RJM

Ruhuna Journal of Medicine

The Official Journal of the Faculty of Medicine
University of Ruhuna Galle, Sri Lanka

http://www.medi.ruh.ac.lk/index.php/research/publicationshome

November 2018 Volume 06 Number 01

Coccinia grandis (Linn.) Voigt (Family: Cucurbitaceae) "Kowakka"

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Proceedings of Faculty of Medicine Academic Session (FMAS) -2018, University of Ruhuna

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Cover Story

Coccinia grandis (Linn.) Voigt (Family: Cucurbitaceae) is an edible perennial climber commonly found in Sri Lanka. The antidiabetic potency of the leaf extract of C. grandis is well documented in traditional medicine applications. Antidiabetic mechanisms of the aqueous refluxed leaf extract of *C. grnadis* were investigated in streptozotocin induced diabetic rats previously[1,2]. At present, a randomized double-blind placebo control clinical trial of the herbal drug of *C. grandis* has been preceded in newly diagnosed patients with type 2 diabetes mellitus (Sri Lanka Clinical Trials Registry number: SLCTR/2018/012). The study is a collaborative research project between Department of Biochemistry and Department of Medicine, Faculty of Medicine, University of Ruhuna, Sri Lanka. The financial assistance for the project has been granted from the National Research Council (NRC Grant 2017/029)

1. Attanayake, A.P., Jayatilaka, K.A.P.W., Pathirana, C., Mudduwa, L.K.B., 2015. Acute hypoglycaemic and antihyperglycaemic effects of ten Sri Lankan medicinal plant extracts in healthy and streptozotocin induced diabetic rats. International Journal of Diabetes in Developing Countries, 35(3), pp. 177-183.

2. Attanayake A.P., Jayatilaka K.A.P.W., Pathirana C., Mudduwa L.K.B., 2015. Antihyperglycaemic activity of Coccinia grandis (L.) Voigt (Cucurbitaceae) in streptozotocin induced diabetic rats. Indian Journal of Traditional Knowledge, 14(3), pp. 876-881.

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Ruhuna Journal of Medicine

Contents

The Ruhuna Journal of Medicine (RJM) is published by the Faculty of Medicine, University of Ruhuna. The Journal publishes original research articles, reviews and case reports.

Types of articles

Original articles

The text of original article encounting up to 2000 words (excluding abstract, references and tables) should be divided into sections with the headings; Abstract (unstructured max 250 words), Key words, Introduction, Material and Methods, Results, Discussion References, Table and Figure legends.

Review articles

It is expected that these articles would be written by individuals who have done substantial work on the subject or are considered experts in the field. The prescribed word count is up to 4000 words excluding abstract, tables and references. The manuscript should have an unstructured abstract (max 250 words) representing an accurate summary of the article.

Case reports

These communications could be of up to 1000 words (excluding abstract and references) and should have the following headings; Abstract (unstructured max 150 words), Key-words (max 5), Introduction, Case Report, Discussion, Reference, Tables and Figure legends.

References

Personal communications and unpublished works should only be mentioned in the text. Reference citations in the text should be identified by numbers in brackets (eg. [1, 2]) before the punctuation marks. References should be numbered consecutively in the order in which they are first mentioned in the text. List all authors when three or less; when four or more, list only first three and add et al.
Examples;

Articles in Journals: Rechel B, Ahmedov M, Akkazieva B, et al. Lessions from two decades of health reform in Central Asia. Health Policy Plan 2012;27(1):281-287. (eg. BMJ type)

Books: Aminoff MJ. Electrodiagnosis in clinical neurology.2005;Elsevier, USA

Books chapters: Kumar P, Clark M. Cardiovascular disease: Camm AJ, Bunce NH, editors. Clinical Medicine, USA:Elsevier;2005;725-872

Tables

Tables should be self-explanatory and should not duplicate textual material. Number tables, in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.

Illustrations (Figures)

Figures should be numbered consecutively according to the order in which they have been first cited in the text.

Authorship Criteria

Authorship credit should be based only on substantial contributions to each of the three components. Mentioned below.

1. Concept and design of the study or acquisition of data or analysis and interpretation of data;
2. Drafting the article or revising it critically for important intellectual content; and
3. Final approval of the version to be published.

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FOREWORD

It is with a great privilege that I send this message to the official journal of the Faculty of Medicine, University of Ruhuna issued marking the 6th Faculty of Medicine Academic Sessions, November 2018.

The theme for this year's academic sessions is "Unresolved Healthcare challenges in Sri Lanka" mainly focusing on the chronic kidney disease. The burden is rising globally and locally, not only resulting in high morbidity and mortality, but also leaving a huge economical burden, particularly for low to middle socioeconomic countries. We believe increased awareness among health care workers would facilitate implementing cost effective management strategies, thus the conference fulfilling a timely need of the country. The conference will also examine research and development of the Medical Faculty, University of Ruhuna and the neighborhood scientific communities. The keynote address, oration, symposium, student`s session and the free paper session will illuminate this year`s session and I hope it will be a source of inspiration to all participants to face different health care challenges of day to day practice.

I am very grateful to Professor Saroj Jayasinghe who delivers the Keynote address at the inauguration ceremony and Professor Mangala C.S. De Silva, Dr Shamitha Dassanayake, Dr. Randula Ranawaka and Professor Rizvi Sherrif who contribute their knowledge and experiences as symphosium speakers.

Also, I take this opportunity to thank all the helping hands behind this endeavor especially the Professor Saman Wimalasundara, Dean of the Faculty of Medicine and the committee members of the FMAS 2018. Finally, I thank all the presenters who contribute their knowledge and experiences by presenting abstracts of their scientific work and wish them all good luck in their future academic career.

I wish you will enjoy the inauguration ceremony and the scientific sessions.

Dr. Janaki Warushahennadi
Chairperson
FMAS 2018

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Message from the Vice Chancellor

It is with immense pleasure that I send this short message to the Ruhuna Journal of Medicine, the official publication of Faculty of Medicine Academic Sessions. (FMAS). Undoubtedly, it is the major academic event organized by the Faculty of Medicine. The theme they have selected for the sessions is "Unresolved Health care challenges in Sri Lanka"

University of Ruhuna is committed to research apart from their teaching and service functions and I as the Vice Chancellor proudly look at the hard work done by academics and students to broaden the scope in diverse fields to ensure the maximum impact. We remain an institution that is highly committed to research, providing a stimulating research environment for staff and students to contribute to the welfare of the society through research.

I extend my sincere thanks to the Chairperson and the members of organizing committee for organizing Faculty of Medicine Academic Sessions for the 6th consecutive year.

I firmly believe that this event will be an intellectual platform for all the academics and students.
I take this opportunity to extend my warmest congratulations!

Professor Gamini Senanayake
Vice Chancellor,
University of Ruhuna.

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Message from the Dean

It is extremely elating to send this message as the Dean of the Faculty of Medicine, University of Ruhuna to the print edition of 6th Faculty of Medicine Academic Sessions (FMAS) journal – "RJM" Ruhuna Journal of Medicine volume 6 being published in November 2018. It is an ideal platform for our academic members especially for the postgraduates to publish their studies.

The field of Medicine is rapidly advancing and the dissemination of knowledge through scientific sessions would be essential for updating the knowledge.

This years academic sessions will span over 2 days with awarding of the Deans awards for the students with outstanding performances.

The theme selected this time by the organizers is "Unresolved health care challenges in Sri Lanka".I firmly believe that it is a very timely topic to discuss over these two days updating all participants with the latest scientific developments. I sincerely thank all dignitaries for accepting our invitation and joining hands with us to enhance the standards and quality of the Sessions. I am fortunate to have a dynamic , dedicated and talented team of academics in the FMAS committees who worked tirelessly throughout the time to make this event a success. I greet all the members of FMAS 2018 team and wish them all the success.

I look forward for a very fruitful Session 2018. Best wishes !

Professor Saman Wimalasundera
Dean,
Faculty of Medicine.

Inauguration

Thursday 15th November 2018

1700 hrs	Guests to be seated
1710hrs	Ceremonial procession
1715hrs	Faculty & University songs
1720hrs	Lighting of the traditional oil lamp
1725hrs	Welcome speech by the Chairperson, FMAS 2018 Dr. Janaki Warushahennadi
1730hrs	Address by the Professor Saman Wimalasundara, Dean, Faculty of Medicine, University of Ruhuna,
1740hrs	Address by the Chief Guest, Senior Professor Gamini Senanayake, Vice Chancellor, University of Ruhuna
1750hrs	Address by the Keynote speaker Unresolved health challenges: the need to cross disciplinary boundaries Professor Saroj Jayasinghe Chair Professor of Medicine, University of Colombo Founder Head, Department of Medical Humanities, University of Colombo Hon. Consultant Physician, National Hospital of Sri Lanka
1840hrs	Cultural event
1850hrs	Deans award ceremony
1905hrs	Oration FMAS 2018 Cleft Palate: Genetic Evaluation and Syndromic Diagnosis - A multi-disciplinary approach in the management Dr. Lande Bandarage Lahiru Prabodha Senior Lecturer, Specialist in Clinical Geneticist, Medical Officer in Charge, Molecular Genetics Laboratory, Department of Anatomy, Faculty of Medicine University of Ruhuna.
1955hrs	Vote of thanks by the secretary, FMAS 2018 Dr. S.K.Y. Iroshani Kodikara
2000hrs	Refreshment & Fellowship

Friday 16th November 2018

0800hrs	Registration	
0830 -0930hrs	Presentations based on completed higher degrees	
0930 -1015 hrs	Tea & Poster session	
	Symposium	
1020 – 1100 hrs	1.	"Development of Novel Biomarkers as Early Detection Tools for Chronic Kidney Disease of unknown etiology (CKDu) in Sri Lanka: Present & Future perspectives". Professor Mangala C. S. De Silva, Department of Zoology, University of Ruhuna, Sri Lanka.
1100 – 1140 hrs	2.	Physicians perspectives at the heart of CKDu in Padaviya, Dr. Shamitha Dassanayake, Consultant Resident Physician, Castle street hospital for Women, Colombo 08, Sri Lanka.
1140 – 1220 hrs	3.	Management challenges in Childhood Nephrotic Syndrome. Dr. Randula Ranawaka, Department of Paediatrics, University of Colombo, Sri Lanka.
1120 – 1300 hrs	4.	Delaying Progression in advancing CKD Vidyajyothi Professor Rezvi Sheriff MD FRCP FRCPE FRACP FCCP FNASSL FSLCGP FIMACGP Senior Professor of Medicine, Kotelawala Defence university Emeritus Professor of Medicine, University of Colombo Consultant Physician and Nephrologist Research Fellow National Science Foundation

The therapeutic value of prokinetic drug domperidone in functional abdominal pain disorders in children

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ABASTRACT

Functional abdominal pain disorders (FAPDs) are common cause for chronic abdominal pain in children. Despite the high prevalence, underlying pathophysiology of this condition is poorly understood and effective treatment options are lacking.

Based on repeated findings, that gastrointestinal motor abnormalities are common among children with FAPDs, we have conducted a double blind, randomized, placebo controlled therapeutic trial to assess the value of prokinetic drug, domperidone in management of FAPDs in children. Hundred children (aged 5-12 years) fulfilling Rome III criteria for FAPDs were randomized into 8 weeks of domperidone or placebo treatment. Primary outcomes defined were cure and patient-reported general improvement. Secondary outcomes were reduction in the severity of abdominal pain and increase in gastric motility. Patients were followed up for 6 months. Using intention to treat analysis, this study indicated that Domperidone is a safe and effective therapeutic modality to achieve a lasting remission of symptoms in children with FAPDs. Domperidone is a cheap drug widely used in developing countries such as Sri Lanka without significant adverse reactions. Therefore, finding such effective treatment modality would have a great advantage in management of functional abdominal pain in the developing word.

This study was conducted at the gastroenterology research lab, Faculty of Medicine, University of Kelaniya and Teaching Hospital, Ragama. The results were included in the thesis, one research papers in peer reviewed index journals and two abstract presented in international forums. The author is the recipient of International Foundation for Functional Gastrointestinal Disorders (IFFGD) award for developing nation investigator. The author delivered the Galle Medical Association oration - 2018.

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Introduction

Recurrent, chronic abdominal pain is one of the most commonly encountered and disabling symptoms in children and adolescence around the world. Available data show that it is highly prevalent (10-12%) across the western hemisphere . Epidemiological studies in Asia have shown similar results .

The majority of children and adolescents with chronic abdominal pain have functional gastrointestinal disorders (FGIDs) . FGIDs are defined as "chronic or recurrent gastrointestinal symptoms, not explained by structural or biochemical abnormalities". Functional abdominal pain disorders (FAPDs), previously called as abdominal pain related functional gastrointestinal disorders (AP-FGIDs) have emerged as an important clinical entity in paediatric practice all over the world. It is a group of disorders diagnosed by Rome criteria and the entities include irritable bowel syndrome (IBS), functional dyspepsia (FD), and functional abdominal pain (FAP). Using Rome III criteria, a recent study in Sri Lanka has reported FGIDs in 93% of patients with chronic abdominal pain , and FAP as the commonest subtype.

