NATIONAL SYSTEMIC LUPUS ERYTHEMATOSUS PROSPECTIVE COHORT.

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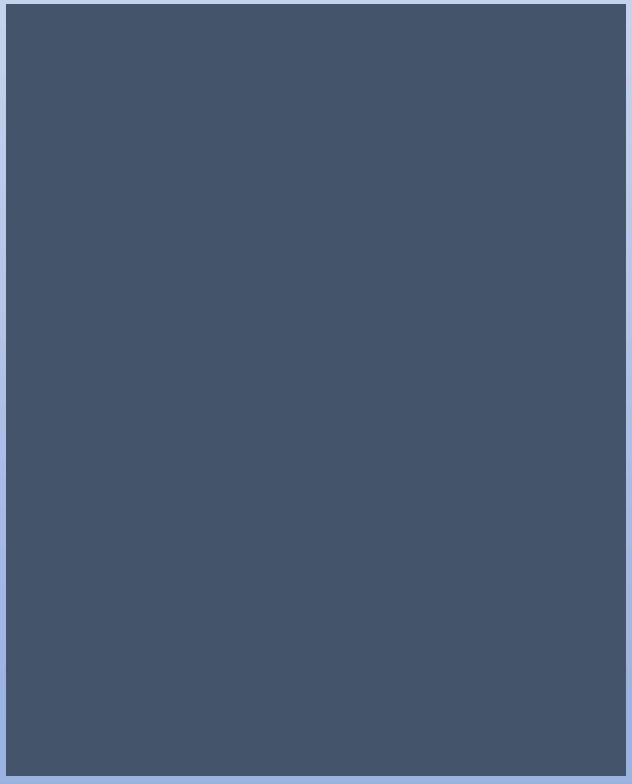
National Systemic Lupus Erythematosus Prospective Cohort, Saudi Arabia

Study proposal

Key Words: SLE, Lupus erythematosus, Observational, Outcomes, Phenotypes.



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ABSTRACT

Systemic lupus erythematosus (SLE) is chronic multifactorial autoimmune disease that affects many organs in human body. SLE patients have three times increased risk of mortality based on international data. Ethnic variation is known feature of the disease with important impact on patients mortality and morbidities. Descriptive studies from Saudi Arabia showed variation in clinical features from one region to another. However reliable inference from these studies is limited by study methodology, study size and lack of prospective cohorts. In addition, translational studies using biological samples to understand clinical phenotypes of SLE and response to treatments are lacking in Saudi Arabia and perhaps in Arab region. This is a proposed longitudinal prospective cohort study of Saudi SLE using open cohort study design to examine clinical characteristics and molecular phenotypes of Saudi SLE patients in relation to local environmental factors and local practices. We also aim to use the cohort as a nest for pragmatic randomised trial comparing efficacy of current treatment regimen of SLE in

Saudi population in the near future.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic multi-system disease in which an underlying aberrant immune system leads to inflammation in various organs, and possibly consequent damage(1). Incidence and prevalence of the disease vary significantly from 1 -7 per 10000-patients to 19- 159 per 10000patients respectively in the united states(2-4). The estimated incidence in the united Arab of emirates is around 3.5 per 100000- patients and the prevalence is 109 per 100000 patients, while in Saudi Arabia the prevalence is

around 19 per 100000 patient. (1, 2) Such variation is likely due to use of different definitions, studies' standardization of age and racial background(2). Ethnicity is an important variable in disease occurrence, severity, phenotypic presentation and prognosis(5). For example; patients with African, Hispanic and Native American tend to have more severe disease and consequently increased mortality(5-7). Unfortunately, national Saudi studies in SLE are sporadic and limited in addressing our unique population in prospective standardized fashion. The majority of the studies use retrospective chart review based designs that are threatened by potential selection and information biases. They also lack molecular and genetic data of Saudi patient with SLE. As a result, our understanding of Saudi SLE patients is limited. The knowledge gap can be categorized in several categories including:

- Descriptive clinical and molecular phenotype along with associated genotype.
  Drug response, side effects and associated signature profile (molecular and genotype)
  Outcomes of disease and its associated complications.
- Patients related research including patient related outcomes and qualitative type of research in Saudi population.

