

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

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

December 2017

Volume 05

Number 01



Female Aedes aegypti, the vector mosquito of potentially fatal diseases including dengue fever



#### Proceedings of Faculty of Medicine Academic Session (FMAS) -2017, University of Ruhuna

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#### Cover Story

Female Aedes aegypti, the vector mosquito of potentially fatal diseases including dengue fever, chikungunya, yellow fever and Zika. Adult female mosquito is a smallish, dark mosquito with conspicuous white markings and banded legs; the proboscis is all black although the palps are white tipped. Adult mosquitoes rest in cool, shaded places such as wardrobes, laundry areas, cabinets and under furniture and their larvae are typically found in small, wet places, including discarded bottle caps, soda cans, cups and tires, as well as potted plants and vases. Their eggs can survive for six months or more without being in water.

Sri Lanka experienced the worst epidemic of dengue in 2017. From 1 January to 7 July 2017, the Epidemiology Unit of the Ministry of Health (MoH) Sri Lanka reported 80 732 dengue fever cases, including 215 deaths. This is a 4.3 fold higher than the average number of cases for the same period between 2010 and 20162.

Approximately 43% of the dengue fever cases were reported from the Western Province and the most affected area with the highest number of reported cases is Colombo District (18 186) followed by Gampaha (12 121), Kurunegala (4889), Kalutara (4589), Batticaloa (3946), Ratnapura (3898), and Kandy (3853). Preliminary laboratory results have identified Dengue virus serotype 2 (DENV-2) as the circulating strain in this outbreak. Although all four DENV have been co-circulating in Sri Lanka for more than 30 years and DENV-2 has been infrequently detected since 2009.

- Identification of Aedes aegypti Long Intergenic Non-coding RNAs and Their Association with Wolbachia and Dengue Virus Infection\_Etebari K, Asad S, Zhang G, Asgari S (2016. PLOS Neglected Tropical Diseases 10(10): e0005069. https://doi.org/10.1371/journal.pntd.0005069
- Dengue update Epidemiology Unit. www.epid.gov.lk (accessed on the 28th Nov 2017)



#### Ruhuna Journal of Medicine

#### Contents

Editorial	II	Instructions to Authors
	Ш	Foreword - Professor Isurani Ileperuma, Chairperson/Organizing Committee
	IV	Message from the Vice Chancellor, University of Ruhuna
		Senior Professor Gamini Senanayake
	V	Message from the Dean, Facult of Medicine, University of Ruhuna
		Professor Saman Wimalasundara
	VI	Inauguration
	VII	Programme – Annual Academic Session – 2017
Reviews	01.	Factors that determine body composition of female systemic lupus
		erythematosus (SLE) patients in Sri Lanka: a comparative study using dual-energy x-ray absorptiometry
		Liyanage P L A N, Lekamwasam S, Disaanayake S P, Munidasa D
	07.	Cembrane type diterpenoids from the leaves of Croton oblongifolius Roxb. and their bioactivities Wijesekera KAKD
		Wijesekela KARD
	13.	Do surveillance cultures help in the management of neonatal infections? A study at a Neonatal Intensive Care Unit (NICU) in a tertiary care hospital in Southern Sri Lanka Weerasinghe NP, Vidanagama DS, Perera B
	18.	A study on hospital admissions with wife battery referred to JMO's office, Teaching
		Hospital, Karapitiya
		Rathnaweera RHAI
Abstracts		FMAS - 2017

#### Instructions to Authors

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), Keywords, Introduction, Material and Methods, Results, Discussion References, Tables 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. Lessons 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.



#### Foreword

It is with pleasure that I send this message on the occasion of the 5th Faculty of Medicine Annual Academic Sessions (FMAS-2017), which all of us have been eagerly looking forward to.

The theme identified for this years academic sessions "Paradigms of Multidisciplinary Research in Medicine", is a pertinent and timely topic that warrants the attention of all stakeholders. The keynote address, symposium, free paper session and the inaugural oration of the FMAS will be a source of inspiration to all participants, to exchange ideas and enrich their knowledge to face the challenges of the future.