Chronic abdominal pain affects child life in many ways. Epidemiological studies have shown that 10% to 15% of affected children have reported the recurrent and chronic nature affects their wellbeing .

Several studies have clearly illustrated that FAPDs reduces quality of life of affected children . In addition, Sagawa and co-workers point out that some FAPDs affect quality of school work as well . Furthermore, one third of the affected children continue to experience pain at least for five years . Increasing age at the time of the diagnosis, waking up during the night due to abdominal pain, and high levels of other somatic symptoms, independently predict the presence of chronic abdominal pain after one year from the initial diagnosis . Finally, poorly managed children with FGIDs continue to have symptoms during adulthood and that is clearly linked with IBS in adults .

There is a very significant impact of FGIDs on health care systems. The majority of children attending these clinics are suffering from FAPDs such as IBS and FAP . Data from children's memorial hospital, Chicago, USA, reported low yield from basic investigations (blood counts, ESR, inflammatory markers), invasive investigations (gastrointestinal endoscopy) and rising costs in assessing children with FAPDs. Patients discharged with a primary diagnosis of abdominal pain yet again had the greatest increase in average cost per hospital stay (from $3558 to 13331) followed by IBS ($5278 to 18853). FD had always been the most expensive FAPD to treat and the percentage of the rise in cost was 183% from $12 674 to $35 898 . These data clearly illustrate the healthcare burden and rising costs of FAPDs on the health budgets of countries . In addition, parents of the children with FAPD are bound to attend their children during the disease. Therefore, paediatric FAPD causes indirect impact on parents' occupation or working hours. This may indirectly have an impact on the economy of the family. Therefore, FAPD are emerging as a group of disorders which warrants more attention in health economical aspects.

Pathophysiology of FAPDs is a grey area in paediatric gastroenterology. The main pathophysiological factors implicated to FAPDs are visceral hypersensitivity, altered motility, immunological dysfunction, altered intestinal microbiota and psychosocial factors . However, even with the cutting edge science including molecular medicine and sophisticated imaging techniques, exact pathophysiological mechanisms of FAPDs are poorly understood. This lack of understanding in pathophysiology is one of the main barriers in developing effective management option for FAPDs. This fragmented knowledge has prevented us from achieving optimal medical care for these children.

Most of the available studies on FGIDs have been conducted in adults , and studies conducted in children are rare. It is suggested that the management approach should be based on the biopsychosocial model for AP-FGIDs in which social, physical and psychological aspect of the child should be taken into consideration .

Currently, available treatment modalities for AP-FGIDs include general health management, pharmacological and non-pharmacological interventions. Main non-pharmacological interventions are dietary modifications, psychological and alternative therapies. Summary of the previous paediatric clinical trials is given in table 1. Adult studies, have shown the positive effects of gastroprokinetic agents in the treatment of FGIDs. Summary of the adult studies is given in table 2.

Table 1 - Summary of randomized controlled trials conducted to assess the value of interventions in the management of AP-FGIDs in children

Reference	Disease	No of patients	Interventions	Outcome/comments
[24]	IBS	33	Amitriptyline or placebo 13weeks	Amitriptyline improves HRQOL
[25]	FAP	115	Mebeverine and placebo	Mebeverine is effective in the FAP
[26]	AP-FGIDs	90	4 weeks of placebo or amitriptyline	Amitriptyline and placebo had a therapeutic response.
[27]	Dyspepsia	25	3 weeks of Famotidine or placebo	Famotidine effective for dyspepsia in children
[28]	AM	14	4 weeks of Pizotifen or placebo	Pizotifen to be clearly superior to placebo in the prophylaxis of AM
[29]	RAP	132	Drotaverine or placebo for 4 weeks	Drotaverine hydrochloride is an effective in the management of RAP
[30]	FAP	115	Citalopram or placebo for 4 weeks	Pain and global severity scores were reduced.
[31]	RAP	52	Fibre or placebo	Fibre decreased the pain attacks.
[32]	FAP	104	Probiotics(LGG) or placebo for 4 weeks.	The LGG moderately success for IBS.
[33]	IBS	50	Probiotics(Lactobacillus GG) or placebo for 6 weeks.	Lactobacillus GG was not superior to placebo in the treatment of pain
[34]	RAP	69	Combination of standard medical care and short-term cognitive-behavioral family treatment (CBT)	CBT reduced the pain.
[35]	RAP	32	received standard paediatric care and CBT	Frequency of pain was reduced

Table 2- Randomized clinical trial studies conducted to assess the effects of prokinetics for FGIDs in adults

Reference	Disease	No of patients)	Intervention	Outcome
[36]	IBS	66	Domperidone or placebo for 4 weeks	Symptoms had disappeared or were at least markedly improved in about 80% of the Domperidone-treated patients.
[37]	Non-nuclear dyspepsia	16	Domperidone or Placebo six weeks	Symptom scores significantly improved in the domperidone group; no significant effect on motility.
[38]	FD	147	2 weeks' treatment with metoclopramide or domperidone (both 30 mg/day); patients unresponsive to dopamine antagonist treatment were randomised to cisapride or placebo	Metoclopramide and domperidone produced comparable alleviation of Epigastric symptoms cisapride appears to be an effective agent in functional dyspepsia unresponsive to other prokinetic agents.

In this study, we studied the therapeutic value of prokinetic drug - domperidone in management of childhood FAPDs.

Ethical approval for this study was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Kelaniya. The trial was registered in the Sri Lanka Clinical Trial Registry (SLCTR) which is the primary registry linked to the WHO International Clinical Trials Registry Platform (WHO - ICTRP). The registration number of SLCTR was SLCTR/2012/008.

The findings of this phase of the study were published in Journal of Pediatric Gastroenterology and Nutrition .

Methodology

One hundred children (aged 5–12 years) fulfilling Rome III criteria were recruited and randomized into two groups (50 in a group) using computer generated random numbers, irrespective of the baseline symptom severity and gastric motility status.

The intervention group received domperidone 10mg 3 times per day, 30 minutes before meals for 8 weeks. The control group received a placebo 3 times per day 30 minutes before meals for the same duration. The placebo was identical to domperidone tablet in physical appearance, colour, taste and packaging. Hundred and sixty eight identical tablets (an 8 week supply) of the drug or the placebo were provided to all children who were included in the study. A

symptom diary was provided to document adherence to treatment, severity, frequency and duration of symptoms and interruption of activities.

After completion of the trial, parents were requested to use only the simple analgesics for pain and not to use any other therapy for FAPDs for 6 months. They were requested to record the drugs used, any diseases or symptoms developed and complications encountered in the diary provided and to report during weekly telephone inquiries. Patients were reviewed at 8th week and 6th month with regards to primary and secondary outcomes.

Primary outcomes defined were cure and patient-reported general improvement. Secondary outcomes were reduction in the severity of abdominal pain and increase in gastric motility.

Primary outcomes

1. *Cure*

Cure was defined when a patient fulfilled all 3 following criteria

- Abdominal pain less than 4 episodes per month
- Average severity of abdominal pain less than 25mm in the visual analogue scale
- None of the pain episodes were severe enough to disrupt the daily activities of the child (e.g. schooling, leisure activities, play and sports etc.)

2. *Patient reported general Improvement*

Patient reported general improvement was defined as overall satisfaction and satisfactory relief of pain following treatment. This was assessed by using two questions.

When he/she indicates positive result for both of the following questions, he/she was considered to have general improvement of FAPDs.

- Overall how do you feel your problem is? Answer was better, same or worse. "Better" was regarded as positive result. "Same" or "worse" was regarded as a negative result
- How did the medication relieve your pain?

Sense of improvement was expressed as excellent, good, fair and poor. Excellent and good was considered as positive result. Fair and poor were considered as negative results.

Secondary outcomes

Decrease in pain severity

The percentage of pain improvement was assessed as the difference of mean pain severity reported on a validated 100mm visual analogue scale before and after the treatment.

Increase in gastric motility

Main gastric motility parameters used as outcomes were gastric emptying rate and antral motility index. The percentage increase in gastric emptying rate and antral motility index at post treatment period compared to pre-treatment assessment was calculated to determine the improvement of gastric motility.

Patients were followed up for 6 months. Data was analyzed using intention to treat analysis.

Results:

Eighty-nine (42 in placebo group, 47 in domperidone group) completed the trial at 8 weeks. Seventy-nine completed the 6-month follow-up.

Primary and secondary following treatment

When primary outcomes were assessed at 8 weeks, 37 (74%) in the domperidone group and 25 (50%) in the placebo group showed patient-reported general improvement (p= 0.013), whereas no significant difference was observed in cure (22 [44%] vs 14 [28%] p=0.09) **(Table 3).** At 6- month follow-up 30 (60%) in the domperidone group and 19 (38%) in the placebo group reported cure (p = 0.028), whereas 44 (88%) in the domperidone group and 33 (66%) in the

placebo group showed patient reported general improvement (p = 0.009).

When assessing secondary outcomes at 8 weeks, the domperidone group reported significant reduction in the severity of abdominal pain (54.1% vs 24.7%, p=0.008) and an increase in the antral motility index (27.5% vs 7.2%, p=0.029) (**Table 4**).

None of the patients reported intervention-related adverse effects.

Table 3- Improvement of primary outcomes after intervention in 8th weeks and 6 months

Outcome	Domperidone group (n=50) Number (%)	Placebo group (n=50) Number (%)	p value*
At 8 weeks			
Cure	22 (44.0)	14 (28.0)	0.096
Improvement	37 (74.0)	25 (50.0)	0.013
At 6 months			
Cure	30 (60.0)	19 (38.0)	0.028
Improvement	44 (88.0)	33 (66.0)	0.009

***Chi-square test**

Table 4- Improvement of secondary outcomes after 8 weeks

Outcome	Domperidone group (n=50)	Placebo group (n=50)	p value*
At 8 weeks			
% reduction of pain severity	54.1	29.7	0.008
%improvement in gastric emptying	14.8	7.4	0 .423
% improvement in antral motility index	27.5	7.2	0.029

***Independent - sample T test**

Discussion and conclusions

This is the first prospective, randomized, double-blind, placebo-controlled clinical trial on therapeutic effects of domperidone, on children with AP-FGIDs. After 8 weeks of therapy, domperidone was found to have a significant patient reported general improvement in children with AP-FGIDs. At 6 month follow up, a significantly higher percentage of children treated with domperidone were able to achieve cure. In addition, significant reduction in the severity of pain and increase in the gastric antral motility index were observed in the domperidone group.

In the current study, the response to the placebo (overall patient reported clinical improvement) was 50%. Similar high rates of placebo responses have been reported in previous studies. In a therapeutic trial assessing the value of mebeverine, the placebo response was 53.4% and it was 75% in a trial assessing the value of amitriptyline . A recent meta-analysis conducted by Hoekman *et al* found a 41% placebo response among children included in clinical trials assessing treatment efficacy of therapeutic agents for AP-FGIDs . It is thereby noted that effects of placebo are contributing towards the therapeutic effect seen in randomized controlled trials on FGIDs . Spontaneous improvement and good patient-practitioner relationships could contribute to the placebo effect .

AP-FGID is a common problem in children and effective therapeutic modalities are not widely available. Interventions such as amitriptyline has shown no benefits over placebo {Saps, 2009 #239}. Mebavarine, famotidine, cyproheptadine, and rifaximin had only shown a modest effects and long term follow up data were not available {Pourmoghaddas, 2014 #243}{See, 2001 #245}{Sadeghian, 2008 #697}{Collins, 2011 #698}. Other interventions such as hypnotherapy and yoga therapy are time consuming and need specially trained professionals and therefore, difficult to implement in busy clinical settings {Rutten, 2013 #277}{Vlieger, 2007 #696}. In such a context, finding a potentially effective, widely available and low cost therapeutic agent has far reaching benefits to children. During the study concealment of allocation was maintained in accordance with current guidelines. Low dropouts and excellent adherence to the protocol provided the final sample size with adequate power to detect the originally proposed differences in the study outcomes. We also used physiological parameters of gastric motility to explore the mechanism of clinical improvement and managed to follow up the majority of patients up to 6 months.

In conclusion, while performing a double blind, randomized, placebo-controlled trial, we have shown that domperidone, is a safe, and effective therapeutic modality to achieve a lasting remission of symptoms in children with AP-FGIDs, specially with FAP. Approximately two third of children treated with domperidone were able to sustain the clinical improvement and were able to achieve our stringent criteria for cure at 6 month follow up.

