokines in these patients

8.4 Susceptibility of *H. Pylori* Infection among Healthy Individuals

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.

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

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

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 levels of immunoglobulins in their serum and these babies will be followed for a period of six month to find out the rate of infection among them.

Aims:

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

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

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.

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

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

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 determined. 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.

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

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

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.

Aims:

a. Compare the effects of adjuvant honey versus conventional therapy alone on T lymphocyte functions in SLE patients
b. Compare the effects of adjuvant honey therapy versus conventional therapy alone on B lymphocyte functions in SLE patients
c. Compare the effects of adjuvant honey therapy versus conventional therapy alone on SLE Disease Activity Index score in SLE patients.

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

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.

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

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

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 $CD3^+$, $CD4^+$,$CD8^+$, $CDB19^+$ 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.

Aims:

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

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

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

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.

Aims:

a. Investigate the role of immunological factors (specific lymphocyte subsets for NK Cell. 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

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

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.

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

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

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.

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.

Microbiology

9.1 Tuberculosis

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 Punjab. The MDR and XDR strains isolated will also be subjected to genotyping by various molecular methods. The study will also focus on the current new second line anti tuberculosis drugs and their activity against *Mycobacterium tuberculosis*.

9.2 Hospital Acquired Infection:

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. 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.

9.3 Helicobacter Pylori:

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. The study will also focus on the antibacterial activity of various natural products against *Helicobacter pylori.*

9.4 Molecular Characterization of Extended Spectrum beta lactamases (ESBL) in Pakistan.

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 genotyping of ESBLs. The objective of the study will be to determine it is chromosomal or plasmid mediated as well as comparison of genotypes of internationally existing enzymes. These enzymes could be the same as ESBL genomes of internationally existing and could be a mutated one.

9.5 Dengue Hemorrhagic fever

The emergence of dengue hemorrhagic fever in Pakistan is of concern not only for the health officials but also for the general public. Dengue hemorrhagic fever has caused many deaths for the last five years. Henceforth there is no concrete data available regarding its prevalence. The objectives of the study are to develop an effective vaccine against the virus. There is also need to develop rapid diagnostic methods for the early diagnosis of dengue haemorrhagic fever.

9.5 Bacteriological Status of Drinking Water from 100 Families of Lahore by Membrane Filtration Technique.

Water is the basic constituent of life. Contaminated water may affect the health of living beings. The pollutants of contaminated water may be pathogenic and cause diseases like “Gastro”. It is not practible to test water for all these organisms, an indirect approach is taken in assessing the risk of drinking water by enteric pathogen e.g.; coliform group especially *Escherichia coli*. Coliform are the members of Enterobacteriaceae which ferment lactose and produce gas at 37^0C but according to UK department it has also the ability to ferment ß-galactoside.

Water can be analyzed by MPN (Most Probable number) method, MF (Membrane filtration method), pour plate method and spread plate method. MF method has advantage over other method as it is requiring less labor and materials and giving results with in 24 hrs. Currently we are working on the topic of “Bacteriological Status of Drinking Water from 100 Families of Lahore by Membrane Filtration Technique.” The samples will be filtered by MF technique and then filters placed on the Chrome agar which has the ability to detect coliform and E. coli at the same time as they produce two different enzymes ß-galactoside and ß-glucoronidase respectively. As most *Escherichia coli* strains are harmless commensals in the human gut, but some strains are known to cause disease. The enterohemorrhagic *E. coli* (EHEC) strains of serotype O157:H7 causes hemorrhagic colitis, which may develop into life-threatening hemolytic uremic syndrome. In future we will use Polymerase chain reaction (PCR) in order to assess the presence O157: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 O157:H7 isolates from the numerous other serotypes that also produce SLTs enterotoxins.

9.7 Honey Research

The Department of Microbiology is very keen and enthuastic in conducting research on honey because Allah Almighty says in Holy Quran;

Your Lord revealed to the bees: “Build dwellings in the mountains and the trees, and also in the structures which men erect. Then eat from every kind of fruit and travel the paths of your Lord, which have been made easy for you to follow.” From inside them comes a drink of varying colors, containing healing for humanity. There is certainly a sign in that for people who reflect. (Surat an-Nahl: 68-69).

