RGC Ref. No.:
UGC/FDS17/M04/16
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:
1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Characterising and Fingerprinting Biomarkers of Urolithiasis: A Case Control Study (Stage 1 of 2)

2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	**Name / Post**	**Unit / Department / Institution**
Principal Investigator	Mayur Danny I. GOHEL / Professor	School of Medical and Health Sciences / Tung Wah College
Co-Investigator(s)	Chi Fai NG / Professor	SH Ho Urology / Surgery / CUHK
Others	N/A	N/A

3. Project Duration

	Original	**Revised**	**Date of RGC / Institution Approval** *(must be quoted)*
Project Start Date	1 January 2017	--	
Project Completion Date	31 December 2018	--	
Duration *(in month)*	24 - months	--	
Deadline for Submission of Completion Report	31 December 2019	--	

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To identify the important biological markers and mediators of inflammation in the urine of patients with renal stones.

2. To test for levels of the selected biomarkers (as identified by Obj. 1) and to validate the results from patients in different groups of stone formers (active and post-ESWL/PCNL), compared to the results from non-stone formers.

3. To conduct a longitudinal study (Stage 2) using the reliable indicators (as identified by Obj.2) showing how advanced warning of the clinically significant recurrence of stones can enable appropriate treatment, thereby bringing both financial and health benefits.

4. To train undergraduate/postgraduate students in biochemical and immunological techniques and knowledge, and to enhance the research capacity of the institution.

5.2 Revised objectives

Date of approval from the RGC: NIL

Reasons for the change:

1.

2.

3. ....

5.3 Realisation of the objectives
(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

The project had set out with 4 Objectives and within 2-years, all the Objectives were achieved with 100%.

The *1st objective* was to evaluate and select a panel of the most promising cytokines, mediators and biochemical markers of blood and urine. The short-list of the biomarkers was made with reference to the available literature, PI's own previous research, feedback received from journal referees and conference presentations from the period of 2012 to 2018. These markers should be easily measured and available on a regular basis when patients come for follow-up consultations so that an effective biochemical work-up can be recommended. Four inflammatory cytokines – IL-1α, IL-6, IL-8 & MIP-1α and two renal biomarkers – hyaluronan (HA) and NGAL / NAG were short-listed for the study within the budget of the grant allocated. Towards the end of the project (Oct – Dec 2018), the PI was invited to give a Plenary lecture at Stone Disease meeting (Experts in Stone Disease, Shanghai Nov. 30 – Dec. 1, 2018), feedback was received from the Clinicians to include Kidney Injury Molecule – 1 (KIM-1) as a more sensitive acute injury marker. Even though the project was ending, there was still sufficient budget to purchase this kit and include this in the panel. (Selection and testing of biomarkers – 100% achieved)

The *2nd Objective* was to delineate the groups of patients and expected numbers within the group to achieve statistical significance (power of 80%) in consultation with the Dr. Ng at Prince of Wales Hospital (co-I of the project). Six groups were decided and the rationale behind these are briefly explained:

(1) **Active stone-formers** (CT/radiographic evidence), before ESWL/PCNL and without evidence of urinary tract infection or pyuria > 5 wbc/hpf.
(2) **Active stone-formers** (CT/radiographic evidence), before ESWL/PCNL and with positive urine culture and bacteria counts.
(3) **Active stone-formers** (following ESWL/PCNL), without urinary tract infection*.
(4) **Non-stone-formers** (CT/radiological and ultrasound evidence) – Patients with urinary tract infections.
(5) **Non-stone-formers** – Patients with bladder cancer undergoing bacillus Calmette-Guérin (BCG) immunotherapy (which is known to stimulate HA production).
(6) **Non-stone-formers** – Normals, who have normal urinalysis, negative urine culture and no known urologic history.

The rationale for evaluating the panel of cytokines and inflammatory mediators in these six groups is to narrow down and select the important markers (in addition to urinary enzymes and HA) that would signal a "forming" stone causing sub-acute inflammation. In all, 166 subjects were recruited and grouped into the above 6 groups equitably. The most difficult subjects to recruit was Group 4, nevertheless, we were able to achieve the required minimum number to reach 80% power. The levels of the selected biomarkers were tested and the results from the above 6 groups of participants had been validated and compared. (The groupings, subjects recruited and biomarkers tested – 100% achieved)

The *3rd Objective* was to design the longitudinal study (Stage-2) based on the successful completion of Stage 1 of the study and achieving the Objectives 1 & 2. The PI is glad to report that a FDS grant for Stage-2 was approved in the 2019/2020 cycle to start in January 2020. (Receipt of further research grant to conduct Stage-2 of the study – 100% achieved).

The 4th Objective was to train undergraduate / postgraduate students in biochemical and immunological techniques and enhance the research capacity of the institution. The PI had recruited two research staff on the project a Medical Science graduate and a Chemistry graduate to train under him. In addition, the PI had incorporated parts of the project (ELISA, Chemical tests, Urinalysis) in training final year Honours project students. Two cohorts (4 students) were trained under this on different aspects and one student did his final year dissertation. The two research staff under this grant will have papers and presentation s

co-published with the PI as a recognition of their work and contributions in the project. (Training of undergraduate / postgraduate students – 100% achieved).