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Investigation of mitochondrial energy metabolism in the liver stage of Plasmodium berghei

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Abstract

Energy metabolism in malaria parasites varies remarkably over the parasite life cycle. Parasites depend solely on anaerobic glycolysis at blood stage but need Krebs cycle, the electron transport chain, and mitochondrial ATP synthase during mosquito stage development. Reverse genetic approaches to study the hepatic stage of Plasmodium have been thwarted because parasites with defects in energy pathways are unable to complete the mosquito stage. This project exploited the malaria parasite's unusual sexual genetics by using a genetic complementation approach to bridge parasites with defects in the β subunit of mitochondrial ATP synthase through the heterozygous mosquito phase; allowing us to assay their performance as knockout haploids in liver stage. ATPase knockouts were indistinguishable from wildtype in in-vitro liver stage assays in terms of the growth size, nuclear content, and merosome production. Robust progression of the knockout parasites in to blood stage confirmed the dispensability of mitochondrial ATP synthesis in liver stages. Hence, we speculate that energy metabolism in the liver stage resembles that in the blood stage, relying predominantly on glycolysis for ATP production.

1. Introduction

The combination of drug resistance, lack of an effective vaccine and ongoing conflict and poverty mean that malaria remains a major global health crisis. Understanding metabolic pathways at all parasite life stages is important in prioritising and targeting novel anti-parasitic compounds. Recently it has emerged that malaria parasites adopt starkly different modes of energy metabolism in different parts of their life cycle. Most eukaryotes produce their ATP using either of two routes: glycolysis, or glycolysis followed by oxidative phosphorylation. Glycolysis takes place in the cytosol. If access to oxygen is limited within the cell, glucose (the main energy precursor) will be converted to either lactate or ethanol after glycolysis. During aerobic conditions however, the end product of glycolysis will be pyruvate, which is then transformed into acetyl CoA inside the mitochondrion. In the next step, acetyl-CoA is modified through a series of chemical reactions to produce ATP through the tricarboxylic acid (TCA) cycle. The cycle also consumes oxygen, reduces NAD+ to NADH, and produces carbon dioxide and water as waste by-products. The NADH generated by the TCA cycle is fed into the oxidative phosphorylation/electron transport chain (ETC), which builds up a proton gradient to drive mitochondrial ATP synthesis. The net result of these two closely linked pathways is the oxidation of nutrients to produce usable chemical energy in the form of ATP.

Whereas animals switch between glycolysis and oxidative phosphorylation depending on oxygen availability, malaria parasites make this switch when changing hosts, seemingly independent of oxygen availability. For example, blood stage malaria parasites mainly depend on anaerobic glycolysis. Despite having access to oxygen, parasites convert glucose scavenged from the host to lactate . Even though glycolysis provides the main source of ATP for blood stage malaria parasites, mitochondrial electron transport is not completely inactive in the blood stage. Rather, parasites utilize a conventional ETC cycle to regenerate ubiquinone for pyrimidine synthesis . In contrast, genetic knockout studies and chemical inhibition assays have demonstrated the essentiality of TCA cycle enzymes during sexual differentiation . Similarly, the majority of the genes associate with TCA cycle and electron transport chain are known to be essential for the mosquito stage but dispensable in blood stage, which is consistent with glycolysis being the main source of ATP . The energy metabolism of the hepatic stage of Plasmodium, on the other hand, is largely unstudied by reverse genetics due to inability of these knockout parasites to survive through the mosquito stage.

ATP synthase, also known as complex V of the ETC (Fig 1), is the enzyme complex that catalyses ATP synthesis from ADP using the proton gradient generated across the inner mitochondrial membrane . ATP synthase consist of two major functional domains: a membrane-extrinsic F1 domain; and a membrane-intrinsic F0 domain, which are joined together by central and peripheral stalks. F1 is the catalytic part of the enzyme where ATP is formed from ADP and Pi, whereas F0 contains a motor to generate rotation . The F1 domain of the ATP

synthase is an assembly of five globular proteins, α, β, γ, δ and ε, with the stoichiometry α3β3γ 1δ1ε1 .

Sturm et al. in 2015 generated a gene knockout of the β subunit of ATP synthase in P. berghei that grew well at blood stage implying that mitochondrial ATP synthesis was dispensable in blood stage parasites, which is again consistent with a primary role for glycolysis in generating ATP . However, the ATP synthase β subunit was shown to be essential during mosquito stage, particularly for ookinete to oocyst development . Given the observed defects in the mosquito stage it was not possible to study the effect of the ATP synthase knockout in liver stage development. Therefore, this

gene was a good candidate to apply the complementation system that was established previously (10) and to explore energy metabolism in the malaria parasite liver stage.

In this study I complemented the ATP synthase β subunit knockout with wildtype ATP synthase β subunit by crossing the red fluorophore tagged gene deletion parasites with green fluorophore tagged wildtype parasites. By carefully analyzing the genetically complemented ATP synthase β subunit knock out sporozoites in vitro using cultured liver cells, I could observe their growth and development in the absence of mitochondrial ATP synthesis. I was also able to study their development in vivo by allowing mosquitoes with complemented ATP synthase mutants to feed on naïve mice and assay for the recovery of a P0 blood stage, which I could genotype, and drug select (the knockouts are pyrimethamine resistant). Complementation allowed me to bridge the ATP synthase β subunit knockout through the mosquito stage and produce sporozoites with no ATP synthase β subunit, which were then used to infect liver cells in vitro and mice by mosquito bites. In this way I explored the viability of ATP synthase β subunit deficient parasites in the liver stage and provided the first evidence that liver stage rodent malaria parasites are not dependent on mitochondrial ATP synthesis, which is the first insight into their energy metabolism. e

2. Materials and methods

2.1 Parasites

A clonal population of P. berghei ATP synthase β knockouts carrying the mCherry fluorescent marker and hDHFR (drug selectable marker) was generated by Dr Angelika Sturm .

The PbsP48/45KO strain was provided by Andy Waters (University of Glasgow, Glasgow, Scotland). Pb ANKA parasite lines expressing GFP and mCherry fluorescence markers were developed by Dr Dean Goodman based on Rathnapala et al .

2.2 Infection of Anopheles stephensi

Adult females of A. stephensi reared under standard insectary conditions (adult mosquitoes were grown at 27 ℃ with humidity of 80% and light: dark photoperiod of 14:10 hours with a 1-hour ramp in) were allowed to feed on anesthetised mice that had been infected with the following combinations (2x105 parasites from each strain were mixed and two independent experiments from each coinfection (total four coinfections) was performed).

- ATPβKOmCh [parasites having ATPβ replaced with, hDHFR gene fused to mCherry fluorescent marker]
- ATPβKOmCh x ATPβWT-GFP [parasites having ATPβ replaced with hDHFR fused to mCherry, mixed with a wildtype ATPβ locus expressing GFP from a different genetic locus]
- ATPβWTmCh x ATPβWT-GFP (parasites with a wildtype ATPβ locus and expressing mCherry from another genetic locus mixed with ATPβ wildtype parasite expressing GFP from another genetic locus)
- Pbs48/45KO [parasites lacking Pbs48 and 45; non-fluorescent strain]
- ATPβKOmCh Pbs48/45WT x ATPβWT P48/45KO (parasites having ATPβ replaced with hDHFR fused to mCherry fluorescents maker, with a wildtype Pbs48/45 locus and mixed with parasites carrying a wildtype ATPβ gene but lacking Pbs 48/45)
- ATPβWTmCh Pbs48/45WT x ATPβWT Pbs48/45KO (parasites with wildtype ATPβ and Pbs48/45 loci and expressing mCherry from another genetic locus mixed with non-fluorescent parasites with a wildtype ATPβ locus but lacking Pbs48/45)

First, the donor parasitic strain ATPβKOmCh was used to infect mosquitoes as a preliminary experiment. Screening of ATPβ knockout and wildtype parasites was performed with the primers listed in Table 1 using the following PCR conditions.

Initial denaturation	95℃	3 min
34 cycles	95℃	15 sec
	55℃	20 sec
	72℃	3 min
Final extension	72℃	5 min

The aim of this trial was to confirm the absence of oocysts in midguts of the mosquitoes and hence the essentiality of ATP synthase β subunit for mosquito stage transmission of parasites (Fig 3).

Next, the oocysts from the mosquito midguts day 12 post infection, and salivary gland sporozoites day 21 post infections for the two cross experiments and equivalent controls were analysed. An immunofluorescence assay (IFA) was used to detect fluorescent expression of ATPβKOmCh Pbs48/45WT x ATPβWT P48/45KO salivary gland sporozoites as described (10) using rabbit anti-mCherry (1: 250; Abcam ab167453)/Alexa Fluor 546 goat anti-rabbit IgG (1:1000; Molecular Probes/ThermoFisher) for ATPβKOmCh x ATPβWT-GFP. The number of oocysts and sporozoites per mosquito was recorded and the ratio of sporozoites/oocysts was compared between parasites of ATPβKOmCh x ATPβWT-GFP and ATPβWTmCh x ATPβWT-GFP from two independent experiments using student t test.

2.3 Sporozoite infections of HepG2 cells

All the sporozoites from each cross experiment were incubated with HepG2 cells (20,000 sporozoites per well) for 68 hours with cells fixed at 68 hours post incubation (hpi). An IFA was carried out (10) for wells fixed at 68 hours by staining parasites with rabbit anti-mCherry (1: 250; Abcam ab167453)/Alexa Fluor 546 goat anti-rabbit IgG (1:1000; Molecular Probes/ThermoFisher) and Hoechst 33342 (5μg/ ml). Next, the number of nuclei per parasite and the area of each parasite were compared to their corresponding control parasites using images taken at 40X magnification of the Leica DM 2500 epifluorescence microscopy. Images were analysed by Image J (1.46a, Wayne Rasband, National Institutes of Health) software. Student t-test (unpaired) was used to compare the average growth size differences 68 hours post infection and $\chi 2$ analysis for nuclear content for parasites pooled from 4 independent experiments. At 70 hpi, detached cells and merosome production were observed and counted using an Olympus CKX41 compound fluorescence microscope at 40X magnification (Table 2).

2.4 Sporozoite infections in mice

Infected mosquitoes (day 21 post infection) were allowed to feed on naïve mice (P0 population by biting) and dissected salivary gland sporozoites were IV injected into naïve mice as 1000 and 10000 sporozoites per mouse (P0 by sporozoite IV). Mice infected with ATPβKOmCh x ATPβWT-GFP were treated with pyrimethamine (70μg/ml: Sigma) once the parasitemia reached approximately 5% and followed up for a further 14 days. In addition to the pyrimethamine treatment, fluorescence microscopy and PCR analysis (primers; Table 1) were used to genotype the resultant parasites from each experiment.

2.5 Statistical analysis

All statistical analysis was done using GraphPad Prism (GraphPad Software, La Jolla California USA).

3. Results

3.1 Parasites with ATP synthase β subunit gene deletion (PbATPβKOmCh) show slightly impaired growth during blood stage but fail to infect mosquitoes All the experiments from generating the PbATPβKOmCh strain, transfection, sub-cloning to generate a single parasitic strain and their blood stage growth experiments were done by Dr Angelika Sturm/ University of Melbourne. This work confirmed that the ATPβKO parasites complete blood stage growth, suffering only a slight growth defect . Further, when the knockout strain was introduced to A. stephensi mosquitoes, parasites failed to produce any oocysts . Hence, it was confirmed the β subunit of the ATPase gene is not essential for erythrocytic development but required for parasite transmission through mosquitoes.

To further verity the above finding, the cloned ATPβKOmCh was PCR genotyped (Fig 3A) using primers listed in Table 1 and fed to mosquitoes. The absence of oocysts in the midgut infected with the knockout compared to the wildtype control (Fig 3B) corroborated the published result .

3.2 Crossing to wildtype parasites complements the ATP synthase β subunit gene knockout defect in mosquito stage development

To determine if the effects of β subunit of ATPase gene deletion (ATPβKOmCh) on parasite growth during mosquito stage could be complemented by crossing to parasites carrying a wildtype copy of the allele (ATPβWT), A. stephensi mosquitoes were fed on mice co-infected with (I) ATPβKOmCh x ATPβWT-GFP and (II) ATPβKOmCh Pbs48/45WT x ATPβWT P48/45KO with their equivalent control strains.