The importance of a national prospective cohort study to address the above mentioned knowledge gap is high, especially in the context of drug development and related clinical trials in which we are underrepresented.

OBJECTIVES

The objectives of this cohort is to study Saudi SLE patients for the following:

1. Understand the molecular and genetic profiles, epigenetic, immunological and microbiome alteration in relation to various SLE phenotypes in Saudi Population.
    a. Conduct whole exome sequencing for SLE patients to identify mendilian phenotypes including novel ones by enriching sequenced patients with patients who have strong family history of the disease.
    b. Develop a baseline Saudi polygenic risk score for our SLE patients and compare it with Caucasian risk score in order to identify novel variations among our population. In addition, trying to explore the relationship of polygenic risk score and important clinical outcomes (disease remission, disease activity, disease damage and mortality).

Identify genetic and molecular signatures. (baseline genetic, proteomic and microbiome that differentiate various SLE clinical phenotypes from one another. By doing so, we might be to better differentiate various phenotypes and understand pathological mechanism behind the difference in clinical phenotypes.

2. To assess effect of environmental, social, medication and comorbidities on SLE important disease related outcomes including disease activity and disease damage.
    a. Examine the relationship of presence of comorbidity (in particular diabetes, HTN and dyslipidemia on disease activity, disease damage and mortality.
3. To assess molecular and genetic/epigenetic signatures of response in relation to various treatment regimens in SLE.
    a. Identify genetic and molecular medications related signatures. (genetic, proteomic and epigenetic modifications and microbiome alteration associated with changes in disease activity states from active to remission). This will allow us to have a holistic approach to SLE pathogenesis and may take us step further toward much more personalized medicine.
4. To assess long-term important outcomes of Saudi SLE population including determinants of disease damage, recurrent hospitalization and mortality.
5. Utilize the cohort as platform for nested pragmatic clinical trials within the cohort using cohort multiple randomized controlled trial design in the near future.

The cohort will focus on addressing all these aims in context of molecular, genetic, epigenetic and mycobiome data that will be collected periodically.

RATIONAL

SLE disease in Saudi Arabia is yet not well defined especially in a population with high consanguinity and high inbreeding coefficient(3). Up until now, there has been no prospective cohort study for SLE patients in Saudi Arabia. As a result, current published literature is focused on retrospective chart reviews which are subjected to many forms of bias(4-6). In addition, these type of studies are difficult to utilize for translational research where clinical data are complemented by biological data collected at the same time. Studying disease characteristics in Saudi will provide better understanding of disease presentation, course

and outcomes especially if complemented by detailed immunological, molecular, genetic and microbiome data.

METHODOLOGY

This proposed prospective registry that will follow open cohort study design. The following section details the inclusion and exclusion criteria, variables to be collected, biobanking and data management.

PATIENTS RECRUITMENT AND ENROLLMENT:

Patients will be recruited from KSU-SLE specialized clinic. The clinic receive general referral from general rheumatology clinics in KSU and general referral from other clinics inside or outside KSU.

Inclusion criteria is the following:

1- Adult patients defined as > 18 years old.

2- Patients should fulfill one of the following classification criteria for SLE (ACR, SLICC or ACR/EULAR criteria).

3- No restriction on time of diagnosis.

Exclusion criteria:

1- Patients who don't fulfill classification criteria mentioned above.

COLLECTED VARIABLES:

Demographics

- Patient's ID (national, research, date, visit) sex, relationship status, educational level, work, gross monthly income, House (apartment, House) and house labor.