We believe that this naturally formulated medicine has got tremendous therapeutic potential and this wonderful product is still grossly underutilized. Alkhamdulillah we have recently evaluated antibacterial activity of honey against multi-drug resistant *Salmonella typhi* and this study has been presented in the 7th International German Apitherapy Congress, held in Passau, 27 - 31 March, 2009, Germany. To go further in this direction we have planned to investigate the role of honey against typhoid fever in mice model and Inshaallah looking forward to do clinical trial of honey against this disease in near future.

Besides we have also designed a clinical trial of indigenous Beri honey versus conventional treatment for the treatment of infected chronic wounds and burn cases. These projects would have Inshaallah social as well as economical implications nationally as well as internationally.

9.8 Research on Natural Products

"Human subtlety will never devise an invention more beautiful, more simple or more direct than does nature because in her inventions nothing is lacking, and nothing is superfluous."

Leonardo da Vinci

The Department of Microbiology has special interest in investigating the antibacterial potential of natural products.

We have already completed a number of projects in this regard and published our findings in national and international peer reviewed journals. Special mention for Black Seed extracts which demonstrated effectives against MRSA and Propolis extract against anaerobic pathogens.

Garlic extract has also been investigated against MDR *Mycobacterium Tuberculosis* and found to be extremely effective against these pathogens. Pomegranate extracts were also shown to have antibacterial activity against MDR *Salmonella typhi.*

Recently WHO has suggested that traditional medicine has been challenged wrongly in the past and it is a time for researcher to explore scientifically the hidden therapeutic potential of natural products. As these products are present every where, affordable and without side effects. The dream of health for all can

be achieved only by integrating the conventional and traditional methods of treatment.

9.9 Molecular Epidemiology of Metallo-Beta-Lactamase Producing *Pseudomonas Aeruginosa*

Pseudomonas aeruginosa is an important bacterial pathogen most frequently responsible for nosocomial infections, especially in immunocompromised patients. Carbapenems, are potent agents for the treatment of infections due to multidrug resistant *pseudomonads*. However, since last ten years, carbapenem resistance has been increasingly reported in *Pseudomonas* and *Acinetobacter species.* 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 ß-lactamases. Class A and D enzymes have a serine-based hydrolytic mechanism (i.e Serine Carbapenemase), while class B enzymes are metallo-ß-lactamases that contain zinc in the active site. Catalytic activity of MBL depends on zinc ions, and is lost if it is sequestered with EDTA. The metallo-ß-lactamases belong to the IMP, VIM, SPM, GIM, and SIM families and have been detected primarily in *Pseudomonas aeruginosa*; however, there are increasing numbers of reports worldwide of this group of ß-lactamases in the *Enterobacteriaceae*.

Metallo-ß-lactamases producing pathogens cause infections that are difficult to treat and have high mortality rates, due to their appearance in multidrug-resistant pathogens such as *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp.

The aim of the study is to describe the molecular epidemiology of metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa.* 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)

9.10 Susceptibility Pattern of Clinical Isolates of *Salmonella Typhi* against Aqueous Extract of Garlic

S. Typhi is the causative agent of typhoid fever, a severe systemic Salmonella infection with high morbidity and mortality, especially in developing countries. Infection occurs by the ingestion of contaminated water or food. The existing estimate of global burden of typhoid fever is 16 million cases and 600,000 deaths annually. Resistant against firstline antibiotics (chloramphenicol, co-trimoxazole and ampicillin) causes the emergence of multi drug resistant S.typhi strains, which is the major health issue world wide. Resistance to second line antibiotics (e.g fluroquinolones & quinolones) has also been detected. Cephalosporins resistance , due to the production of extended-spectrum β-lactamases (ESBLs), is an ever increasing problem. Poverty, which is major force that has been cited by theWorld Health Organization is actually involve in the development of antimicrobial resistance. To resolve this issue, we have diverted our attention towards the herbal and natural products. These products are effective as well as harmless. The efficacy of product will be monitored both by invitro method (agar incorporation technique) and by invivo studies (injecting in mice).