Table 1: List of primers used for screening of genes

Gene		5'-3'	Expected size (bp)
ATPβ			
KO	SP	CCGCTCAGGAACGAATTTAG	2670
	ASP	TGTGAATTCCGTCCCAACAAACAATTAACA	
WT	SP	TGATTTTAGTGGTTCAAAAGCTG	807
	ASP	TGTGGATCCCATTTGTTTAGCCATTTC	
P48/45			
KO	SP	CATTCATACATACATGTGTATGG	1300
	ASP	ACGCATTATATGAGTTCATTTTAC	
WT	SP	TTTTGGGAACAGCCGTTTT	689
	ASP	TTTGTTTGCACGCTAGCACT	
GIMO GFP			
GFP	SP	AACTGAAAAAGAGCGCAAATG	1570
	ASP	CCCAGAATTCCCATGAACTG	
No GFP	SP	GCGGCCGCGTTTGTATTATGCACGCAAC	1070
	ASP	TCTTGAGGAACGGATAGACAC	

Table 2: Number of mCherry expressing merosomes generated by each strain at 70 hpi in the ATPβKOmCh study

Strain	Number of mCherry expressing merosomes (number of trails=4)
$ATP\beta^{KOmCh}$ x $ATP\beta^{WT}$-GFP	45±11
$ATP\beta^{WT}$mCh x $ATP\beta^{WT}$-GFP	25±34
$ATP\beta^{KOmCh}$ $Pbs48/45^{WT}$ x $ATP\beta^{WT}$ $P48/45^{KO}$	31±34
$ATP\beta^{WT}$mCh $Pbs48/45^{WT}$ x $ATP\beta^{WT}$ $Pbs48/45^{KO}$	55±9

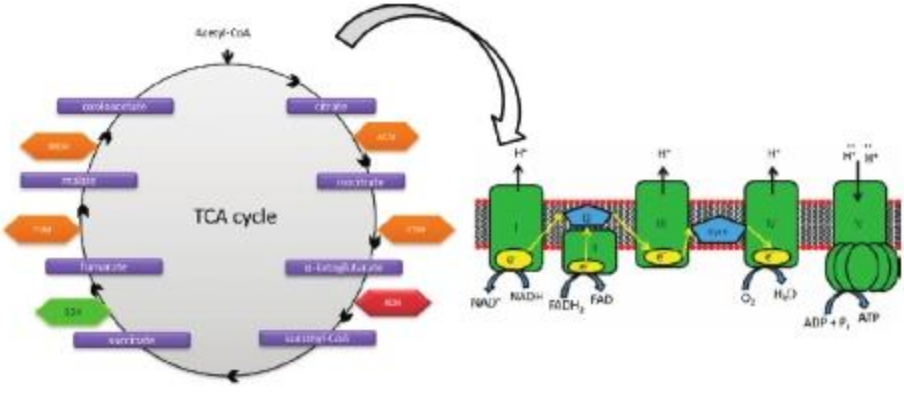

Figure 1: A diagram showing mitochondrial tricarboxylic acid cycle (TCA) and electron transport chain (ETC) to represent the gene candidate of the study. ATP synthase is the complex V of ETC which harvest ATP, generated through the mitochondrial membrane proton gradient.

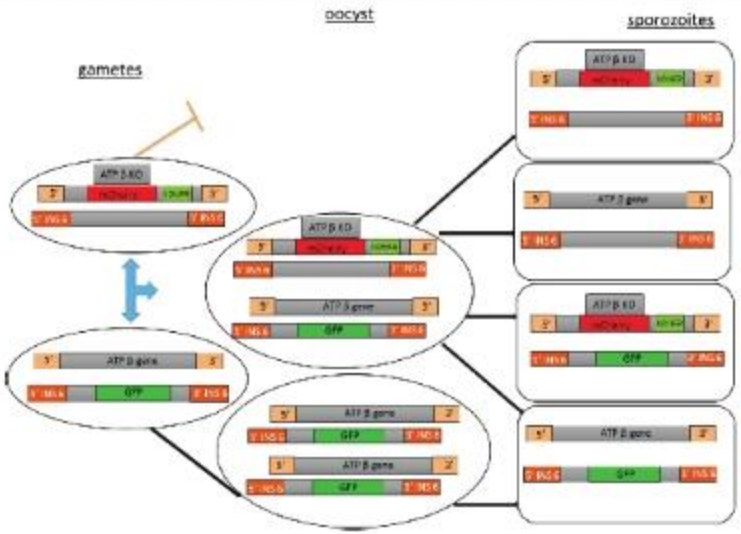

Figure 2: Schematic representation of the predicted parasite genotypes at the polyploid oocyst stage and haploid sporozoite stage after allowing mosquitoes to feed on mice dually infected with ATPβKOmCh and ATPβWT-GFP parasites. The ATPβKO mutation prevents self-fertilization of this parasite line, so two genetic combinations are possible in the polyploidy oocyst: outcrossing creating an oocyst with both ATPβKOmCh and the ATPβWT-GFP alleles (orange phenotype) and self-fertilization yielding oocysts with only the ATPβWT-GFP allele (green phenotype). Following sporogony, the parasite returns to the haploid state yielding four possible genetic combinations of the ATPβKOmCh and the ATPβWT-GFP genes: The original ATPβKOmCh (red) or ATPβWT-GFP (green) alleles alone, a combination of both the ATPβKOmCh and ATPβWT-GFP alleles (orange) or parasites carrying neither allele (colourless).

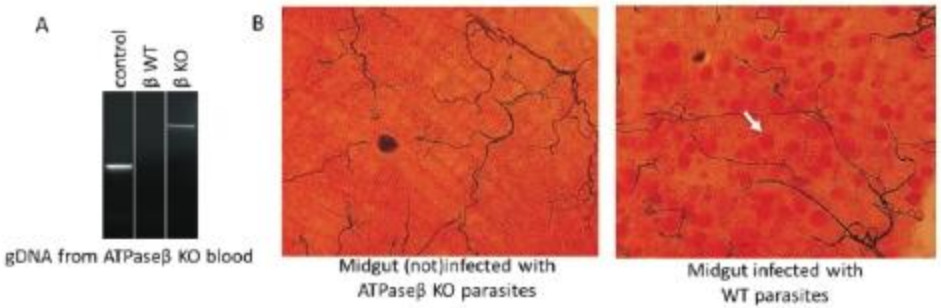

Figure 3: Confirmation of ATPaseβ knockout clone (A) PCR reaction showing the ATPaseβ gene knockout/deletion of the ATPase β gene. Primers used are, control; to amplify a known region in the parasite genome, β WT; to amplify the wildtype gene, β KO; to amplify the knockout gene (Table 1) (B) Mosquito midguts infected with (I) ATPaseβ KO (II) control wildtype P. berghei to compare the absence of knockout oocysts in the mercurochrome stained midguts (arrow head shows an oocyst)

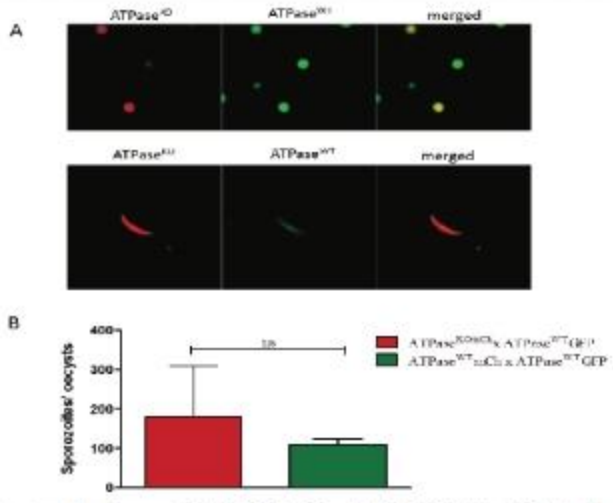

Figure 4: Mosquito stage development of ATPβKOmCh x ATPβWT-GFP dual infected parasites (A) Orange colour oocysts at day 12 post infection expressing mCherry (ATPaseKOmCh) with GFP (ATPβWT-GFP) and oocysts with GFP alone (ATPβWT-GFP). The salivary gland sporozoites day 21 post infections are stained with anti-mCherry antibody to show the presence of ATPβKOmCh (B) Graph showing a comparison of the ratios of salivary gland sporozoites/ oocysts between cross and control strains (P=0.6448; student t-test) ns; not significant

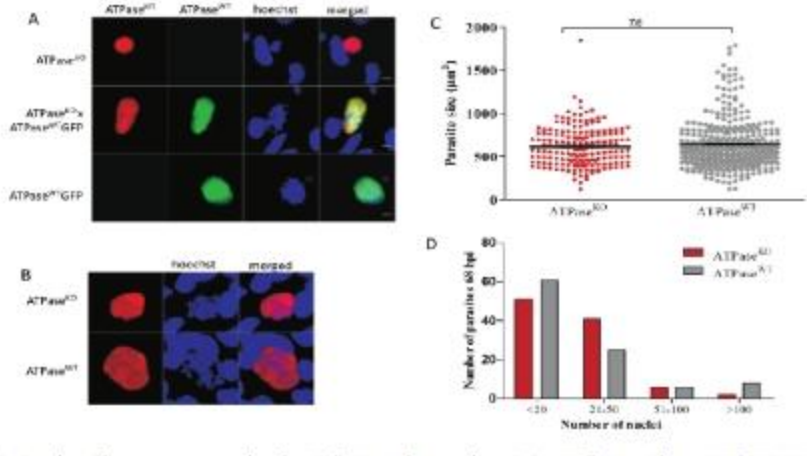

Figure 5: In-vitro liver stage growth of ATPβKOmCh parasites (A) ATPβKOmCh x ATPβWT-GFP; the parasites are stained with anti-mCherry antibody and DNA stain Hoechst (68 hours post infection (hpi) to show the presence of, parasites having only red colour, both red and green colours and only green colour. The red colour parasites contain the ATPβKOmCh allele and are originated from the recombination (Fig 2). Similarly, the orange colour parasite which has both ATPβKOmCh and ATPβWT-GFP alleles is a recombinant. The green colour parasites come from either self-fertilized green colour oocyst or from the heterozygous orange colour oocyst which contains the ATPβWT-GFP (Fig 2) (B) ATPβKOmCh Pbs48/45WT x ATPβWT P48/45KO; represented as ATPaseKO and their equivalent control parasites (represented as ATPaseWT, a wildtype P. berghei strain expressing mCherry mixed with P48/45KO) stained with anti-mCherry antibody and Hoechst, 68 hpi. Scale bar 20 µm (C) Graph showing a comparison of average size (in µm2) of β knockout parasites and the equivalent control strain 68 hpi where the differences is not statistically significant (P=0.2292; student t-test) ns; not significant (D) A comparison of number of nuclei produced by each strain of parasites 68 hpi. Both ATPβKOmCh and wildtype lines producing parasites with more than 100 nuclei (P value=0.0389; $\chi 2$).

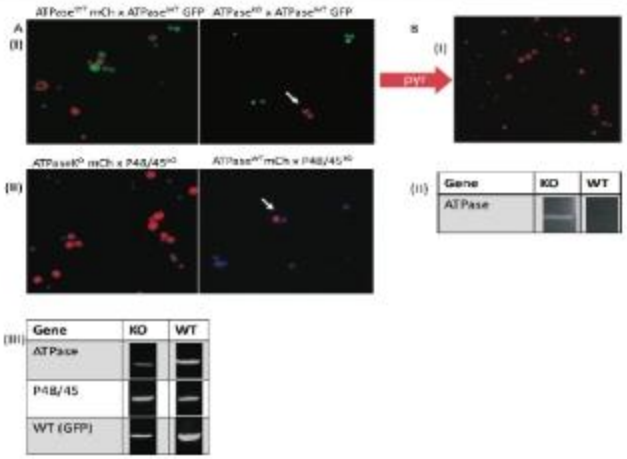

Figure 6: P0 blood stage growth of ATPβKOmCh parasites (A) Fluorescence microscopy images showing (I) recovery of ATPβWTmCh x ATPβWT-GFP parasites with both GFP and mCherry expression and knockout parasites expressing mCherry (arrow head) and GFP, from ATPβKOmCh x ATPβWT-GFP (II) images of red colour ATPβKOmCh parasites (arrow head) mixed with P48/45KO and the equivalent control. The P48/45KO strain has no intact fluorescent marker hence only the red colour expression can be seen. (Parasite DNA has been strained and seen in blue colour) (III) genotypes of parasites recovered as P0 which include all the expected genotypes post recombination at mosquito stage; ATPβKO and ATPβWT, P48/45KO and P48/45WT and parasites with (KO) and without (WT) GFP. Primers in Table 1 (B) (I) pyrimethamine selection of P0 ATPβKOmCh x ATPβWT-GFP parasites showing the elimination of drug sensitive GFP expressing wildtype parasites while the number of mCherry expressing ATPβKOmCh parasites show a drastic increase over 2-3 days post treatment (II) genotype of parasites recovered through the drug treatment where all the parasites have knocked-out ATPaseβ gene.

The replacement of the ATPβ coding region with mCherry fused to hDHFR in ATPβKOmCh parasites allows tracking of ATPβKO genotype after sexual reproduction. Following sexual recombination in mosquitoes and encystment, oocysts were seen 12 days post infection. As expected (Fig 2), there were only two phenotypes of oocysts present; orange colour recombinants carrying both ATPβKOmCh and ATPβWT-GFP genes and green colour self-fertilized oocysts with ATPβWT-GFP (Fig 4A). Due to the growth defect of ATPβ gene deletion, self-fertilized ATPβKOmCh parasites expressing only the red colour did not complete their oocysts development, which explains why I saw no red only oocysts . Nevertheless, salivary gland sporozoites with mCherry expression could be seen (Fig 4A) confirming the survival of parasites having knockout ATP synthase β gene in mosquito stage. Hence, complementation of the ATPβKO in the oocyst restored sporozoite production in both co-infections. In addition, there was no significant difference (P=0.6448; student t-test) between the ratio of sporozoites/oocysts developed by the ATPβKOmCh x ATPβWT-GFP compared to the control (Fig 4B). Due to the low level of mCherry expression seen in ATPβKOmCh-Pbs48/45WT x ATPβWTmCh-Pbs48/45KO cross; the number of red colour oocysts could not be counted. However, all the sporozoites from the ATPβKOmCh-Pbs48/45WT x ATPβWTmCh-Pbs48/45KO cross showed mCherry fluorescence despite having a mix of ATPβKOmCh and ATPβWT genotypes. This suggests significant carry-over of transcripts and/or protein from the shared cytoplasm of the oocyst that could mask any phenotype resulting from the loss of ATP synthase β subunit activity in sporozoites .