I am confident that participants would benefit immensely from the wealth of knowledge and the expertise of the world renowned panel of speakers who have been invited for the Sessions. It is my sincere hope that deliberations of the two-day academic sessions, while providing insights into the current status will explore new ideas and approaches that will result in practical and implementable research proposals. This event also provides an opportunity for the academics to socially interact and network with one another.

I would like to extend my sincere appreciation to Professor Saman Wimalasundera, Dean of the Faculty of Medicine, PICs and all the members of the FMAS committee, distinguished speakers and each and every person who assisted us in numerous ways to make this event a reality.

I take this opportunity to extend my best wishes for the success of the sessions.

Professor Isurani Ilayperuma Co-Chairperson/FMAS 2017



#### Message from the Vice Chancellor

It is with great pleasure that I send this brief message to the proceedings of the Faculty of Medicine Academic Sessions (FMAS 2017). Undoubtedly, every year, it is the major academic event organized by the Faculty of Medicine.

University of Ruhuna is committed to research and I am proud that our academics have worked hard to broaden the scope in diverse fields to ensure the maximum impact. We remain an institution that is highly committed to enhancing outcomes for all students, to providing a stimulating working environment for staff and to contributing to the welfare of the society.

As the Vice Chancellor of the University of Ruhuna, I extend my sincere thanks to the Dean of the Faculty of Medicine, Chairperson and the members of organizing committee for organizing Faculty of Medicine Academic Sessions for the 5<sup>th</sup> consecutive year.

I firmly believe that this event will be an intellectual platform for academics, researchers and clinicians alike. Further the proceedings of the FMAS will reflect the diverse fields of research that are being conducted by our academics in the medical faculty.

I take this opportunity to extend my warmest congratulations!

Professor Gamini Senanayake Vice Chancellor, University of Ruhuna.



#### Message from the Dean

It gives me immense pleasure to send this message as the Dean of the Faculty of Medicine, University of Ruhuna to mark the 5<sup>th</sup> Faculty of Medicine Academic Sessions (FMAS 2017).

As we are all aware of the rapid progress, advancement and development taking place in the field of Medicine, it is our obligation to update and disseminate knowledge as well as to exchange expertise among our members.

This year's academic sessions will span over 2 days including a keynote address on Digital Medicine and a symposium on Global Health thus blending well with the theme "Paradigms of Multidisciplinary Research in Medicine".

I sincerely thank foreign and local faculties for promptly accepting our invitation and joining hands with us to enhance the standards and quality of the sessions. It is also encouraging and happy to have presentations based on completed higher degrees by our academics.

I am fortunate to have the support of dedicated and talented team of academics in the FMAS committees who worked tirelessly throughout the year in organizing this event. I take this opportunity to thank the Chairperson and the committee for their efforts to make this event a success.

I look forward to meet all of you at the sessions and I am confident that the participants will be richly benefitted in your academic and research endeavors.

Best wishes!

Professor Saman Wimalasundera Dean, Faculty of Medicine.

#### Inauguration

### Thursdays 7th December 2017

1700 hrs. Guests to be seated

1710 hrs. Ceremonial Procession

1720 hrs. Lighting of the traditional oil lamp

1725 hrs. Welcome speech by the Co-Chairperson, FMAS 2017

Professor Isurani Ileperuma

1730 hrs. Address by the Dean, Faculty of Medicine, University of Ruhuna

Professor Saman Wimalasundara

1740 hrs. Address by the Chief Guest

Senior Professor Gamini Senanayake Vice Chancellor, University of Ruhuna

1750 hrs. Address by the Deputy Vice Chancellor, University of Ruhuna

Dr. A M N Alagiyawanna

1800 hrs. Address by the Keynote speaker

Digital Health - How Genomics and Information and Communication Technology

will reshape healthcare of the future Professor Vajira H W Dissanayake Chair, Professor of Anatomy Director, Human Genetics Unit,