MSc Nursing

10.1 Workplace Stress in Nursing in Tertiary Care Hospitals in Lahore

Sress 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.

10.2 Educational Issues in Oral Care

Oral complications of cancer therapy influence both patient outcomes and fiscal outcomes. Patient awareness of the importance of oral health improves adherence to the oral care standard. However, the lack of knowledge of health professionals is a barrier to implementing patient education and oral care standards.

Objective of the study is to discuss the importance of patient, family and health care professional education in enhancing oral care and adherence to treatment regimens.

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

All patients admitted to a 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

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.

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

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

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

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 fluid 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.

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

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.

10.7 The Competencies and Preparedness of Nursing Students in Clinical Settings

Competent performance by health care professionals is expected throughout society. However, defining what it is and teaching students how to perform competently faces many challenges that nurses may confront in rapidly changing health care delivery system require that health care workers possess practice-defined competencies. With the rapid changes in health care, nurses are not only required to possess adequate knowledge or skills for their job, but also to be able to transform competencies to effective performance in new situation. The purpose of the study is to describe factors influencing nursing students' competencies and preparedness in clinical settings. The hypothesis of this study is that there is association between competencies and preparedness of nursing students in clinical settings.

The objectives are :

- To identify the relationship between the clinical settings and competencies, preparedness of nursing students.
- To examine the factors that contributes to a positive clinical experience.
- To explore differences in satisfaction, competencies and preparedness of nursing student in clinical settings.

Pharmacology

RESEARCH AGENDA

Focus point of the Pharmacology lab is to explore the molecular mechanism and pathways involved in disease development & persistence and ultimately search out the compounds having therapeutic effects. Most of the drugs being used today for the treatment of different ailments have been reported to possess many side effects, so there is an urgent need to switch towards alternative medicines, identify new compounds and to check their potency & efficacy in different diseases. Moreover, the goal is to design and conduct the mechanistic studies for the determination of mode of action of new and already proved alkaloids/compounds.

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

Cardiovascular diseases are one of the major health challenges and hyperlipidemia ia one of the predisposing factors. It 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 the present study is designed to investigate the antihyperlipidemic effect of Pakistani berry honey in patients with hyperlipidemia.

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.

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

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. In this study, 4-day old male albino rats will be divided into 3 groups, I, II. Group I will serve as control, Group II will receive DPP suspension for 35 days starting at 4^{th} day of age. At the end of experimental period 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.

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

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. The 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 and ginger 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.

If we 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.*

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

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. In this study, rats will be divided into four groups. 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. 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 *Carum carvi* seeds. 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.

11.5 Transdermal Drug Delivery System of Glipizid in Rats

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

$A_{1:}$ 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.

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

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. In the study 4 groups of rats will be taken. 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 for following.

- Serum Alanine Amino Transferase (ALT)
- Serum Aspartate Amino Transferase (AST)
- Serum Creatinine Kinase (CK)
- 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.

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

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. The study will be carried out on 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.

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

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. The 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 is 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 down regulating their expression.
4- BSO can inhibit the expression of CD28 molecule on T-cells
5- BSO may up regulate the expression of CTLA4 which might result in attenuation of T-cell activation and proliferation hence attenuating Th2 type inflammation.

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

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. Using a two-treatment, two-period, two-sequence, randomized crossover design, test and reference formulations will be administered as individual single doses to healthy male rabbits under non-fed conditions, with 4 days washout period between dosing. The 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.

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

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. 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.

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

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.

The 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.

Future prospects are:

1. Explore the therapeutic values of the indigenous flora of different parts of Pakistan and extend collaboration with different institutions in this regard.
2. Mechanism of development of drug resistance and measures to overcome drug resistance
3. Development of the efficient and cost-effective techniques for commercial drug testing & bio- Assays
4. Development of a fully equipped laboratory to test the commercial preparations on different Pharmacological parameters.