Exposures

- Family history (of SLE, other seropositive diseases or autoimmunity)
- Environmental (smoke inhalation, smoking, sun exposure, fasting, level of activity).
- infections (Bacterial (T.B, PJP, C.diff, altered gut microbiome, H-pylori), Viral (hepatitis B, C, HIV,

  CMV, EBV, HSV, VZV) fungal (candida), protozoal)

- medications/supplements (conventional DMARDs, Biological DMARDs, Synthetic DMARDs and other medications used for comorbidities)

Examination related variables

- The measurement of weight
- Hight
- Blood pressure
- 66 Joint count (swollen, tender) Back exam.
- Skin and mucosal exam
- CVS
- Respiratory
- Abdominal
- Neurological.

Disease outcomes

- Disease activity (system based from head to toe) (see standardized data collection form.
- Disease related damage.
- Cardiovascular disease (MACE), surrogate outcomes (carotid artery plaques using U.S), PAD.
- Osteoporosis (DEXA, fragility fracture (radiographic and symptomatic)).
- Malignancy.
- Renal failure requiring renal replacement therapy/renal transplant.
- Cognition (MME, MOCA) Death/mortality.

Patient reported outcome

- PROMIS (version PROMIS-29 Profile v2.1) which include the following the assessment domains:
    - Physical Function o Anxiety o Depression o Fatigue o Sleep Disturbance
    - Ability to Participate in Social Roles o Pain Interference
- LUPSQUAL

DATA COLLECTION PROCESS AND STORAGE:

Data will be collected using standardized data form (soft version is in the process of development). The form will be filled by trained individual (clinician or nurse joining the site) upon their joining of the clinical site. Training session will be conducted in standardized fashion using the glossary key for collected variables. Upon completion of data collection in each clinical visit, data will be transferred and stored in data storage program (Redcap, access or CAISIS). Data entry, cleaning, confirmation and retrieval will be handled by hired research coordinator.

Data will be collected regularly every 3 months (2-4 months). Research coordinator will ensure patients adherence to follow up protocol including attendance, data collection completion and rescheduling for missing visits.

Loss of follow up will be handled by direct communication with patients. Reasons of loss or missing follow up will be obtained using standardized data collection form through phone. Reasons include: death(reason of possible), being sick (type?), hospital admission(reason and where, records of admission should be retrieved), work, study, forgetfulness, travel, moving from city, loss of interest (due to symptoms remission, perception of poor management, follow up with another rheumatologist (why?), no specific reason).

DATA QUALITY AND DATA VALIDATION:

Data will be validated for each variable using chart reviews, blood workup or hospital reports to ensure accuracy regularly (at least every 3 month). Data checkup will be conducted yearly by choosing random sample of participants, comparing data with patient chart and measuring percentage of missing data.

DATA STORAGE AND MANAGEMENT:

All patients data will be collected from the clinics through our research coordinator and then transferred to unique patient file that will be labeled by patient study number. Data will be entered in data manager program (KSU based – CASIS) through a collaboration with college of medicine research center-clinical research support unite. The primary investigator will have the key for patient identification. In the absence

of the primary investigator, data management will be transferred to KSU-CASIS database manager until a new Investigator is recruited or decision about study termination is taken by the coinvestigator.

DATA AND AUTHORSHIP RIGHTS:

All patient data is govern by protocol committee. This committee is consistent of principle investigator and co-investigators. Each member is entitled for his own patients' information (clinical and biological stored samples), however for all cohorts participants, data retrieval will be granted by the committee once study proposal is submitted and reviewed. A primary investigator vote along with one more coinvestigator votes will be required for data to be retrieved. Turnover for study review and approval is two weeks. Principle investigator will be required to choose a successor if he decided to leave the institute.

GLOSSAEY DEFFINTIONS:

Please see the attached appendix for definition of variables in data collection form.