Chairperson, Specialty Board in Biomedical Informatics

University of Colombo

1850 hrs. Vote of thanks by Secretary, FMAS 2017

Dr. Janaki Warushahennadi

1900 hrs. Oration FMAS 2017

Cardiovascular Risk Assessment in Diabetes: Challenges and Opportunities

Dr. H M M Herath

Senior Lecturer in Medicine, Faculty of Medicine

University of Ruhuna

1945 hrs. Refreshment & Fellowship

#### Faculty of Medicine Academic Session (FMAS) - 2017, University of Ruhuna

#### **Programme**

#### Friday 8th December 2017

08.00 - 08.30 am	Registration
08.30 - 10.30 am	Presentations based on completed high degrees
08.30-09.00	A study on hospital admissions with wife battering referred to Judicial Medical Officer's (JMO's) office, Teaching Hospital, Karapitiya  Rathnaweera RHAI
09.00-09.30	am Cembrane type diterpenoids from the leaves of Croton oblongifoliusRoxb. and their bioactivities Wijesekera KAKD
09.30-10.00	Factors that determine body composition of female systemic lupus erythematosus (SLE) patients in Sri Lanka: a comparative study using dual-energy x-ray absorptiometry  Liyanage PLAN

10.00–10.30 am Do surveillance cultures help in the management of neonatal infections? A study at a Neonatal Intensive Care Unit (NICU) in a tertiary care hospital in Southern Sri Lanka

Weerasinghe NP

10.30 - 11.00 am	Tea & Poster Session
11.00 - 01.00 pm	Symposium on Global Health

Prof. Frank Cobelens (MD MSc PhD)

Professor of Global Health, Department of Global Health, Academic Medical Center, University of Amsterdam.

Dr. Guus ten Asbroek (MSc PhD)

Education Coordinator, The Amsterdam Institute for Global Health and Development, University of Amsterdam.

01.00 – 02.00 pm	Lunch
02.00 – 03.00 pm	Free Paper Session
03.00 - 03.15 pm	Awards ceremony
03.15 - 03.45 pm	Tea

## Factors that determine body composition of female systemic lupus erythematosus (SLE) patients in Sri Lanka: a comparative study using dual-energy x-ray absorptiometry

#### Liyanage P L A N, Lekamwasam S, Disaanayake S P, Munidasa D

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#### ABSTRACT

Studies on body composition and its regulating factors among SLE patients are limited. Estimation of body composition, analysis of determinants and associations of different body compartments are important in planning long-term care of these patients. The aim of the study was to identify the changes in body composition among SLE patients and assess the effect of corticosteroid use, patient and disease-related variables on body composition.

We compared lean mass, fat mass, bone mineral density (BMD), and bone mineral content (BMC) determined by dual-energy x-ray absorptiometry technology, in a group of premenopausal women with SLE (n=27) and an age-matched healthy group of women (n=27).

The median (IQR) duration of SLE was 3 (2-5) years while median (IQR) duration and dose of prednisolone therapy were 108 (88-172) weeks and 9730 (6160-15360) mg, respectively. No significant difference was observed in body mass index (BMI) or total fat mass between the two groups.

SLE patients, however, had significantly lower lean mass (p<0.001), BMD (p<0.001) and BMC (p<0.005) than healthy controls. Among cases, compared with lean mass, total body fat content showed stronger associations with total body BMD (r=0.49, p<0.01) and total body BMC (r=0.63, p<0.01). When a stepwise regression model was fitted, lean mass among controls and total fat mass among cases emerged as the best predictors of BMC/BMD. No significant correlations were found between the disease duration or cumulative glucocortico steroid dose and total body BMD, total body BMC, lean mass or total fat content in SLE patients.

This study was done in Teaching Hospital Karapitiya as a partial requirement for MD in Dermatology in January 2012, Results were published in Lupus journal, and received National Research Council merit award for scientific publication in year 2013. Abstract presentation was done in 25th Dermatological Society of Singapore Annual scientific meeting 2012.

#### Introduction

Systemic lupus erythematosus (SLE) is an autoimmune multi-systemic disease with an unpredicted clinical course. Females are more frequently affected than males at a ratio of almost 9 to 1(1). Over the past decades, the prevalence of SLE has increased rapidly, possibly due to the improvements in treatment and survival (2). The disease prevalence has a wide geographical variation and has estimated 1 in 250 among Afro Caribbean women in the United States and 1 in 4200 white women in New Zealand are at risk of SLE (3-6). Asian patients with SLE have shown a higher prevalence of systemic involvement as well as higher mortality (7). SLE predominantly occurs between the ages of 20 and 40 years, which is in the economically productive age group. The cumulative prevalence of physical disability among SLE patients is 23 % (8) and is essentially multifactorial. Low bone mineral density (BMD), and bone mineral content (BMC) are recognized risk factors of fractures.