3.3 The role of parasite-synthesized ATP synthase β subunit gene in liver stage development

Sporozoites recovered from the test and control crosses were used to infect in vitro cultures of HepG2 liver cells, and parasite development was assessed by IFA at 68 hours post infection. By 24 hours, any residual fluorescent mCherry protein seen in the sporozoites was lost and the parasites could be genotyped by fluorescence expression . As seen in images (Figs 5A,B), there were red colour ATPβKOmCh parasites present 68 hours post infection as fluorescence expression allowed us to

correlate liver stage parasite size with the presence of an active ATPase β subunit gene. In the ATPβKOmCh x ATPβWT-GFP cross, parasites with only the red colour as well as orange colour parasites were products of recombination of orange colour oocysts which came from cross fertilization of ATPβKOmCh and ATPβWT-GFP (Fig 2). Similarly, parasites which express only green colour could be a result of either self-fertilization of ATPβWT-GFP or from the recombination of orange colour oocysts (Fig 5A). A similar pattern was seen in the parasites from the ATPβKOmCh Pbs48/45WT x ATPβWTmCh Pbs48/45KO co-infection 68 hpi (Fig 5 B). In the absence of a second fluorophore however it was not possible to distinguish ATPβKOmCh Pbs48/45WT self-fertilization from ATPβKOmCh Pbs48/45WT x ATPβWTmCh Pbs48/45KO recombinants. Depending on the size of each parasite according to the red colour they expressed, there was no significant difference (P value=0.2292; student t-test) in between the cross and control parasite development at 68 hours of infection (Fig 5C). The similarity in parasite size correlated with similar numbers of nuclei present (Fig 5D) in ATPβKOmCh parasites at 68 hpi compared to their wildtype equivalent (P value=0.0389; $\chi 2$). Both ATPβKOmCh and wildtype lines produced parasites containing more than 100 nuclei.

Production of detached cells and merosomes was assayed at 70 hours post infection (Table 2) and the presence of mCherry expressing detached cells and merosomes in both cross infections confirmed ATPβKOmCh ability to complete the hepatic phase replication in in-vitro.

The ability of ATPβKOmCh parasites to complete liver stage in naïve mice in vivo was tested using both bite-back and injection of purified salivary gland sporozoites. Mice infected by either strategy became infected within the expected period for a wildtype parasite (day 4-5), and fluorescence microscopy revealed the presence of mCherry expressing parasites in the infected mice, demonstrating that parasites carrying ATPβKOmCh could complete the liver stage (Figs 6A I and II). However, the proportion of red colour parasites was very low in comparison to the wildtype parasites. Nevertheless, all gene combinations expected as P0 following recombination, were detected by PCR screen, including the Pbs48/45KO allele, presumably transmitted via the male ATPβWT Pbs48/45KO gamete (Fig 6A III).

To further verify the presence of ATPβKOmCh parasites, and to enhance the number of parasites present in the population, I selected for mutant parasites using pyrimethamine resistance linked to the ATPβKOmCh locus (Fig 6B I). Following infection with sporozoites from the ATPβKOmCh x ATPβWT-GFP cross, the mice were treated with pyrimethamine after the infection was established (7 days post infection). All parasites except mCherry expressing parasites were cleared after 4 days of pyrimethamine treatment and did not reappear over further days of observation as confirmed by the PCR (Fig 6B II).

4. Discussion

In this study I have used a reverse genetic strategy to make one of the first explorations of energy metabolism in the liver stage of malaria parasites. Previously, energy metabolism has been difficult to assay in liver stage malaria parasites. The liver is difficult to access, and the number of parasite is relatively miniscule. An additional problem is that the liver cells are metabolically active, so teasing out the parasite metabolic activity from that of the host is problematic. To address this, I applied my genetic complementation strategy (10) to bridge knockout alleles of mitochondrial ATP synthase through mosquitoes and then on to liver stages to assess essentiality of this gene in liver stage parasites.

The ATP synthase β subunit has been the subject for genetic manipulation in both P. falciparum and P. berghei strains. An early report concluded that the β subunit of ATP synthase was essential in P. falciparum blood stage on the grounds that no knockout could be obtained after concerted effort . In contrast, a knockout of the β subunit of ATP synthase was obtainable in P. berghei parasites and caused only a minor growth defect , which is consistent with a global knockout program for several ATP synthase subunits in which most were found to be dispensable . What can we make of this apparent difference between reliance on mitochondrial ATP synthase in rodent and human malaria parasites? One possible explanation is that P. falciparum preferentially invades mature erythrocytes whereas P. berghei utilises reticulocytes, which can perhaps provide more metabolic resources than mature erythrocytes. Reticulocytes have a complex, enriched metabolic profile than mature erythrocytes and thus a greater metabolic overlap between parasites occupying reticulocytes and mature erythrocytes has been observed from multiple knockout studies . A potential confounding factor is the reliance on absolute gene knockouts in P. falciparum rather than conditional knockouts. Indispensability is often inferred by the lack of ability to procure a knockout. However, if a gene knockout incurs a growth rate cost, it can be difficult to recover knockout parasites. It will be interesting to learn if mitochondrial ATP synthase is dispensable or not in human malaria parasites at blood stage.

When the β subunit of ATP synthase knockout P. berghei parasites were introduced into the mosquito stage, progression from ookinete to oocysts was found to fail . Similar to many of the genes related to either TCA cycle or electron transport chain, these mitochondrial ATP synthase knock-out parasites appear unable to scavenge sufficient resources from the mosquito to sustain their development. Trehalose rather than glucose becomes the key sugar molecule in the mosquito hemolymph and the accessible quantity may be limiting in comparison to the erythrocyte stage . Indeed, a picture is gradually emerging that parasite maturation and proliferation in the mosquito is constrained by the accessibility of limited metabolites, which is manifested by parasite death at various stages in the mosquito midgut for different metabolic knockouts . In addition to any limitation of resources in the mosquito hemolymph, it is also possible that the protective cyst wall is a substantial barrier for the exchange of metabolites. Whatever the case, it is clear that the parasites require a robust, functioning mitochondrial energy generating system to sustain themselves within mosquitoes .

Reliance on a complete system of mitochondrial ATP synthesis machinery in malaria parasite mosquito stages creates an obvious blockade for reverse genetic studies of liver stages. I presented a genetic complementation strategy that allowed me to produce gene of interest deficient sporozoites despite the fact that the gene was essential for mosquito stage development of sporozoites . In this work I applied the same approach to observe liver stage growth of the ATP synthase β subunit deficient P. berghei strain. By crossing to two different strains, one a wildtype P. berghei having GFP inserted into a null chromosome [chromosome 6;] and second a male sterile line Pbs 48/45 , the lack of the active subunit was compensated. By using two fluorophores in the ATPβWTmCh x ATPβWT-GFP cross, I could easily follow up the self-crossed parasites and recombinants. The ATPβ knockout parasites become female sterile once introduced into the mosquito therefore I used the male sterile strain to cross with, with the aim of increasing the number of recombinant parasites in the resulting progeny.

With the cross of ATPβ knockout parasites into the parasites with wildtype locus of ATPβ, I could restore the growth of oocysts and the observed progeny had no detectable difference in terms of production of oocysts or salivary gland sporozoites (Fig 4). Similarly, mCherry expressing sporozoites were readily recovered from both crosses. Since the reported phenomena of "carry-over" of oocysts proteins into the sporozoites , all the sporozoites I recovered were red in color. Therefore, I did not attempt to further analyze the motility of the sporozoites as it can be misleading with the carry-over of foreign products.

I observed normal growth of ATPβ knockout parasites (genotyped by their red fluorescence) in in-vitro liver cells (Fig 5), with no significant difference in average size, nuclear content nor merosomes produced between ATPβ deficient and wildtype parasites (Table 2) strongly suggesting that a functional ATP synthase gene is not required for liver stage development.

To further confirm normal growth of these knockout parasites in in-vivo liver stage, I next studied the capacity of mutants to progress to the next blood stage/P0 by infecting naïve mice with the complemented sporozoites from the cross. Parasites were recovered from mice infected with both bite-back and IV sporozoite injection (1,000 and/or 10,000 sporozoites per mouse) within time frames (d 4-5) equivalent to wildtype parasites, strongly suggesting that the ATPβ deficient parasites successfully completed liver stage development in vivo as well as in vitro. Fluorescent microscopy observations revealed the presence of only a small proportion of red fluorescent parasites with a higher proportion of parents and recombinants recovered (Figs 6A I,II). The fact that the knockout parasites have a slight growth delay in blood stage perhaps explains the low numbers of red fluorescent parasites present in the P0 progeny. To increase the knockout parasite population in the ATPβKOmCh x ATPβWT-GFP cross, I treated infected mice with pyrimethamine once they reached approximately 5% parasitemia. Since the GFP expressing parasites are pyrimethamine sensitive, the treatment enriched red color parasites (Fig 6B. I). It was not possible to treat ATPβKOmCh Pbs48/45WT x ATPβWT P48/45KO infected mice with pyrimethamine as both ATPβKO and Pbs48/45KO have hDHFR inserted and are thus pyrimethamine resistant. Phenotyping of parasites by PCR further confirmed the presence of ATPβKOmCh parasites (Figs 6A III and B II) as well as the recovery of other genetic combinations of parasites post recombination (Fig 6A III).

With careful analysis of ATPβKOmCh parasite growth in liver stage and further into the P0 blood stage, I here confirm the dispensability of the presence of a functional ATP synthase complex in the inner mitochondrial membrane during liver stage development. It has been previously reported by Boysen and Matuschewski that the complex I of electron transport chain, the NADH:ubiquinone dehydrogenase complex is also not essential for liver stage growth of P. berghei. They used a similar approach to generate sporozoites with the gene knockout and to recover the P0 knockout parasites without my refinement of fluorescently tagging knockout parasites. Nevertheless, my findings are in line with this early work suggesting mitochondrial energy metabolism is dispensable in rodent malaria

parasite liver stage.

In the absence of the complex V of electron transport chain, the parasites must either use a different approach to yield ATP driven by the proton gradient across the inner mitochondrial membrane or a different mechanism rather than depending on the ATP synthase complex. The most likely hypothesis is that rodent malaria parasites rely primarily on glycolysis for their ATP production in liver stages just as they do in blood stages.

This first foray into the reverse genetics of malaria parasite liver stage energy metabolism provides a finding that hepatic stages of the rodent malaria parasite probably don't require mitochondrial ATP synthesis. My tentative conclusion is that liver stage parasites resemble blood stage parasites in relying on glycolysis to generate most of their ATP. Nevertheless, I demonstrate the excellent utility of my complementation strategy to undertake reverse genetic studies of liver stage malaria parasite energy metabolism for which there has previously been an impasse.

Acknowledgement

The author acknowledges the University of Melbourne, Australia for: Melbourne International Research Scholarship, Melbourne International Fee Remission Scholarship, Faculty of Science RHD travel grant, Science Abroad Travel Scholarship and Albert Shimmins award. The author further expresses sincere thanks to Dr. Angelika Sturm (University of Melbourne) for generating and cloning the P. berghei ATPase knockout strain, Dr Dean Goodman (University of Melbourne) for providing The PbANKA parasite lines expressing GFP and mCherry fluorescence and Oliver Billker for the Pbnek-4ko strain (Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK). The PbsP48/45KO strain was provided by Andy Waters (University of Glasgow, Glasgow, Scotland). The author is grateful to Dr. Alex Johnson, Prof. Stuart Ralph, Vanessa Mollard, Anton Cozijnsen and all the McFadden lab members of University of Melbourne for their innumerable support throughout her PhD.

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Cardiovascular Risk Assessment in Diabetes; Challenges and Opportunities

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Introduction

Diabetes mellitus (DM) is a major public health problem worldwide. Current global estimates indicate that this condition affects 415 million people and is set to escalate to 642 million by the year 2040. A further 193 million people with diabetes remain undiagnosed due to the often mild or asymptomatic nature of this condition.