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. *To identify the important biological markers and mediators of inflammation in the urine of patients with renal stones.*	✓	100%
2. *To test for levels of the selected biomarkers (as identified by Obj. 1) and to validate the results from patients in different groups of stone formers (active and post-ESWL/PCNL), compared to the results from non-stone formers.*	✓	100%
3. *To conduct a longitudinal study (Stage 2) using the reliable indicators (as identified by Obj. 2) showing how advanced warning of the clinically significant recurrence of stones can enable appropriate treatment, thereby bringing both financial and health benefits.*	✓	Funding for Stage 2 Approved to start on 1 January 2020.
4. *To train undergraduate /postgraduate students in biochemical and immunological techniques and knowledge, and to enhance the research capacity of the institution.*	✓	100%

6. Research Outcome

6.1 Major findings and research outcome
(Maximum 1 page; please make reference to Part C where necessary)

The first major finding was investigating urinary glycosaminoglycans (GAGs) and Hyaluronan (HA). The presence of HA is now well established, as a secondary effect to a forming stone. HA is suggested to be one of the significant mediator of inflammation as it activates a host of cytokines such as IL-1β, TNF-α and insulin-like growth factors. In this study, urine samples from 166 in total from stone-formers (SF) and normal controls (NC) were collected and urinary GAGs were isolated and HA was determined as a fraction of total GAGs by HPLC. It was found that and re-confirmed from previous PI's study that active stone-formers and post-treated stone formers had lower total GAGs content but increased proportion of HA than that of normal controls which affirms our previous findings that HA is a potential biomarker to monitor patients of an impending silent stone forming in the renal tract. (*Global Journal of Urology; Gordon Research Conference*)

Lithogenic ions relevant renal stones were also measured in both urine and serum samples of the groups. The ions measured were calcium, oxalate, citrate, and phosphate. It was found that oxalate levels were significantly lower in active SFs compared to NC and those SFs who had stones removed. Citrate levels followed similar trends suggesting that those active SFs will move the soluble oxalate to the forming stones and the resulting calcium ion is chelated by the citrate in the urine. (*Global Journal of Urology; 38th Congress of the Societé Internationale d'Urologie; 4th Experts in Stone Disease Conference*)

The second major finding was the investigation of the six patient groups that were recruited for an initial pilot study to investigate the levels and significance of cytokines (IL-1α, IL-6, IL-8 & MIP-1α) that had been studied and reported before. It was found that IL-6 and IL-8 were significantly increased in active SF compared to other groups (Normals and those stone-formers who had their stones removed). At the same time, monitoring of the lithogenic ions such as calcium, oxalate, citrate, phosphate revealed that both citrate and oxalate remained high in stone-formers (after removal of stones) compared to normal and stone-formers who have not been treated. (*Urolithiasis; 4th Experts in Stone Disease Conference*)

The third major finding was investigating renal markers, particularly, Neutrophil gelatinase-associated lipocalin (NGAL) – an early biomarker for renal tubule-interstitial injury and kidney injury molecule – 1 (KIM-1) – a transmembrane molecule that is upregulated during upper kidney (proximal) injury. It was found that NGAL in active SF prior to removal of stones had significantly higher levels of NGAL compared to those SFs who had their stones removed and Normals. Regarding the KIM-1 molecule, which was added late in the study, we did not see any significant differences between the SFs (pre- and post-removal of stones) and the normal controls. (*Urolithiasis; 4th Experts in Stone Disease Conference*)

This stage-1 study conclusively narrows down the potential biomarkers that can be used to predict a forming stone or monitor the progression of the patients following removal of the stones. HA, IL-6, IL-8 and NGAL would be suitable predictor of stone forming within the urinary tract whilst other markers such as IL-1α, MIP-1α and KIM-1 were not found to be useful under the current study.

6.2 Potential for further development of the research and the proposed course of action *(Maximum half a page)*

This study conclusively narrows down 4 potential markers, namely, HA, IL-6, IL-8 and NGAL that could be used routinely to assess stone-formers. Ideally, 1-2 marker would be would be best as it would lower the costs of tests and burden on the patients and healthcare providers. At the beginning of this research, 9 potential candidate biomarkers were short-listed and 5 were used in this study of which 4 are potentially useful, but still that is too many.

The next stage-2 of the study, is the confirmation part, to follow cohorts of subjects (active stone formers, pre- and post- removal of stones, normals) using these 4 markers in a longitudinal study over 36-months, who are recruited to come to the clinic every 3-months for follow-up investigations. The outcome from this study would be the selection of 1 to 2 most promising marker that could be used to predict a forming stone and be part of the biochemical work-up for the patient.