SLE patients have low bone mineral density (BMD) and bone mineral content (BMC) and with high incidence of fractures (9). Even though the higher fracture incidence is explained by the reduced bone mass, little is known about the unfavorable changes in other body composition parameters affecting disability in SLE patients. Reduced muscle mass increase the fracture risk further by increasing the tendency to fall and on the other hand high fat mass may provide a protection from fractures. The ability of fat tissue to dissipate the energy generated locally at the site of impact following a fall is well established (10,11). Studies in this area require estimation of body composition and analysis of associations and interactions of different body compartments. Evaluation of this important fact is important because body composition is potentially modifiable, and interventions promoting healthy body composition in patients with SLE may offer promise in attenuating disease related disability and improving long-term care.

Previous studies examining body composition have revealed a lower lean mass and a higher fat mass among patients with juvenile onset SLE when compared with age- and sex-matched controls (12). The fat-free mass is a significant predictor of total body BMD among Australian female SLE patients and there is a significant association between reduced fat-free mass and SLE severity (13). Furthermore, the role of glucocorticoids, disease activity and menstrual status on body composition of SLE patients is not clear (12-14). Kipen et al. concluded that the disease severity and corticosteroid exposure are independently and inversely associated with both total body BMD and fat-free mass (14). Regio et al. however, showed that corticosteroid-related variables do not have harmful effects on body composition (12). While studies examining body composition and its determinants among SLE patients are limited, studies involving patients in the Asian region are sparse. As SLE prevalence and disease activity have a geographical variation, SLE patients in the Asian region may be materially different from their European or North American counterparts. Furthermore, the geographical differences in access to health care, food pattern, physical activity and environmental factors may have an influence on their body composition and its determinants. Hence, this study was designed to identify the changes in body composition among SLE patients and the effect of corticosteroid use, patient and disease-related variables on body composition.

#### Methods

Female SLE patients who had been classified according to the American College of Rheumatology revised criteria (15,16) and registered in medical and rheumatology clinics at the Teaching Hospital, Galle in Sri Lanka were invited to participate in a comparative cross-sectional study. Only those aged 18 years or more were included while patients with other diseases such as primary anti-phospholipid syndrome, rheumatoid arthritis, mixed connective tissue disease, thyroid or parathyroid diseases, malabsorption and other chronic inflammatory diseases were excluded. Patients with metal implants and those who were pregnant or breast feeding were also excluded.

Written informed consent was obtained from all participants and the study protocol was approved by the Ethics Review Committee of the Faculty of Medicine, University of Ruhuna. Age-matched (to the nearest 5 years) healthy females were selected from the community in the Galle district. Galle district is the service area of the Teaching Hospital, Galle and these subjects were selected in random manner using the most recent voters' registers and with the help of field health workers.

Data were collected using a pre-tested interviewerbased questionnaire and perusing medical records. All patients underwent a detailed physical examination. Data recorded included age, menstrual status and drug usage. The cumulative corticosteroid dose was calculated from medical records. The disease duration was calculated from the time of the diagnosis to the time of interview, and the disease severity was assessed clinically and categorized to mild, moderate and severe according to the systems involved during the course of the disease (17). The activity of the disease was assesed using Systemic Lupus Disease Activity Index (SLEDAI) at the time of recruitment (18). In the SLEDAI, serum complement levels and DNA binding were not assesed due to limited resources.

Anthropometric measurements such as body weight were measured to the nearest 0.1kg without foot wear and height was recorded using portable stadiometer (Weight Master International, Japan) to the nearest 0.5cm. Body mass index (BMI) was calculated by dividing weight (kg) by square value of height (m2). Body composition was measured by dual-energy x-ray absorptiometry (DXA by Hologic Inc, Bedford, USA) and the following measurements were taken: total fat mass, truncal fat mass, lean mass, total body BMD and total body BMC. All DXA measurements and anthropometric measurements were taken by the same technician adhering to the standard protocols. The analytical software (version 12.4) provided by the Hologic manufacturer was used to estimate absolute total body BMC, total body lean and fat masses. The same software was used to estimate the regional fat content in the abdomen (truncal fat).