South Asia is one of the worset affected area with diabetes and it is estimated that 8-12% of people living in South Asia have type 2 diabetes mellitus (T2DM). Accumulating evidence suggests that the prevalence of diabetes is rising in almost all parts of the world, but this rise is reported to be much higher in South Asia compared to the other parts of globe. Similar to rest of the South Asian countries, diabetes is rising in Sri Lanka too. Fernando et al showed that the prevalence of diabetes was 5% in 1994 , however, it had increased by almost three folds in some part of the country within next two decades. It is interesting note that the prevalence of diabetes can very depend on factors such as geographical area, study population (e.g-rural versus urban) , ethnicity and the diagnostic tool used to diagnose diabetes. American Diabetes Association (ADA) has recommended three diagnostic tools namely plasma glucose criteria, either the fasting plasma glucose (FPG) or the 2-h plasma glucose (2-h PG) value during a 75-g oral glucose tolerance test (OGTT), or glycosylated hemoglobin (HbA1c). However, previous studies conducted in particular in Asia suggest that diagnostic performance of these tests is not uniform.

HbA1c as diagnostic test offer several advantages over fasting blood sugar. Firstly, HbA1c is less affected by day-to-day variation in plasma glucose and secondly it doesn't require fasting and dietary preparations. It is also now formally endorsed in many countries as a diagnostic test for T2DM. However, debates still continue regarding its applicability for diagnosis of diabetes. Even though, HbA1c is found to be effective in predicting diabetes especially in Caucasians there is limited evidence of its diagnostic utility in South Asians.

Considering different phenotypes of diabetes and other characteristics such as genetic differences in the concentration of hemoglobin, and the rates of glycation , more evidence and data are needed to evaluate HbA1c cut-off points in diagnosing diabetes and pre-diabetes in Sri Lanka. The first study presented in this oration was done with aims of determining the optimal HbA1c cut-off points for detecting diabetes in Sri Lanka and to study the sensitivity and specificity of cut-off levels of HbA1c recommended by the ADA against the gold standard oral glucose tolerance test (GTT).

Study 1

Use of HbA1c to diagnose type 2 diabetes mellitus among high risk Sri Lankan adults

Aim:

Even though, glycosylated hemoglobin (HbA1c) was found to be effective in predicting diabetes especially in Caucasians there is limited evidence of its diagnostic utility in high risk Sri Lankan adults. This study aimed to determine the optimal HbA1c cut-off points for detecting diabetes in a high risk population in Sri Lanka.

Materials and methods:

This community based study consisted of 254 previously healthy adults with history of diabetes in one or more first-degree relatives. Fasting plasma glucose (FPG) , glucose tolerance test (GTT) and HbA1c were measured in all and GTT was used as a reference to diagnose T2DM. Receiver operating characteristic curve was created to find the optimum HbA1c cut-off value to predict diabetes.

Results:

The mean age of the study population was 50.3 (12.1) years, and 48.6% were females. The mean FBS, GTT (2 h glucose value) and HbA1c were 102 mg/dL, 145 mg/dL and 6.1% respectively. Of the total number of 254 subjects, 12.2% (n = 31) were diagnosed with diabetes based on FPG criterion of _126 mg/dL and 16.1% (n = 41) were diagnosed based on GTT (two h value of _200 mg/dL). Significantly higher number of subjects (n = 70, 27.6%) were diagnosed with diabetes based HbA1c criteria of _6.5% ($P < 0.01$). Nearly 10% of subjects (25/254) were detected to have diabetes based on all three tests. Twenty seven (11%) were diagnosed to have diabetes by both FPG and GTT. Out of 41 subjects diagnosed with diabetes based on GTT criterion, 8 were found to be non-diabetic based on both FPG and HbA1c criteria. Out of 70 subjects diagnosed with diabetes based on HbA1c, 34 (48%)

were found to be non-diabetic based on both FPG and GTT. Overall less than half of the subjects diagnosed as T2DM by HbA1c fulfilled the criteria for diabetes based on FPG (35%) and GTT (41%). Interestingly, all patients detected to have diabetes based on FPG, were found to have diabetes either by HbA1c or GTT . The prevalence of pre-diabetes based on FPG and GTT was 27%, and 19% respectively. With the HbA1c, the prevalence of pre-diabetes rose 39% and it was significantly higher than the both GTT and FPG ($p < 0.01$). Considering GTT as the gold standard, HbA1c at cut-off value of $\geq$6.5% had 78% sensitivity and 82% specificity. In comparison, FPG had a comparatively lower sensitivity (65%), but had a higher specificity (98%). When FPG and HbA1c were used in combination, the sensitivity improved to 82% and the specificity remained at 82%. The diabetic group with Hba1c >6.5% were further categorized into two groups based on 2-h value of GTT (presence of T2DM or not) . Results showed that both age and BMI were not different between subjects with or without T2DM. However there is significant difference of mean FBS values between the two groups even though all of them have T2DM based on Hba1c. ROC curve was performed to assess the discriminative capacity of HbA1c for detection of T2DM. The area under the ROC curve for the ability of HbA1c to predict T2DM based on GTT was 0.911 (SE 0.02) . The best cut off point of HbA1c value to predict T2DM occurred at 6.35 (sensitivity of 80.5%, specificity 79%). When the lower cut-off value of 6% was used, the sensitivity improved to 95%, but it was at the expense of reduced specificity(67%). HbA1c had a positive and significant correlation with GTT ($r = 0.78, P < 0.001$) and FBS ($r = 0.70, P < 0.001$).

Conclusion:

Our study showed that optimum HbA1C cut-off for detecting diabetes was 6.3% and it had better sensitivity, but lower specificity than FPG. This study further showed that the prevalence of diabetes would become double if HbA1c is used over FPG to screen this high risk population in Sri Lanka.

After knowing that Hba1c is better in many ways than FBS in detecting diabetes in high risk individuals we were interested to know which cardiac risk score is better in predicting cardiac risk in diabetic individuals in Sri Lanka. This is important due to the fact that patients with T2DM have a considerably higher risk of developing cardiovascular disease (CVD) compared with age- and sex-matched patients without T2DM. They have a two to fourfold increase in risk of having ischemic heart disease (IHD) and a 1.5 to 3.6-fold increase in mortality. Due to the heterogeneous nature of the disease, prevalence and pattern of CVD risk factors vary among individuals with T2DM and therefore the risk of developing cardiovascular events such as coronary artery disease and stroke is also different. Recognizing CVD early at asymptomatic stage is important as several primary prevention strategies are proven to be effective in reducing future cardiovascular events in patients with T2DM. In particular, lipid-lowering therapy with statin, blood pressure control with anti-hypertensives, and antiplatelet therapy with aspirin have been shown to be effective in patients with T2DM. Indications of these primary preventive strategies are decided based on the cardiac risk. For an example, aspirin therapy is recommended by the American Diabetes Association (ADA) for patients with T2DM with a 10-year cardiac risk of 10% or above. Although interventions focused on individual CVD risk factors have proven benefits in patients with T2DM, a previous study has shown that adopting total risk approach, in comparison to treatment decisions being based on the level of a single risk factor, could lead to reductions in expenditure. However, it is unclear whether these risk assessment tools which were primarily developed using data of different ethnic group and geographical location can be used for our population.

Over the past few decades, several risk assessment tools have been developed to estimate the total CVD risk in individuals with T2DM. The Framingham risk score is one of the most widely used risk assessment tools globally. It is based on the findings of the Framingham study conducted predominantly among the Caucasian population. The UK Prospective Diabetes Study (UKPDS) risk engine is another risk assessment tool, which was developed based on data from this study. Unlike other risk assessment tools, the UKPDS risk engine is diabetes-specific and it incorporates glycemia, systolic blood pressure (SBP), and lipid levels as risk factors, in addition to age, sex, ethnic group, smoking status, and time since diagnosis of diabetes. Based on the findings of epidemiological surveys on the prevalence and magnitude of CVD risk factors in the South Asian region, the World Health Organization/International Society of Hypertension (WHO/ISH) has developed a risk assessment tool suitable for use in individuals with diabetes in the region. These WHO/ISH charts use five parameters that can be measurable at low resource, primary care setting and include sex, age, SBP, smoking status, and serum total cholesterol (TC). Using the WHO/ISH charts, an individual's risk of developing a vascular event during the next 10 years is predicted as a probability. However, the major modifiable CVD risk factors in diabetes such as low-density lipoprotein (LDL) cholesterol and diastolic blood

pressure (DBP) for which therapeutic interventions have shown proven benefits have not been included in the WHO/ISH charts in order to reduce the cost of its application in the resource poor setting.

The Ministry of Health in Sri Lanka has recommended the use of WHO/ISH charts for screening of individuals in the primary care setting and professional organizations such as the Ceylon College of Physicians, have endorsed this approach. However, the validity of WHO/ISH risk assessment tool in identifying high risk individuals among Sri Lankans with T2DM has not been studied yet.
The second study present in this oration was done with the aims of estimating the prevalence of CVD risk factors in patients with T2DM, to find out CVD risk estimated by two different tools (WHO/ISH risk prediction charts and UKPDS risk engine), and to assess the validity of two risk prediction tools by their ability to detect individuals with raised LDL and DBP based on their cardiac risk.

Study 2

Cardiovascular risk assessment in type 2 diabetes mellitus: comparison of the World Health Organization/International Society of Hypertension risk prediction charts versus UK Prospective Diabetes Study risk engine

Introduction

Patients with type 2 diabetes mellitus (T2DM) are at higher risk of developing cardiovascular diseases, and assessment of their cardiac risk is important for preventive strategies.

Aim

The Ministry of Health of Sri Lanka has recommended World Health Organization/International Society of Hypertension (WHO/ISH) charts for cardiac risk assessment in individuals with T2DM. However, the most suitable cardiac risk assessment tool for Sri Lankans with T2DM has not been studied. This study was designed to evaluate the performance of two cardiac risk assessments tools; WHO/ISH charts and UK Prospective Diabetes Study (UKPDS) risk engine.

Methods

Cardiac risk assessments were done in 2,432 patients with T2DM attending a diabetes clinic in Southern Sri Lanka using the two risk assessment tools. Validity of two assessment tools was further assessed by their ability to recognize individuals with raised low-density lipoprotein (LDL) and raised diastolic blood pressure in a cohort of newly diagnosed T2DM patients (n=332).

Results

WHO/ISH charts identified 78.4% of subjects as low cardiac risk whereas the UKPDS risk engine categorized 52.3% as low cardiac risk ($P<0.001$). In the risk categories of 10%–<20%, the UKPDS risk engine identified higher proportions of patients (28%) compared to WHO/ISH charts (7%). Approximately 6% of subjects were classified as low cardiac risk (<10%) by WHO/ISH when UKPDS recognized them as cardiac risk of >20%. Agreement between the two tools was poor (κ value =0.144, $P<0.01$). Approximately 82% of individuals categorized as low cardiac risk by WHO/ISH had higher LDL cholesterol than the therapeutic target of 100 mg/dL.

Conclusion

There is a significant discrepancy between the two assessment tools with WHO/ISH risk chart recognizing higher proportions of patients having low cardiac risk than the UKPDS risk engine. Risk assessment by both assessment tools demonstrated poor sensitivity in identifying those with treatable levels of LDL cholesterol and diastolic blood pressure.

After knowing that WHO risk score perform poorly in detecting cardiac risk , we were interested in validating these risk assessment tools for Sri lankan settings. The gold standard way of validating a risk tool is assessing the cardiac risk at baseline using cardiac risk tools and by observing cardiac events over many years. However, this requires lot of resources and long duration of follow up. Since Carotid Intima-Media Thickness (CIMT) has been shown to predict Cardiovascular (CV) events in multiple large studies, ability of the risk prediction tools to predict CIMT can be used to assess the accuracy of these tools . The amount of lesion in the Common Carotid Artery (CCA) has been reported to correlate to the extent of atherosclerotic lesions elsewhere in the body including the heart. Several large, research-based cohort studies have clearly indicated a relationship between CIMT and CV events. In the third study presented in this oration, we study the associations between CIMT and the CVD risk estimates obtained using the three risk assessment tools in a cohort of patients with diabetes mellitus.

Study 3

Association of Risk Estimates of Three Different

Cardiovascular Risk Assessment Tools with Carotid Intima Media Thickness in Patients with Type 2 Diabetes

Introduction

Risk assessment tools used to calculate the Cardiovascular Disease (CVD) risk such as the Framingham Risk Score (FRS), United Kingdom Prospective Diabetes study (UKPDS) risk engine and the World Health Organization (WHO) risk score have not been tested on their ability to detect subclinical atherosclerosis in most developing countries.

Aim

To study the association between the calculated CVD risk scores using each of these tools and Carotid Intima Medial Thickness (CIMT), a surrogate marker of atherosclerosis, in a group of patients with Type 2 diabetes (T2DM) in Sri Lanka.

Materials and Methods

We calculated CVD risk scores of 68 randomly selected patients with T2DM with no history or symptoms of CVD and measured their CIMT using B-mode ultrasonography (USS). Carotid USS was considered positive when the maximum carotid IMT was 0.9mm or when arteriosclerotic plaques were detected. The 10-year CVD risk was calculated using the FRS, the UKPDS risk engine and the WHO risk score. Pearson correlation was used to study the association between CVD risk scores with CIMT.

