The Wael Al Mahmeed & IAS Research Training Grants and Fellowships

Project Title: Atherogenic Markers in Podocyte Injury Model of Focal Segmental Glomerulosclerosis.

Background and Study Aims:

Focal segmental glomerular sclerosis (FSGS) is a chronic kidney condition characterized by fibrosis of the filtration unit of the kidney, the glomerulus. It is the primary glomerular disease in the USA (reported in over 40% of biopsies), and in Asian Arab countries[1,2,3]. The disease most often terminates in renal failure and reoccurs in 25% of transplant receivers [4,5].

It is has becoming increasingly recognized that chronic kidney diseases, including FSGS, are commonly associated with an increased risk for atherosclerosis (AS) and cardiovascular disease (CVD). FSGS patients have an augmented risk for developing cardiovascular disease and a higher prevalence of cardiovascular burden compared to the general population. A diagnosis of FSGS is often associated with clinical findings of classic CVD risks including dyslipidemia and hypertension [6]. Additionally, several studies have reported non-classic CVD risk such as altered cardiac geometry and function including increased intimal media thickness, a surrogate marker for developing atherosclerosis [2,7,8]. Collectively, clinical implications of FSGS and associated risks place the FSGS cohort at grand risk for overt cardiac outcomes and clinical complications.

Typically, screening for atherosclerosis requires high infrastructure and specialized personal to perform mildly invasive procedures. As an extension of a larger project, we aimed to investigate molecular markers for atherosclerosis in an induced mouse model of FSGS. Circulating adhesion molecules; soluble intracellular adhesion molecule-1 (sICAM-1) and soluble vascular adhesion molecule-1 (sVCAM-1) have long been reported as excellent diagnostic markers due to their elevated levels in AS due to their significant association with its development and progression [6,8,9,10]. This would provide a simple, and cost-effective method to diagnose AS in a heterogeneous population.

Research Objectives:

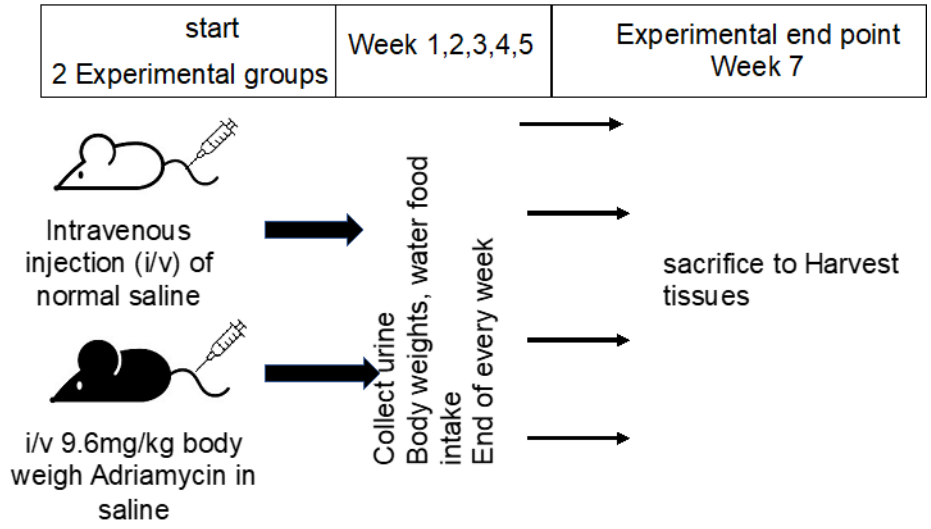
- Determine levels of soluble forms of adhesion molecules in the serum of FSGS model mice in comparison to healthy controls.
- Present findings in an international meeting.

Research Question:

- Can plasma levels of sICAM-1 and sVCAM-1 be used as predictive and diagnostic markers of atherosclerosis in FSGS?

Methodology Brief:

- Animal Study:



- Mouse-specific ELISA kits were run to quantify serum levels of the molecular markers.

Results

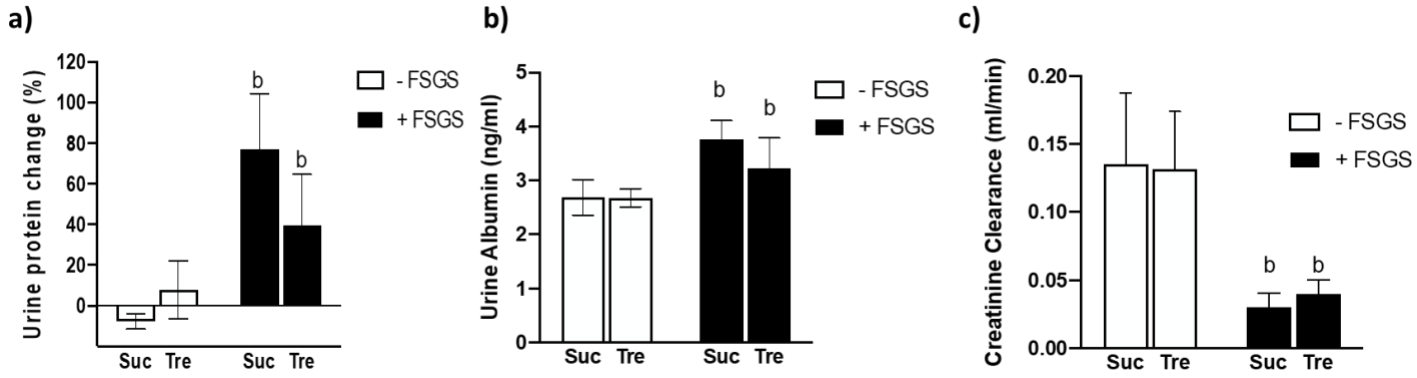


Figure 1. *Percentage protein change in urine of control and treatment mice (a) levels of tested kidney function markers albumin (b) and creatinine clearance (c).*