#### Statistical analysis

The descriptive data are given as either mean (SD) or median (interquartile range) depending on the data distribution. Indices of body composition were normally distributed and they were compared, between cases and controls, using the unpaired ttest. The associations between BMD, BMC, fat content and lean mass were tested using Pearson correlations and regression models. Similarly, the associations between body compartments and disease or treatment-related factors among cases were examined by either a Pearson correlation or Spearman rho. P<0.05 was considered statistically significant for all analyses.

#### Results

Among SLE patients (n=27), the median (interquartile range; IQR) disease duration was 3 (25) years. Twenty-two of them (81.5%) were positive for anti-nuclear antibodies, while 23

(87.5%) had positive anti-ds-DNA antibodies at the time of the diagnosis. None of the patients had complement levels measured during their disease course. The median (IQR) duration of prednisolone therapy was 108 (88-172) weeks while the median (IQR) cumulative prednisolone dose was 9730 (6160-15,360) mg. The median SLEDAI (IQR) score was 4 (0-8) while the median SLICC/ACR damage index was 2 (0-3). None had fractures, vertebral collapse or avascular necrosis. As controls (n=27) were age matched to SLE cases there was no significant age difference between the two groups. Compared to controls, cases were lighter but no significant difference was observed in BMI between the two groups (Table 1).

**Table 1:** Comparison of indices of anthropometry and body composition between cases and controls

	Casesa	Controlsa	Mean	
	(n=27)	(n=27)	difference	Pvalue
Measurement	8 8			
Age (years)	32.2 (8.9)	33.2 (8.2)	1.1	0.67
Height (m)	1.53 (0.69)	1.57 (0.67)	0.04	0.053
Weight (kg)	49.5 (10.6)	56.0 (9.9)	6.7	0.021
BMI (kg/m <sup>2</sup> )	21.2 (4.9)	22.9 (4.5)	1.8	0.17
Total fat mass (kg)	17.51 (8.1)	20.0 (5.9)	2.08	0.29
Truncal fat mass (kg)	7.09 (4.24)	7.66 (2.83)	0.58	0.56
Lean mass (kg)	29.66 (4.93)	35.11 (6.23)	5.45	0.001
Total body BMD (g/cm <sup>2</sup> )	1.010 (0.099)	1.093 (0.073)	0.083	0.001
Total body BMC (g)	1662.5 (374.7)	1893.3 (169.9)	230.8	0.005

<sup>&</sup>lt;sup>a</sup>Given values are mean (SD). <sup>b</sup>p contracts the mean differences between cases and controls.

The body fat contents, both total and truncal, were not different between cases and controls. However, cases, compared with controls, had significantly low lean mass, total body BMD and BMC (Table 1). Compared with total fat mass, lean mass showed a stronger association with total body BMD (TBBMD) and total body BMC (TBBMC) among controls (Table 2). Among cases, compared with lean mass, total body fat content showed stronger associations with TBBMD and TBBMC (Table 2).

Table 2 : Correlations between measures of body composition among SLE patients and controls

	Lean mass	Total body BMC	Total body BMD
Total fat mass	Cases=0.15	Cases=0.63**	Cases=0.49**
	Controls=0.37	Controls=0.18	Controls=0.03
Lean mass	_	Cases=0.13	Controls=0.28
		Cases=0.28	Controls=0.44*
Total body BMC	_	_	Cases=0.87**
5			Controls=0.74**

Values are Pearson correlation (r). \*p<0.05; \*\*p<0.01.

When separate step-wise regression plots were fitted for the two groups with lean and fat masses as the independent variables and BMD or BMC as the dependent variable, lean mass among controls and total fat mass among cases emerged as the best predictors of BMC/BMD (data not shown). Among patients with SLE we did not find significant correlations between the disease duration or cumulative glucocorticosteroid dose and TBBMD, TBBMC, lean mass or total fat content (data not shown).