Results

Of the 68 patients studied, 50% were males and their mean age (SD) was 56.9 (±9.6) years. The mean age at onset and duration of diabetes were 44.3(±9.1) and 12.2(±7.6) years respectively. Of the scoring methods, UKPDS tool had weak, but significantly positive ($r = 0.26$, $p < 0.05$) and FRS had positive but not significant association ($r = 0.21$) with CIMT. There was a negative association between CIMT and WHO risk score ($r = -0.07$).

Conclusion

Of the three CVD risk assessment tools, both UKPDS risk engine and FRS have almost equal ability (former being marginally superior) in predicting underlying atherosclerotic vascular disease in patients with T2DM. Negative association of the WHO risk score with CIMT argues against its utility for CVD screening. These findings highlight the need for developing more sensitive and reliable CVD risk assessment tools for developing countries. Findings of this study revealed that FRS and UKPDS risk engines are better CVD screening tools to detect subclinical atherosclerosis than the WHO risk score in a cohort of Sri Lankan patients with T2DM. Ability of a CVD risk scoring tool to detect individuals with high risk of CVD in a given setting depends on several factors. Out of them, variables (risk factors) used in each tool to calculate CVD risk and the relative weightage given to each of them are important. Three CVD risk screening tools compared in this study differ in the variables included in them. Age, gender, smoking status and systolic blood pressure are common to all three tools but the UKPDS risk engine in addition include ethnicity, duration of diabetes, glycosylated haemoglobin level and the FRS include high density cholesterol level. Findings of this study revealed that inclusion of additional parameters have improved the detection rates of individuals at all cut-off levels in both FRS and UKPDS tools.

The possible explanation for both UKPDS and FRS to reveal stronger associations with CIMT than the WHO tool could be due to inclusion of additional variables such as HDL cholesterol, glycated haemoglobin and duration of diabetes in them. Studies have shown higher prevalence rates of lower HDL cholesterol among Indian Asians . Higher CVD morbidity and mortality in the South Asian region is postulated to be associated with lower HDL level . Markedly lower percentages of individuals in the CVD risk categories of > 10% with WHO risk score suggests the relative lack of sensitivity of including the total cholesterol level alone in predicting the CVD risk in Asian ethnicity.

Summary

Studies presented in this oration showed that the prevalence of diabetes could vary depending on the screening test used, with a higher prevalence of diabetes was observed with HbA1c and comparatively lower prevalence was seen with FBS. The prevalence of diabetes gets double when HbA1c (27%) is used over FBS (12%) to screen diabetes. Therefore, we recommend HbA1c over FBS to screen diabetes at community level/ asymptomatic individuals in Sri lanka.

The second study presented in this oration revealed that the WHO cardiac risk assessment tool tend to classify most patients (>80%) to low risk category and it performed poorly when compared to UKPDS risk tool in detecting adverse LDL. The third study confirmed that CVD risk estimates obtained with FRS & UKPDS tools positively predict

atherosclerosis (CIMT) while WHO chart has no predictive ability and hence WHO risk chart doesn't recognise individuals with established atherosclerosis. Therefore, we can conclude that the performance of WHO chart is poor in our setting and we suggest Ministry of Health, Sri Lanka to revisit the recommendation of using WHO risk tool as the primary risk tool in assessing cardiovascular risk of individuals with diabetes at the community level.

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Abstracts

FAMS 2018

Factors associated with Health Related Quality Of Life (HRQOL) of patients with stroke

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Introduction

Stroke has been associated with poor HRQOL. This study was aimed to assess the factors associated with HRQOL of patients with stroke.

Methods

A descriptive cross-sectional study was conducted in Teaching Hospital Karapitiya with the participation of 241 patients with stroke. The proxy version of SAQOL-39g scale was used to assess the HRQOL and the Barthel Index was used to determine the level of dependence. The SPSS version 20 was used to analyze data. The significantly correlated variables with Pearson and Spearman rank order correlations were entered into the multivariate regression analysis model. The weak correlations were excluded by applying stepwise manner. The p-value < 0.05 was considered as significant.

Results

The mean age of the participants was 66.1 years (range 33-80), (SD±11.7) with males comprising two-thirds of the study sample [61% (n=147)]. The level of dependence, having aphasia, age, type of stroke, side of the lesion and level of education were the significant predictors of HRQOL of patients with stroke $F(6,234) = 42.6$, $p < 0.05$. The most significant predictor was level of dependence ($r=0.72$, $R2=0.52$, $SEE=0.67$). The correlations of the QOL scores with Barthel index scores and higher education level were significant in the positive direction $p < 0.05$.

Conclusion

Severe level of dependence, having aphasia, older age, haemorrhagic stroke and left side lesions was associated with low HRQOL scores. Higher education level associated with higher HRQOL scores. The level of dependence, having aphasia, age, type of stroke, side of the lesion and level of education were independent predictors of post stroke HRQOL.

Association between stress and non verbal intelligence of female adolescents in Galle educational zone: a cross sectional study

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Background

Age range from 11 to 14 years is regarded as early adolescence. This period is characterized by physical, psychological, cognitive and social changes. The brain continues to develop into adolescence and these developmental changes in brain are also sensitive to environmental stresses.

Objective

To determine the association between stress scores and non verbal intelligence test scores of early female adolescents in Galle education zone.

Material and methods

A school based cross - sectional study was conducted on female adolescents (11 - 14 years, n = 218). After obtaining baseline data, Test of Non Verbal Intelligence (TONI) was administered to measure abstract reasoning and problem solving ability. The educational performance was determined by Mathematics, Sinhala and Science subject scores. Self administered socio demographic questionnaire and psychosocial adversity scale were used to assess socioeconomic state and psychosocial adversities respectively.Adolescent Stress Questionnaire (ASQ), which measures 9 dimensions of stress, was used to assess stress. Bivariate Pearson correlation coefficient test applied to determine the associations.

Results

Perceived stress score ranged from 88 to 208 (142.01±19.9). Many adolescents were stressed as n=139 (63.7%) and n=79(36.3%) belonged to stress group and non stress group category respectively. Mean TONI raw score of stress group participants 16.75(5.54) is significantly lower compared to counterpart 23.78(5.75). There was a negative correlation between ASQ total score with TONI raw score ($r=-0.35; p<0.01$), TONI quotient ($r=-0.22: p<0.01$) and TONI percentile ($r=-0.34; p<0.01$) values. However ASQ total score was not significantly correlated with mathematics, science and sinhala subject performances.

Conclusions

Stress is negatively associated with scores of Test of Non Verbal Intelligence, indicating poorer cognitive function amongst the stressed adolescents. Urgent measures to decrease stress levels amongst these adolescents need to be implemented.

Association between social activity participation and cognition of elderly people in elderly care institutions in Southern Province, Sri Lanka

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Introduction

Population ageing is a characteristic of 21st century. Decline in cognitive functions in ageing is becoming a personal and societal burden. The exploring of factors that reduce this cognitive decline is vital for preventive measures and has attracted the interest of researchers. This study was aimed to assess the association between social activity participation and cognition among a sample of elderly people living in elderly care institutions.

Materials and methods

A descriptive cross sectional study was carried out with 421 elderly people living in elderly care institutions in Galle & Matara Districts in Southern Province. Cognitive status was assessed using Mini Mental State Examination (MMSE). Independent sample t test was used to assess the association.

Results

The mean age of the study sample was 71.9+6.7, of which 65.8% (n=277) were females. Mean MMSE score was 22.9+4.9. Out of total study population, 56.3% (n=237) had normal cognition while 43.7% (n=184) had mild to moderate cognitive impairment. Nearly 76% (n=319) had social interactions, 44.2% (n=186) had participated in activity groups, 11.2% (n=47) had engaged in voluntary work while 6.7% (n=28) had participated in community related organizations. Those who had social interactions (23.7+4.6 vs. 20.4+5.0), participated in activity groups (24.4+4.6 vs. 21.8+4.8), did voluntary work (25.1+3.8 vs. 22.6+4.9) and participated in community related organizations (26.4+2.7 vs. 22.7+4.9) had significantly higher MMSE score than their counterparts ($p<0.01^{**}$).

Conclusion

There is a significant association between social activity participation and cognition among elderly people living in elderly care institutions in this study population.

Impact of physical activity pattern on quality of life of pre and postmenopausal women: a cross-sectional study

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Background

Menopause is associated with impaired quality of life (QOL). This cross-sectional study was aimed to evaluate the QOL and impact of physical activity (PA) pattern on QOL of a group of premenopausal (PrMW) and postmenopausal women (PMW) in Bope-Poddala MOH area.

Materials and methods

Healthy community-dwelling women (PrMW=184 and PMW=166) were selected randomly. Self-administered short-form-36 survey was used to evaluate the QOL under 08 domains; physical-functioning (PF), role-limitations due to physical-health (RLPH), role-limitations due to emotional-problems (RLEP), social-functioning (SF), energy/fatigue (EF), emotional-wellbeing (EWB), pain (P) and general-health (GH). PA pattern was evaluated using international PA questionnaire (IPAQ) short-version, which evaluates walking, moderate and vigorous intensity PAs and total PA score (MET-min/week).

Results

Mean (SD) age of PrMW and PMW was 42.4(6.0) and 55.8 (3.8) years. Mean (SD) overall QOL of PrMW and PMW were 66.82(17.94) and 57.49(18.83) ($p<0.001$) respectively. Significantly lower QOL was observed in PF ($p<0.001$), RLPH ($p<0.001$), RLEP ($p=0.005$) and P ($p=0.001$) domains in PMW. In PrMW, QOL was not correlated with PA pattern even after the possible confounders (age, sociodemographic status and menopausal symptoms scores) were controlled. In PMW, QOL was correlated significantly with vigorous (r; 0.19, $p=0.01$), moderate (r; 0.26, $p=0.001$) and total PA score (r; 0.27, $p<0.001$). These significant correlations were remained or improved even after controlled for possible confounders (vigorous; r; 0.28, $p<0.001$, moderate; r; 0.26, $p=0.001$ and total PA score; r; 0.34, $p<0.001$) in PMW.

Conclusion

Level of PA is positively correlated with QOL in PMW. The interventions focused on PA as a lifestyle modification can be considered in improving QOL of PMW.

Psychological care domains among institutionalized elders in Sri Lanka: a qualitative study.

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Background

"Elderly" has been defined as a chronological age 65 years old or more. Presently the population of people aged 60 years and above is increasing rapidly in the world. Residential care or personal care homes offer personalized service to small groups of elders. The quality of life of the institutionalized elders depend on quality of physical care they received.

Objective

To identify the psychological care domains among institutionalized elders in Sri Lanka.

Methodology

Phenomenological study design was used to conduct the study. In-depth interviews (IDI) were conducted by the investigator in order to obtain the perceptions of psychological care received by elders at the elderly homes after obtaining ethical approval from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka. 50 participants were recruited by using convenient sampling technique at all the elderly homes in Galle Municipal area. Informed consent was obtained from the participants before starting data collection. IDIs were conducted in Sinhala according to the pre tested structured interview guides to maintain the uniformity. Data collection was done up to data saturation is achieved. 50 IDIs were conducted with elders. IDIs were transcribed verbatim. Data analysis was done in manually using thematic analysis at the same day of the data collection.

Results

Three domains of psychological care were identified; care for the anxiety; care for the social interactions and care for the hopelessness.

Conclusion

There are three domains to address when providing psychological care in order to improve the quality of life among institutionalized elders in Sri Lanka.

Two-dimensional ultrasonographic volume assessment of globular and non-globular objects: a preliminary study

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Introduction

Ultrasonography is a widely used imaging modality to measure volumes of normal organs and pathological structures. The volume is auto generated by the scanner assuming the structure as a globe. However, there were arguments regarding the accuracy of sonographically measured organ volumes compared to the actual volumes (AV). There is scarcity of studies assessing ultrasonic volume accuracy for different shaped objects. The objectives of the study were to assess the accuracy of auto generated sonographic volume measurements of different shaped objects and to compare the measurement accuracy between two different scanners.

Methodology

Hollow plastic objects; 6 globular (Gb) and 6 non globular (NGb), were filled with known water volumes (10-250 ml) taken as AV. Height, length and width of the objects were measured ultrasonically using Affinity (VP) & Volusion (VV) scan machines with 3.5Mz probe, by a single Radiologist. Auto generated volumes of the objects were recorded. AV were compared with VP and VV.

Results

Average volumes of Gb(SD) and NGb(SD) were 128.56(102.3)ml and 159.94(177.74)ml respectively ($p<0.05$). No statistically significant difference was found between AV and auto generated volumes of Gb shaped objects ($p=0.178$). A significant difference was found between AV and auto generated volumes in NGb shapes ($p=0.019$). No statistically significant differences between VP and VV of both Gb and NGb objects ($p<0.05$).

Conclusion

Although, auto generated ultrasound volume measurement is accurate for globular shaped objects, there seems to be a significant error for non globular objects. No statistically significant differences in volume calculations are observed between two scanners.