Physiology

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

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. 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. 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.

12.2 Expression of Circadian Clock and Cell Cycle Genes in Chronic Lymphocytic Leukemia

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. 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.

This cross sectional study will involve 60 adults; including 30 CLL patients and equal number of age and sex matched healthy individuals comprising the control group. 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. 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.

12.3 Predictive Role of Pulmonary Function Test among Asymptomatic Rheumatoid Lung Disease Patients

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 present in about 50 percent of patients. 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.

This cross sectional study will involve 80 known rheumatoid arthritis patients, who have no pulmonary symptoms and 40 age and sex matched healthy controls will also be taken. After taking complete history, physical and systemic examination, spirometry will be done. Three milliliters of blood will be taken from each patient, it will be centrifuged to obtain serum which 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 function test 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. This study will highlight the pulmonary involvement in rheumatoid arthritis patients

and pre-clinical decline in pulmonary functions among rheumatoid lung disease patients. This study will be of great benefit to both patients and physicians in the management of rheumatoid lung disease.

12.4 Comparative Study of Serum Anti-mutated citrullinated Vimentin Antibody and Rheumatoid Factor for Diagnosing Rheumatoid Arthritis

Rheumatoid Arthritis is a systemic autoimmune disease of unknown etiology characterized by chronic joint inflammation that often leads to joint destruction. Once established Rheumatoid Arthritis is a lifelong progressive disease that produces significant morbidity and premature mortality in many patients. Rheumatoid Arthritis has a worldwide estimated prevalence of 0.5-3%, 2-3 times greater in women than in men, most frequent during fourth and fifth decades of life. Rheumatoid Arthritis typically produces symmetrical swelling of peripheral joints of hand and feet, but may affect the large joints as well. Extra-articular manifestations of Rheumatoid Arthritis include cardiopulmonary disease, ocular disease, neurologic disease, Felty's syndrome, vasculitis, Sjogren's syndrome. As it is a systemic disease, constitutional symptoms like fatigue, low grade fever, malaise, anorexia and weight loss are common. 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. Disease progresses rapidly during the first two years of onset and can lead to irreversible joint destruction. Early therapeutic intervention results in earlier disease control and consequently less joint damage. Rheumatoid factor is the only serological marker included in the American College of Rheumatology criteria for the classification of Rheumatoid Arthritis. It has low specificity as it can be detected in sera of patients with 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) and anti-MCV antibody titers shall

be compared to evaluate the validity of these markers in diagnosis of the disease. The subjects will be selected from public sector hospitals and instituitions. After obtaining a written informed consent, thorough examination will be performed. The blood samples will be taken and secured in vacutainers. Serum will be extracted by centrifugation and stored at -20˚C till analysis. Rheumatoid factor and anti-MCV antibody titers will be determined by ELISA method. The data obtained to be analyzed by using SPSS version 16.0. It is expected that the results of the present study will help the clinicians in early diagnosis and timely management of this debilitating disease.

12.5 Serum Hyaluronic Acid as Biomarker of Disease Severity in Osteoarthritis

Osteoarthritis (OA) is a crippling disease of old age that is characterized by pain in the joints with resulting disability and progressive impairment of routine physical activity. Although before 45 years of age it is more common in men but its prevalence significantly increases in women after this age due to menopause. Generally obese individuals are more likely to develop osteoarthritis but other risk factors, such as trauma or congenital defects of joints, are also involved.

Osteoarthritis is conventionally diagnosed on patient's history, examination and x-ray of the involved joint. These methods, although easy and routinely performed to diagnose the disease and to predict its severity, are less sensitive and do not determine overall disease burden in patients of osteoarthritis. Moreover, frequent exposure to radiations by x-rays done for the diagnosis and determination of disease severity & therapeutic response, makes the patient vulnerable to potentially carcinogenic mutations in the body. Recently many biomarkers have been recognized in the serum of OA patients, which not only detect osteoarthritic changes even before they are evident on x-ray, but also determine the severity and overall disease activity of OA with much more accuracy. Serum hyaluronic acid (HA), which forms an important part of connective tissue in articular cartilage and synovial membrane, has been found to be one of the most important biomarkers in assessing disease severity in OA and its levels rise with the advancing disease, as determined by some recent studies. The aims of this study are to find an association between serum

hyaluronic acid levels & OA and to assess serum hyaluronic acid as biomarker of OA disease severity in local population.