#### Discussion

This analysis shows that Sri Lankan women with SLE are lighter compared with age- and sex matched controls. Furthermore, SLE patients have low lean mass, low total body BMD and BMC, compared with the matching controls. In addition, among SLE women the fat mass is the major predictor of BMD and BMC. Among healthy controls, lean mass predicts BMD/BMC better than the total fat mass.

The fact that SLE patients have lower lean mass and BMD/BMC compared with normal subjects is understandable and somewhat expected. SLE is a chronic inflammatory disease with intermittent relapses and a disease of this nature could lead to poor overall growth. In addition, among SLE patients, reduced physical activities and nutrition which are well-known determinants of muscle growth and bone accrual could partly contribute to the findings. Drugs such as glucocorticoids have negative effects on both BMD/BMC and skeletal muscles. Hence, SLE patients can be expected to have lower lean mass as well as BMC and in turn, lower body weight and shorter stature compared with normal subjects.

We found the lean mass to be the main predictor of BMD/BMC among normal controls. Previous studies examining body composition among healthy premenopausal women in the same study area (19) and also among healthy black, white, and Hispanic premenopausal women(20) showed similar results. This association appears to have changed in SLE patients to the extent that the total fat mass emerges over the lean mass as the major predictor of BMD/BMC. This may be due to altered ratio of fat to lean mass allowing fat mass to be dominant over the lean mass. While glucocorticoids have a negative

effect on lean mass (13,21) its positive influence on fat tissue is well documented (21).

The difference of total fat mass/lean mass ratio does not appear to be the plausible explanation as we did not find this ratio to be significantly different between the two groups (cases 60.4% vs control 58.5%, p=0.83). The effect may entirely be due to the difference in the lean mass. Apart from glucocorticoids, SLE by its multi-system nature has negative effects on muscles. Although our patients had no special food habits, poor intake due to anorexia and upper gastrointestinal abnormalities may have led to a negative nitrogen balance in the body and contributed to lower lean mass. In addition, restricted physical activities among cases also may have contributed.

Several studies have described the changes in the body composition among SLE patients because of its relevance to prognosis and survival. The findings, however, have not been uniform. In a study conducted on the Chinese SLE population, Mok et al (5) demonstrated a significant correlation between total body BMC and fat mass over lean mass. In the same analysis, body BMC did not correlate with prednisolone dosages. In contrast, Kippen et al. (14) reported fat-free mass and not the fat mass to be predictive of change in total body BMD among Australasian premenopausal SLE patients. In this analysis too, corticosteroid-related variables were not found to have deleterious effects on body composition. In a separate analysis, Kippen et al. (13) demonstrated fat-free mass to be the main predictor of total body BMD.

We were unable to find associations between disease or drug-related variables and indices of body composition. Previous studies examining the relationship between the dose of glucocorticoids and BMC/BMD have generated conflicting results. Kalla et al. (22) showed that SLE causes significant trabecular bone loss, which is not due to corticosteroid therapy. In addition, according to Li et al. (23) SLE patients had significantly low BMD compared with healthy controls but there was no relationship between dose and duration of corticosteroid treatment as well as activity and duration of the disease. However, Kipen et al. (24) demonstrated a strong inverse relationship with BMD and steroid usage. Thus our results were keeping with results of most of previous

studies. But unfortunately we only assessed cumulative corticosteroid dose and not the steroid use at enrolment, indications of steroid use and calcium supplementation. A follow-up study would provide more information than our cross-sectional comparative study.

There are limitations and strengths in our study. Our study population was comparatively young (age 32+/- 8.9 years) and most were premenopausal and in the reproductive age group. This allowed us to interpret BMD changes independent of the influences of reduced gonadal hormone status. Some of the previous studies included a significant proportion of postmenopausal women and their BMD/ BMC could have been altered due to lack of gonadal hormones. In our study cumulative corticosteroid dose was calculated by perusal of patients' clinic notes. As these patients were on longterm therapy, some details were not easily recollected. The disease activity was assesed at the time of recruitment of the study by using SLEDAI score without assessing serum complement levels and DNA binding due to limited resources. This could have made the scoring system incomplete in assessing the disease activity. British Isles Lupus Assessment Group (BILAG) index was not used as we have not assessed disease activity comparing with prior visits (25). Further, we collected data in a cross-sectional manner and more information could have been gathered if we followed them up with repeated scans. However, we had a control group, matched for age and sex and collected from the same study area. As the number of SLE patients we could recruit to the study was limited to 27, we did not calculate the sample size at the beginning of the study. The post-hoc power calculation, however, showed that the study had more than 85% power to detect differences in BMD, BMC, and lean and fat masses.