The PI is pleased to report that support for funding for the Stage 2 of the study was approved through the Faculty Development Scheme (FDS) of the Research Grants Council 2019/2020. This project is planned and scheduled to start on 1 January 2020 to 31 December 2022 for 36-months. It is optimistically anticipated that a biomarker will be put forward as a regular test in the biochemical work-up for renal stone patients on conclusion of this study.

7. Layman's Summary

(Describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

The main goal of this project is to allow renal stone patients to be monitored with a simple blood / urine test, which will give advanced warning of clinically significant recurrence of a stone. This study is designed to narrow the biomarkers that can be readily tested and validated during Stage 2 of the longitudinal trials through a double-blinded study. The results of this study may allow follow-up treatment to be instigated at the most appropriate times, thereby bringing both financial and health benefits. Annual health checks that include assessments (blood / urine) for cardiac, liver, renal, cancer and general health is not unusual nowadays and this study envisages the addition of one more test to be included so that it (together with the electrolyte profile already on renal panel) can provide useful information to the clinicians. This study has narrowed down to 4 potential biomarkers from a list of 9 <u>and</u> in the second stage of the study it is envisaged to pick the 1-2 most promising, cost-effective biomarker to be recommended.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project
(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				**Author(s)** *(denote the corresponding author with an asterisk*)*	**Title and Journal / Book** *(with the volume, pages and other necessary publishing details specified)*	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
2019	2019			MDI Gohel*, HYH Or, MCK Lau, CF Ng	Hyaluronan as a predictive biomarker in recurrent renal stone formers, *Global Journal of Urology*.	No	No (Not received PDF yet)	Yes	Yes
2020/2021			2019	MDI Gohel*, HYH Or, MCK Lau, CF Ng	Preliminary detection of biomarkers in recurrent renal stone formers, *Urolithiasis*.	No	No	Yes	Yes

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered
(Please attach a copy of each conference abstract)

Month / Year / Place	**Title**	**Conference Name**	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
07 / 2018 New London, USA	Distinguishing Urinary Biomarkers of Renal Stones	Gordon Research Conference on Biomineralization	No	Yes	Yes	Yes
10 / 2018 Seoul, SOUTH KOREA	Identifying Important Biomarkers in Recurrent Stone Formers (***Best Moderated ePoster Award***)	38th Congress of the Societé Internationale d'Urologie	No	Yes	Yes	Yes
12 / 2018 Shanghai, CHINA	Calcium Stones – Etiology and a Biochemical approach to understand Biomarkers (***Invited Plenary Lecture***)	4th Experts in Stone Disease Conference	No	Yes	Yes	Yes

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning
(Please elaborate)

Yes, undergraduate final year project students benefited to conduct tests and learn techniques in ELISA, HPLC, AAS and obtain data and do statistical analyses. Incorporated and updated teaching of Clinical Chemistry and Research Methods in Medical Science.

11. Student(s) Trained
(Please attach a copy of the title page of the thesis)

Name	**Degree Registered for**	**Date of Registration**	**Date of Thesis Submission / Graduation**
	BSc (Hons) Medical Science	2013	2018

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

Awards

- Best moderated Poster for the paper "Identifying Important Biomarkers in Recurrent Renal Stone Formers, presented at the 38th Congress of Societe Internationale d'Urologie in Seoul, South Korea, October 4-7, 2018.

Invited Plenary / Keynote Speaker

- "Calcium stones: Etiology and diagnostics", Plenary lecture presented at the 4th Experts in Stone Disease Conference, Shanghai, China, November 30 – December 1, 2018. (**INVITED PLENARY LECTURE**)
- "Traditional herbal medicines in the treatment of Urolithiasis – fake news or a reality for the future?", Plenary lecture presented at the 4th Experts in Stone Disease Conference, Shanghai, China, November 30 – December 1, 2018. (**INVITED PLENARY LECTURE**)

Collaboration with other research institutions

- Prof. Anthony Ng Chi Fai, Dept. of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong.
- Dr. John Yuen Wai Man, School of Nursing, The Hong Kong Polytechnic University, Hong Kong.
- Prof. Allen Rodgers, Dept. of Chemistry, University of Cape Town, Cape Town, South Africa.

Editorial Board

- Appointed to the Editorial Board of "Urolithiasis", Springer-Verlag, Heidelberg, Germany.

Teaching Enhancement

- 2 research staff recruited and one of them was a graduate of Tung Wah College Bachelor of Medical Science programme interested in postgraduate work.
- Trained undergraduate students to conduct ELISA, HPLC and AAS tests alongside the research staff during the processing and testing of samples.
- Incorporated the project proposal, statistics and literature review in the course "Research Methods in Medical Science" which is led by the PI.

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	**Conference Papers**	**Scholarly Books, Monographs and Chapters**	**Patents Awarded**	**Other Research Outputs (please specify)**	
No. of outputs arising directly from this research project	2	2	--	--	Type	No.
					Invited Plenary Lecture	2

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons
N.A.	