It will be a cross-sectional study, for which 85 diagnosed volunteer patients of OA will be selected randomly from the hospitals of the city. Their blood samples will be drawn and the sera will be tested for the levels of hyaluronic acid by ELISA technique. The values obtained will be compared with the clinical stage of OA as well as BMI and Lipid profile, which is biochemical determinant of obesity (the 2nd most common risk factor for OA). The clinical stage of the disease will be determined by patient's history, examination an x-ray findings in the involved joint. The whole data will be entered and analyzed using computer software SPSS (version 17.0) and validation of serum HA as biomarker of disease severity will be assessed. A P-value of <0.05 will be considered statistically significant.

12.6 Growth, Obesity and Pubertal Development Pattern in School Girls of Province of Punjab

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 limits 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.

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) and Group III (14-16 yrs). 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. 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.

12.7 Iron Stores and Insulin Resistance in Healthy off Spring of Type-2 Diabetics

Type 2 diabetes mellitus is the predominant public health concern world wide, accounting for 90% of cases of diabetes globally. Most of the patients with type 2 diabetes are middle aged, not elderly and 80% of the cases are found in low and middle income countries. Currently type-2 diabetes is thought to occur in genetically predisposed individuals who are exposed to various environmental factors that precipitate the onset of clinical disease. Pathogenesis of type-2 diabetes involves insulin resistance, defective insulin secretion and increased glucose production by the liver. Insulin resistance is present several years before the onset of type-2 diabetes in genetically predisposed individuals and it results from multiple derangements in signal transduction in liver, muscles and adipose

tissues. Recent studies have revealed that insulin resistance compensated by hyperinsulinemia is positively correlated with increased 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 different redox reactions in the cells.

In the present study relationship between various iron parameters and insulin resistance of healthy off springs of type-2 diabetics will be determined and compared with the similar parameters of healthy off springs of non diabetics. Subjects will be selected by convenient sampling from registered cases of type-2 diabetes in different diabetic centers of Lahore. Written informed consent will be taken. Thorough examination will be done; blood samples will be taken and stored at -80º C. Blood tests will be conducted following standard procedures. Data will be analyzed using SPSS-16 software.

12.8 Comparative Study of Non-Invasive Parameters in Predicting and Grading Esophageal Varices

The chronic ongoing injury to the liver due to multiple reasons which 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 consumption in the west. Cirrhosis causes irreversible damage and commonly accompanied by portal hypertension leading to development of esophageal varices. Esophageal varices are present 60% in decompensated patients and 30% in compensated cirrhotic patients with a 17-57% mortality rate. 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. It will comprise 196 diagnosed patients of liver cirrhosis

irrespective of etiology and categorised on the basis of Child Pugh classification as A, B and C. 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 endoscopic findings. Statistical analysis of obtained data will be carried out with the SPSS version 16.0. The results of the study will help to determine the more precise non invasive parameter for the detection and grading of esophageal varices. This study will help the clinicians to pin point cost effectively those patients who are at high risk of bleeding, need strict follow up, hospitalization, prophylaxis and emergency interventions. This will help to prevent bleeding and in turn a drastic number of complications and will also help to reduce the anxiety and pain of patient which they suffer during endoscopy.

12.9 VDR Polymorphisms (*FokI, BsmI*) and Breast Cancer Risk in an Asian Pakistan Population.

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 two polymorphisms namely *Fok*1 and *Bsm*1 in the *VDR* gene and their relationship to breast cancer in Pakistani population. 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. 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. The study may find applications in risk assessment of breast cancer and may assist in modulating treatment therapy.