Our findings have a clinical relevance. The combination of low BMD and lean mass we found among our SLE patients will make them more vulnerable for fractures. Sarcopenia has been found to be a factor related to recurrent falls. Although high body fat content can reduce the chance of fracture by reducing the impact of fall, our patients did not have a higher body fat content when compared with controls. Only the ratio between fat mass and fat free mass was altered. It is important to know the

morbidity attached to the combination of low bone mass and low lean mass seen among our patients. They need to be assessed for their postural instability and incidence of falls and fractures. Further, the role of physical in intervention in mitigating the morbidity should be assessed.

#### Acknowledgement

The authors would like to thank Ms Anuradha Wickramasinghe and Ms Malini Kariyawasam for performing DXA scans

#### Conflict of interest

The authors have no conflicts of interest to declare.

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## Cembrane type diterpenoids from the leaves of Croton oblongifolius Roxb. and their bioactivities

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#### Abstract

Current study focused on the isolation, characterization and evaluation of biological activities of secondary metabolites from *C. oblongifolius*. Two cembrane type diterpenes; crotocembranal and crotocembraneic acid were isolated. Structures of the compounds were elucidated by analyzing spectroscopic data. Cytotoxicity, cancer chemopreventive properties antibacterial activities were tested. Both compounds showed mild cytotoxicity towards the cancer cell line tested. Crotocembraneic acid exhibited minimum inhibitory concentration of (MIC) of 25 µg/mL for *Mycobacterium tuberculosis H37Ra and* 100 µg/mL for *Staphylococcus epidermidis*. Crotocembranal was inactive as an anti-infective agent.

#### Introduction

Croton is a genus which belongs to the family Euphorbiaceae covering over 1300 species widespread in tropical regions of the world. Several species have an extended role in the traditional medicine systems in Asia, Africa and South America.

The plant C. oblongifolius Roxb., is a middle-sized tree and grows in India, Sri Lanka and Thailand. It has been used in the traditional Thai medicine to alleviate dysmenorrhea (fruits), as a purgative (seeds), and to treat dyspepsia (bark) and dysentery (roots). Even though Seshadri et al extensively investigated the metabolites of C. oblongifolius, Roengsumran et al reported that the same plant when collected in Thailand contains different constituents, possibly due to geographical variation-. Two cembrane type diterpenoids namely, crotocembranal and crotocembraneic acid were isolated from the current study. Hundreds of cembranoids from plants, insects as well as of marine origin have been isolated to date. The notable biological property of this group of compounds is the cytotoxicity. In addition, they

have also been reported to have numerous biological activities such as anti-inflammatory, neuroprotective, calcium-antagonistic, anti-arthritic and antimicrobial effects. Herein report the isolation, characterization and biological activities of two cembrane type diterpenoids crotocembranal and crotocembraneic acid which were isolated from the leaves of *C. oblongifolius* Roxb.

#### Materials and Methods

#### General experimental procedures

UV spectra were obtained from a Shimadzu UV-1700 PharmaSpect UV-Vis spectrophotometer. IR spectra were acquired with a Perkin Elmer Spectrum ONE spectrophotometer using Attenuated Total Reflectance (ATR) technique. NMR spectra were recorded on a Bruker Avance 300 (<sup>1</sup>H at 300 MHz, <sup>13</sup>C at 75 MHz). HRESI-MS spectra were obtained from a Bruker Micro-TOF mass spectrometer. Column chromatography was packed with Sephadex LH-20 (GE Health Care Bio-Sciences AB).