1. **Model Confirmation.**

Urine samples were collected weekly. Protein concentration in urine was measured by Bradford assay. Changes in protein levels in urine of study animals were monitored to inspect proteinuria development, a hallmark of FSGS. Protein levels in urine were found to progressively increase in FSGS mice compared to the control, denoted by the overall percentage urine protein change in Figure 1a.

Further, kidney function was assessed by measuring well known markers including albumin and creatinine clearance. As shown in Figure 1b and 1c, the elevated urine albumin levels and contrarily low clearance of creatinine in treated mice are indicative of compromised integrity of renal filtration and kidney damage.

2. **Serum levels of proposed atherogenic markers in FSGS.**

Mouse specific-ELISA kits were used to measure levels of sICAM-1 and sVCAM-1 in serum samples from study animals following protocols provided by the manufacturer (Invitrogen). Levels of both markers were found to be lower in AD-mice (FSGS+) compared to the control group (Figure 1). This rejects our hypothesis and suggests that levels of the adhesion molecules are potentially weak atherosclerotic markers in FSGS. Further experiments are needed to provide a better statistically supported conclusion.

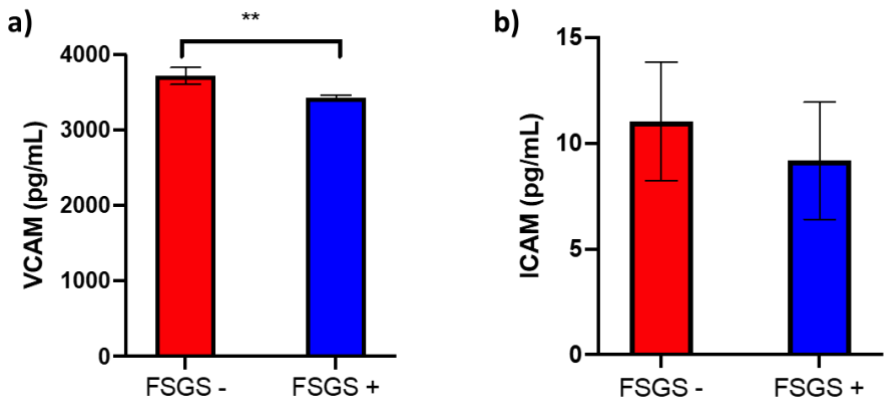


Figure 2. *Measured serum levels of sVCAM-1 (a) and sICAM-1 (b) in serum of control and treatment mice.*

Poster presentation

Findings of the study were presented at the 91st European Atherosclerosis Congress in Mannheim, Germany in May 2023.

Outcomes and Conclusion

Successful induction of FSGS in Balb/c mice post injection with Adriamycin was confirmed by progressive development of proteinuria and abnormal kidney function markers in urine of FSGS mice. Contrary to our prediction, our data shows higher levels of sICAM-1 and sVCAM-1 in control mice compared to FSGS+ mice. This rejects our hypothesis and suggests the adhesion molecules as weak prognostic markers in FSGS.

Prospects

Further studies running more replicates and experiments are needed to provide a statistically supported conclusion of our findings. Following experiment will aim to measure levels of lipid markers (low-density lipoprotein and very low-density lipoprotein cholesterol) to test for correlation.

Additional Work

As mentioned previously, the described worked was an extension of a larger project lead by the P.I. Dr. Fahad Al Zadjali (supervisor). The project was designed to determine the effect of

Trehalose treatment on FSGS. The project was an excellent chance to expand my personal skillset as several laboratory investigations were used throughout the duration of the study. These included working with mice, sample collection and processing, and running Bradford assays, which were run weekly to monitor disease progression.

Further, after sacrifice, harvested tissue samples were used to extract RNA, DNA and protein for further investigations. Over 20 PCRs were run to test the effect of the treatment on genes related to kidney function, inflammation, metabolic and other cellular pathways, etc. Further investigations were conducted using colorimetric assay kits, ELISAs, and western blot analysis. Additionally, kidney sections were analyzed by electron microscopy and immuno-histological assessment. Results of the study will be published soon.

Acknowledgment

I would like to acknowledge and thank Dr. Wael Al Mahmeed and the International Atherosclerosis Society for the opportunity and continuous support. Additionally, I would like to extend my acknowledgment and thanks to Dr. Fahad Al Zadjali for his kindness and generous support as well as staff members of the Biochemistry Department at Sultan Qaboos University.

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