BIOLOGICAL SAMPLES AND BIOBANKING PROCESS:

Each patient will have two comprehensive visit during which 4 heparin blood tubes will be collected from the patient for biobanking storage (exception will be for pregnant ~~SLE~~ patients who will have biological sample collection at the beginning of each trimester along with postpartum sample (six weeks from delivery). Once the blood is withdrawn, it will be sorted into: plasma separation, (immune cell sorting using flow cytometry to into major cell line including T-cells, B cells, mononuclear cells and granulocytes/versus storage as PBMC – the decision will be guided by available fund), RNA and DNA then stored at appropriate temperature using patient unique research identification number.

For DNA processing; blood will be collected using standardized phlebotomy technique directly into PAXgene Blood DNA tubes. Sample will be processed in 24 hour in the collaborated lab using PAXgene Blood DNA Kit (please refer to the DNA purification protocol in appendix). After completion of purification step, DNA will be stored in -80 until further utilization. Note that labels with patient study number will be written on the tubes and entered in data management program with location and time of processing in addition to storage location.

For RNA processing; blood will be collected using standardized phlebotomy technique directly into PAXgene Blood RNA Tube. The sample will be then stored in -20 until further utilization in future projects. PAXgene RNA kit will be used to purify RNA and then analysis will be carried using RT-PCR.

For plasma processing; blood will be collected using standardized phlebotomy technique directly into EDTA tube, sample will be centrifuged and buffy coat will be isolated, washed and stored as PBMC while remaining plasma will be transferred to cryovials to be stored into -80 until further use.

Please note that total amount of blood is 5 EDTA tubes that will be collected from the patient in the two visits, two will be used for routine clinical work and rest will be utilized for biobanking as detailed above. In addition, microbiome data will be collected and stored using RNALater. Patient self-collected fecal samples will be stabilized in RNAlater supplied container which contains RNA and DNA stabilizers. Material will be mixed and allocated in several aliquots that will be stored in -80 until further use.

Sample processing will be handled in collaboration with prince Nayef immunology lab and CMRC core lab facility in complete adherence with standard operational procedure that include sample labelling using patient study number and date collection and processing. Once college of medicine biobank open, all samples will be transferred there for storage and future handling. Sample location, date of separation will be recorded by research coordinator in the data management program.

CONSENT FORM AND CONSENT PROCESS:

Patients will be consented using patient centered consent form. In this process, patient will be consented by the research coordinator using standardized form (please see supplementary appendix).
Once consented, data will be collected and stored as described above.

The consent form includes three parts:

- Consenting on enrollment of observational cohort and use of data for future research.
- Consenting on collection of biological samples (serum, immunological, genetic and microbiome) for future research projects.

BUDGET AND SUPPORTING SERVICES

Estimated cohort enrollment is 40-50 new patient/year. Each patient will have two biological data collection for biobanking and four clinical data collection/year. The budget of this prospective cohort will be divided into:

- Research coordinator. (fulltime annual salary: 72,000)
- Data management and storage program. (in collaboration with Deanship of electronic communication, KSU innovation hub and CASIS, estimated coast is 10,000)
- Biobanking (Freezer, coast of blood withdraw, tubes, serum and cells separation, cells sorting, samples storage and microbiome collection and storage. (estimated coast is 70,000/freezer, 5000/year for samples biobanking).

Upfront coast for the first year is estimated to be 150,000 Saudi riyals. Estimated annual coast is around 80,000 Saudi riyals. Multiple grant approach will be used to maintain the fund until more secure mean of fund is allocated (such as a research chair).

NATIONAL AND INTERNATIONAL COLLABORATION

The cohort aims to be part of national and international cohorts collaborations. In setting of collaboration, only primary investigator will be included in authorship from this cohort unless other coinvestigator contributed to study in literature review, design, analysis, interpretation of the results or preparation of manuscript. Ideally, authorship should be discussed in advance among all investigators to avoid any conflict. Data sharing agreement will be required before the initiation of any collaboration in accordance to KSU-IRB standard procedure.

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