#### Plant material

Leaves of *C. oblongifolius* were collected from Nakhonsawan province, Thailand, in October 2014. The plant, *C. oblongifolius*, was previously authenticated by Panarat Charoenchai, and the specimen (no. CRI 285) was deposited at the Laboratory of Natural Products Chulabhorn Research Institute, Bangkok, Thailand.

#### Extraction and isolation

Powdered, air-dried leaves (0.8 kg) of *C. oblongifolius* were macerated with dichloromethane to yield a crude extract of 37.6 g. The crude extract was subjected to silica gel column chromatography (SGCC) (10×56 cm), a stepwise gradient elution was performed with hexane:CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>2</sub>Cl<sub>2</sub>:MeOH stepwise gradients and yielded 19 fractions (A-1 to A-19). Fractions A7 and A8 which had similar TLC patterns and proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were combined (658.8 mg) and separated by SGCC (2x44

cm), eluted with hexane:CH<sub>2</sub>Cl<sub>2</sub> (4:6) to obtain 8 fractions (B1-B8). Fractions B4 and B5 which had similar TLC patterns and <sup>1</sup>H NMR spectra were combined (57.1 mg), and further separated by SGCC (2x44 cm) eluted with a gradient solvent system of hexane:CH<sub>2</sub>Cl<sub>2</sub> giving crotocembranal (compound 1) (20.2 mg). Fraction A11 (840.2 mg) was separated by Sephadex LH-20 column chromatography (3x100 cm), to obtain 11 fractions (C1-C11). Fractions C-10 and C-11 were combined (395 mg) and further separated using SGCC (1.5×17 cm), eluted with a gradient solvent system of hexane:CH<sub>2</sub>Cl<sub>2</sub>, to yield 14 fractions (D-1 to D-14). Fraction D5 to D7 gave crotocembraneic acid (compound 2) (77.7 mg).

#### Biological activity

Biological activities of the isolated compounds were evaluated at the Central Research Facility for Bioassays-Chulabhorn Research Institute, Natural Products Laboratory-Chulabhorn Research Institute, and Ramathibodi Hospital, Mahidol University, Thailand. Procedures for the determination of respective biological activities are briefly describe below.

#### Cytotoxic activity

Cytotoxic activities against adhesive cell lines; HepG2 (human hepatocellular carcinoma cell line), HUCCA-1 (cholangiocarcinoma cell line), and A549 (human adenocarcinomic alveolar epithelial cell line) were evaluated using the MTT assay. XTT assay was used for the assessment of cytotoxicity of non-adhesive cell line MOLT-3 (human acute lymphoblastic leukemia-T cell type). Etoposide and doxorubicin were used as the reference drugs.

#### Chemoprevention activity

## Scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals

The potential for scavenging free radicals was determined photometrically by reacting the test samples (dissolved in DMSO) with DPPH, as described by Gerhauser *et al*. Ascorbic acid (vitamin C) was used as the reference compound, exhibiting an  $IC_{50}$  value of  $21.2 \,\mu\text{M}$ .

## Measurement of oxygen radical absorbance capacity (ORAC)

Peroxyl or hydroxyl radical absorbance capacity of test sample was tested by a modified ORAC assay, following the method previously described by Gerhauser et~al. Results were expressed as ORAC units, where 1 ORAC unit equals the net protection of  $\beta$ -phycoerythrin ( $\beta$ -PE) produced by 1  $\mu$ M trolox ( $\delta$ -hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), a water soluble vitamin E analog. Only scavenging capacities of more than 1 ORAC unit were considered as positive.

#### Aromatase inhibitory assay (AIA)

The inhibition of aromatase was performed according to the method previously described by Stresser *et al.* using a CYP19/methoxy-4-trifloromethyl-coumar-in (MFC) high throughput inhibition screening kit. Ketoconazole, which typically has IC<sub>50</sub> value of 2.4 μM, was used as the reference compound.

## Inhibition of superoxide anion radical formation by xanthine/xanthine oxidase (X/XO assay)

The formation of superoxide anion radicals by X/XO was performed by following the method described by Gerhauser et al. Superoxide dismutase (30 U/mL) was used as the control. Allopurinol, a known inhibitor of xanthine oxidase, was used as a positive control with an  $IC_{50}$  value of 3.0  $\mu$ M.

#### