Project Check-1

1. **Disease & Population**

Lupus Nephritis is a systemic autoimmune disease. Systemic means that it can potentially affect all body organs and tissues. Autoimmune means that the immune system instead of fighting infections and tumours, it starts to attack body tissues. Genetic problems, sunlight exposure, hormonal reasons, and infections may be the reason for the immune system dysfunction.

Autoimmune B-lymphocytes produce autoantibodies such as antinuclear antibodies (ANA), anti-dsDNA, anti-Smith and antiphospholipid antibodies. These are often used by doctors to help make the diagnosis of lupus.

Autoantibodies bind self-particles like DNA, and cell proteins, The complexes they form (immune complexes) travel in the blood circulation and go to different organs. There they cause inflammation by different mechanisms including the activation of "complement factors" C4 and C3.

Systematic lupus erythematosus, or SLE, is an autoimmune disease that can affect nearly every organ system and has a wide range of clinical presentations. One of the most dangerous side effects of SLE is kidney involvement. There is still much to learn about the mechanisms of action underlying SLE and its complex etiopathogenesis. The risk of SLE is an excessive generation of radioactive debris as a result of aberrant and massive apoptotic events that are mistaken for foreign bodies. This abnormal antigen presentation eventually results in a loss of tolerance to B and T cells. Due to this loss of tolerance, T cells become hyperactive, which in turn causes the production of inflammatory cytokines. B cells also become hyperactive, which results in the massive production of autoantibodies and the development of immune response

Most common symptoms:

- Joint pain, stiffness in the morning, and swelling (mostly hands)
- Skin rash after exposure to the sun (butterfly rash)
- Chest pain with deep breathing (pleurisy or pericarditis)
- Leg swelling due to lupus nephritis

Less common symptoms:

- Muscle weakness,
- Low blood count (anaemia), bruising (low platelets), blood clots Seizures, psychosis, stroke

Lupus nephritis (LN) is the most important organ involvement in SLE.Up to 30-50% of lupus patients may develop nephritis (usually within 3 years from diagnosis). Inflammation in the kidney causes protein leakage in the urine (proteinuria), leg swelling (edema due to fluid retention), and worse kidney function (measured by serum creatinine test) Lupus nephritis tends to come back even after it gets better Repeated flares cause scarring in the kidney (not reversible) Up to 20-30% of patients with Lupus Nephritis may develop renal failure and will require a kidney transplant or dialysis(haemodialysis or peritoneal dialysis) Only rarely it can happen again after kidney transplantation

Diagnosis of Lupus Nephritis symptoms

- New/worse high blood pressure (hypertension)
- Edema of feet and/or around eyes.
- Polyuria ornocturia
- Frothy urine (proteinuria); Urine discolouration (haematuria)
- It may have no symptoms(Urinalysis is critical)

Blood tests:

- Serum creatinine (normally <1 in the average woman; it may be higher in muscular men as it reflects muscle mass).
- Albumin (normal>3.5) which may be decreased due to loss of protein in the urine.
- Electrolytes: sodium, potassium, bicarbonate, fasting lipids (often high), glucose, vitamin D
- Estimated Glomerular Filtration Rate (eGFR). Calculated by using creatinine, age, and gender. Normally 90-120 ml/min/1.73m2
- Complete blood count (CBC) to check for anaemia or low counts of white blood cells and platelets
- Tests for exposure to Tuberculosis , hepatitis B/C, or HIV

Urine tests:

- Urinalysis. Normally 0-trace protein, no red and white blood cells (<5 RBC, <5 WBC)
- 24-h urine protein (we also measure creatinine to assess whether the collection was performed properly): normally <150 mg/24h. In Lupus by definition>500 mg/24 hour. If the urine protein is >3,500 mg/24 hours, we call this nephrotic range proteinuria
- Urine protein/creatinine ratio. Either spot or 24-hour urine collection.

Renal Ultrasound: to exclude urine flow obstruction, scarring of the kidney or other causes of kidney disease

Kidney Biopsy :

- It helps confirm the diagnosis of lupus nephritis and exclude other causes of kidney disease
- It helps establish the class of lupus nephritis as management is different
- It helps assess the amount of activity/inflammation (reversible) versus the amount of chronicity/scarring (irreversible): Activity index (Al) and Chronicity index (CI)

Risks:

- Kidney biopsy is a low-risk procedure. Patients can go home the same day if no complications
- Under ultrasound or CT scan imaging, a needle is inserted into the lower part of one kidney and a tiny tube-like piece is taken for microscopic analysis
- Blood pressure should be well controlled and anticoagulants held to minimize the chance of bleeding around the kidney.
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Classification of Lupus Nephritis :

- Class I. Minimal mesangial lupus nephritis (LN)
- Class II. Mesangial proliferative LN
- Class III. Focal LN (less than 50% of glomeruli are involved)
- Class IV. Diffuse LN (more than 50% of glomeruli are involved):
- Class V. Membranous LN
- Class VI. Advanced sclerosing LN

A patient might have either:

- Class III/IV LN, or Class V LN or
- both Class III/IV+Class V
- Class III or IV nephritis (with or without Class V) is worse than Class V LN because it often affects kidney function. It requires more aggressive treatment
- Class V nephritis (membranous) without Class III/IV LN causes proteinuria without affecting kidney function in the beginning.