A case of fatal elder abuse and neglect in Sri Lanka

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Introduction

Abuse of elderly people is a hidden problem. According to the World Health Organization data around 1 in 6 people 60 years or older experience some form of abuse in community setting. Elder abuse is predicted to be increased as many countries are experiencing rapidly ageing populations. The real extent of the problem however is not adequately researched in Sri Lanka.

Case Detail

73 years old lady who was living with her daughter's family in a tea estate home was found dead. She was partially incapacitated due to complete blindness for last two years.

A medico legal autopsy was performed. The body was severely emaciated. There were multiple, bilateral, comminuted rib fractures with bleeding into surrounding muscles. The left pubic bone was fractured with bleeding into surrounding soft tissues extending to the anterior abdominal wall. There was severe coronary atherosclerosis in the left anterior descending coronary artery and there was an area of fibrosis in the wall of the myocardium. The cause of death was concluded as multiple rib fractures, pelvic fractures following blunt force injuries.

Conclusion

The multiple, comminuted rib fractures can be interpreted as a sign of a significant trauma. As she was a partial incapacitated person, severe accidental trauma could be excluded and the pattern of injuries was suggestive of non-accidental injury. The ischemic heart disease may have contributed to the death.

Elder abuse can lead to serious physical injury and psychological consequences or even to death. Elder abuse and neglect may co exists where death may due to natural causes. All medical professional especially forensic pathologists should play a major role in identification and documentation of abuse in both living and dead.

Awareness of practical performance regarding first aid and safety measures on sports related injuries among school athletes in Anuradhapura educational zone , Sri Lanka.

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Introduction

Sports injuries are gradually increasing all over the world. School children are the first stepping stone in improving knowledge, attitude and skills in the community, therefore understanding and improving their knowledge of first aid and safety measures are of great importance. Proper first aid helps to minimize the extension of injuries, prevention of occurrence injuries and reduce further complications.

Objectives

Assess practical performance of first aid and safety measures by using different methods and protocols during sports related injuries among school athletes in Anuradhapura educational zone.

Methods

A descriptive cross sectional study was conducted among school athletes in Anuradhapura Educational Zone. Sample of 168 students aged between 12 -17 years were selected from three schools in this Zone and recruited for the study. A self-administered questionnaire which consist of both open ended and closed ended questions .The results were analyzed using SPSS 20 for the descriptive and analytical statistics.

Results

There were 168 school athletes of which 81were females and 87 were males in this target population. There were 73 subjects in the age group of 14 – 15 years. Out of all subjects only 145 had previous exposure to first aid training. However, Out of them in the study population only 1.2% had knowledge regarding the correct method of using the sling for bone fracture. Out of all, 7.1% had awareness of proper bandage protocol. Overall practical performances of first aid among school athletes were insufficient.

Conclusion

According the results of the study overall knowledge regarding first aid and practical protocol were poor. Health care professional should improve the first aid skills among school children in this education zone.

Domains of quality of domiciliary care for the patients with oral cancer in Sri Lanka: a qualitative study

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Background

Cancer is a disease which is widely spreading in the world. Oral cancer is the most common type of cancer among Sri Lankans. Domiciliary care is important to enhance the quality of life of oral cancer patients as in other chronic conditions. Identification of the domains of quality domiciliary care is important in order to provide a quality domiciliary care to patients with oral cancer.

Objective

To identify the domains of quality of domiciliary care received by the patients with oral cancer attending oncology clinic, Teaching Hospital Karapitiya.

Methodology

Phenomenological study design was used to conduct the study. In-depth interviews (IDI) were conducted by the investigator with the patients having oral cancer in order to obtain the perceptions on domiciliary care after obtaining informed written consent. Participants were recruited by using convenient sampling technique at the Oncology Clinic, Teaching Hospital Karapitiya. IDIs were conducted in Sinhala according to the pre-tested structured interview guide to preserve the uniformity. Data collection was done until data saturation was achieved. Thirty IDIs were conducted. Notes were takedown and analysis was done manually using thematic analysis on the same day. Ethical approval was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna.

Results and conclusion

Seven domiciliary care domains were identified; self-care deficit, impaired swallowing, disturbed sleeping pattern, disturbed energy, chronic low self-esteem, impaired role performance and impaired social interactions. These seven domains should be considered when attempting to improve the domiciliary care of patients with oral cancer.

Frequency of partner directed violence in the context of couple relationships of delusional jealousy patients reported to selected mental health services in Sri Lanka

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Introduction

Jealousy is a typical precipitant of violence. Violence is likely in any relationship tainted with jealousy. Violence of jealousy is primarily vented on the partner and often takes the form of intimidation followed by assaults.

Material and Methods

A descriptive cross sectional survey was conducted among fifty patients with delusional jealousy for duration of three months at National Institute of Mental Health, University Psychiatry Units of National Hospital and Teaching Hospital Karapitiya, and Norris Clinic. Data were collected using a self-administered general information sheet and a structured interview.

Results

30 (75.00%) out of 40 partners and 32 (64.00%) out of 50 patients had experienced partner directed violence. 23 (82.14%) out of 28 female, 6 (54.50%) out of 11 male partners and 11 (68.75%) out of 16 female and 21(63.63%) out of 33 male patients had experienced violence. 10(58.8%) partners and 7 (46.70%) patients experienced violence every day. Difference between partners and patients on exposure to partner directed violence was non-significant, $\chi 2$ (1) = .794, p = .373. Difference between male and female partners on exposure to violence was non-significant, (Fisher's exact P = .109). There was no significant difference between male and female patients on exposure to partner directed violence, $\chi 2$ (1) = .001, p = .974.

Conclusions

Violence is a common occurrence in jealous relationships. Violence was however not confined to partners of delusional jealousy patients. A majority of partners and patients had experienced partner directed violence. Exposure to violence was higher among partners than patients and females than males; however, differences among groups were not significant.

Homocystinuria: a paediatric case series

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Introduction

Homocystinuria is an autosomal recessive disorder with the prevalence of 1;200000. It is due to the defect in the Methionine metabolism which results in accumulation of homocysteine in the body. It is well known that early detection and initiation of treatment would prevent most of the complications in Homocystinuria. We report a series of patients with Homocystinuria followed up at university paediatric clinic Teaching Hospital Karapitiya.

Methods

Clinical profiles of patients with Homocystinuria were reviewed using clinic records and physical examination findings.

Results

There were four children with Homocystinuria from 7 to 18 years, of them, 2 were males. None of them were born to consanguineous parents and two were siblings. The age at diagnosis ranged between 6 to 17 years. All children had delayed cognitive development with the IQ level between 40-77. The condition was suspected with the onset of ophthalmic manifestations such as Ectopia lentis glaucoma and ptosis. Marfanoid boy habitus (tall stature, high arch palate and arachnodactyly) and scoliosis were present in all four children. Three children had low bone mineral density and 2 of them had fractures. None of them had thromboembolic events. Serum homocysteine and Methionine levels were high, ranged 145-373µmol/L (4.6-8.1) and 79-177 µmol/L (6-60) respectively. All had normal vitamin B12 levels between 150.0 and 216.0 pmol/L (140-650).

Conclusion

All children in this case series were diagnosed at an advance stage of the illness. Therefore, it is important to suspect Homocystinuria in children with intellectual impairment, visual defects and marfanoid body habitus.

Morphometric evaluation of renal dimensions: a preliminary cadaver study

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Introduction

Renal dimensions are considered as important parameters for clinical assessment of patients with congenital anomalies, neoplasia, renal artery stenosis and also for the assessment of renal transplant candidates. The aim of the present study was to determine the renal dimensions of adult cadaveric kidneys in a group of adult Sri Lankan population.

Materials & methods

Present study was conducted using a total of 52 kidneys (28 male and 24 female) obtained from formalin fixed 26 adult cadavers aged 58–72 years from the Department of Anatomy, University of Ruhuna, Sri Lanka. Subjects with any history of renal and vascular impairments were excluded from the study. Maximum renal length, thickness, width at superior pole, hilum & inferior pole were measured by a vernier caliper capable of measuring up to 0.01mm (Mitutoyo, Japan).

Results

The maximum renal length (cm) was male: 7.92±1.20; female: 7.74±0.93, maximum renal width at the superior pole: male: 3.39±0.52; female: 3.06±0.24, maximum renal width at the inferior pole: male: 3.29±0.46; female: 3.05±0.41, maximum renal width at the hilum: male:3.48±0.38; female: 3.52±0.25 and the maximum renal thickness male: 2.65±0.41; female: 2.73±0.48. Statistically significant gender differences were observed in superior pole renal width.

Conclusions

Renal dimensions possess significant clinical importance. The results of this preliminary study provides the first step in establishing the reference set of data on renal dimensions for an adult Sri Lankan population. Such population specific data will be of value in diagnosis as well as in the evaluation of progression of renal disease.

A death of a teenage due to a firearm injury

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Introduction

Deaths due to firearm injuries are becoming a major problem in Sri Lanka. The deaths may be due to suicidal, homicidal or accidental. It is very important to determine the manner of death during the medico legal investigation of these deaths.

Case report

A body, of a 16 year old boy was found in his back yard. A shot gun was fixed on to a small coconut tree and a suicide letter was found near the body. The scene was not disturbed. There was single perforated laceration with blackening and burning on the front chest. Blacking was wider towards the left side. The injury has perforated the left lung and the heart. There were nine split lacerations on the left back chest.
It was revealed that the mother has punished him in public before the incidence for misbehavior and victim had a past history of suicidal attempts.

Discussion

Perforated laceration on the front chest was the entry wound and nine split lacerations back of the chest were exit wounds. Single entry wound and multiple exit wounds are compatible with firing with a shot gun. The presence of burning and blackening indicates close range firing. Cause of death was concluded as a firearm injury to chest.

The presence of the causative weapon and a suicidal note at the scene, close range firing, stressful situation faced by the child and presence of past history of suicidal attempts were supportive of suicidal firearm death.

Students Abstracts

Awareness and compliance regarding food based dietary guidelines in pregnancy and the associated factors among pregnant mothers in Bope-Poddala area

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Introduction

Pregnancy is a vulnerable period in which many changes happen in the mother's body. Nutrition plays an important role during this period as these changes affect the wellbeing of both the child and the mother. It is known that nutritional requirements are increased during this period. This study aimed to assess the awareness of Food Based Dietary Guidelines (FBDG) in pregnancy, compliance with FBDG and the associated socio-demographic factors among pregnant mothers in Bope-Poddala area.

Materials and methods

A cross-sectional survey was conducted among 183 pregnant mothers attending antenatal clinics in Bope-Poddala area. Data were collected using a self-administered questionnaire. A 24-hour dietary recall was taken to assess their dietary intake. Data analysis was done using SPSS statistical software. Ethical approval was obtained from the Ethical Review Committee of the Faculty of Medicine, Galle.

Results

The Mean ageSD of the sample was 28.94.9 years and approximately 85% had completed GCE (O/L). Majority of the mothers (55.7%) had a satisfactory level of awareness about FBDG, though the compliance with FBGD among the mothers was poor (35.6%). No significant associations were found between age, education level or social class of the pregnant mothers and the level of awareness/compliance. However, a positive association was observed between the level of awareness of FBGD and the level of compliance among the mothers ($p<0.05$).

Conclusions

Even though the level of awareness of FBGD among the pregnant mothers was satisfactory, the level of compliance was poor. Nutrition education should be coupled with other measures to encourage compliance with FBDG in pregnancy.

Factors affecting pre-hospital delay in patients with stroke

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Introduction

Stroke requires urgent interventions like thrombolytic therapy (TT) within 4 ½ hours, to prevent mortality and permanent disability. This is limited by pre-hospital delays. This study aimed to find out the reasons for delay in care seeking at a thrombolytic facility among stroke patients attending a tertiary care setting.

Materials and methods

A hospital based descriptive study was conducted among 168 patients attending Teaching Hospital Karapitiya (THK). Ethical clearance was obtained from the Ethical Review Committee of Faculty of Medicine, University of Ruhuna. Data was collected using a questionnaire and analyzed with IBM SPSS Statistics version 23.

Results

Out of 168 patients, 33.9% have arrived after 4 ½ hours of onset of symptoms. Significantly more patients who are females ($p=0.049$), who live farther away from THK ($p=0.002$), who have taken more time to leave home ($p=0.008$), who chose a place other than THK as the first point of care seeking ($p<0.001$), have arrived after 4 ½ hours. Delay was not associated with the presenting symptoms, onset during sleep, being alone, mode of transport, difficult road access, prior knowledge about symptoms and risk factors of stroke. Only 37% knew about the free ambulance service ("Suwaseriya").

Conclusions

Pre hospital delay makes one third of stroke victims, not to be considered for TT even if otherwise indicated. Associated factors identified were, female gender, distance from THK, delay in leaving for hospital, seeking care from centers other than THK. Health education on symptoms and need for early care seeking at a thrombolytic facility, promoting "Suwaseriya", initiating TT in General Hospitals, may improve this situation.

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