- However, Class V can cause severe (nephrotic) proteinuria, with often very bad leg edema, and high cholesterol, and may cause a blood clot. This needs to be treated more aggressively.

Population:

- Lupus is much more common in women than in men and most often strikes during the childbearing years. Nine out of 10 people who have lupus are women.
- Lupus is also more common in people of African or Asian background. African Americans and Asian Americans are about 2 to 3 times more likely to develop lupus than Caucasians. In the United States, 1 out of every 250 African American women will develop lupus.
- SLE (systematic lupus erythematosus) is a disease of young or middle-aged women. About 1 per 1,000-5,000. The ratio of females to males affected is 9:1
- The disease is more frequent and often more severe in non-Caucasians.

DATA ACQUISTION

"Data for this project was obtained from the NCBI Gene Expression Omnibus (GEO) public repository. We searched GEO using keywords ‘lupus nephritis’, ‘chronic kidney disease’ , ‘Homo Sapiens’and certain criteria such as a sample size of above 50 and gene expression profile. The selected dataset (GEO Series accession number: GSE200306) profiled gene expression in glomerular tissues and controls. The series matrix file was downloaded for analysis and normalised glomeruli expression data was also obtained from database.

The overall design used in the dataset was 58 paired kidney biopsies from patients with proliferative lupus nephritis (class III, class IV or class III/IV+V) that were initially evaluated. This is a well-defined, well-curated dataset with clinical and histologic data available for all patients. Glomeruli and tubulointerstitium (TI) were isolated separately and samples with adequate RNA were submitted for nanostring analysis. Overall, 70 glomerular samples and 92 TI samples were submitted for nanostring analysis (35 glomerular and 46 TI pairs). Pre-implantation donor kidney biopsies (n=10) served as healthy controls.

Data processing:For nanostring data, raw counts were normalized to the positive spike-in controls and then log2 transformed. To reduce technical bias, genes with an expression level below the mean plus two standard deviations of the negative controls were filtered out. Quantile normalization was applied to the remaining transcripts (522 for glomeruli and 502 for TI). Linear mixed-effect models were used to identify differentially expressed genes by taking into account the correlation between repeated measures before and after treatment. To improve the stability of variance estimation, moderated t-tests were employed for differential expression detection.

PROJECT MANAGEMENT:

The data chosen has 181 samples out of which 79 glomeruli tissue samples were taken for this analysis 28 class 3 Lupus Nephritis and 31 class4 Lupus Nephritis and 9 control samples of glomeruli tissues were further considered for Data preprocessing and exploration to find potential targets for Lupus Nephritis by finding genes up or downregulated in the pathway leading to calcineurin activation. A phosphatase called calcineurin is involved in the synthesis of inflammatory mediators by T cells as well as the upkeep and appropriate operation of the glomerular filtration membrane, both of which are critical for the development of lupus nephritis. As a result, calcineurin inhibitors (CNIs) are a promising tactic for blocking T cells, and in recent years, CNI-based research and clinical trials have grown dramatically. The use and mechanisms of action of CNIs as a therapeutic approach for LN are the main topics of this review, along with the role of calcineurin in the pathogenesis of SLE.

1. Data preprocessing

- Data Manipulation:The following R packages were used:
  - Biobase
  - dplyr
  - tidyr
  - GEOquery.

- Data Normalization:
  - limma
  - Biobase

- Data Visualization:

  - ggplot2
  - RColorBrewer
  - geneplotter
  - pheatmap.
  - enrichplot
  - EnhancedVolcano
  - umap

    Others used:
    clusterProfiler (analysis): Designed for functional enrichment analysis of gene sets.
    org.Hs.eg.db (annotation): Provides gene annotations for the human genome.
    AnnotationDbi (database access): Facilitates access to annotation databases.

2. Exploratory data analysis:
   - Volcano Plots
   - Pheatmaps
   - Functional enrichment analysis

3. Differential gene expression analysis:

   - linear model statistical testing
   - multiple testing through FDR control.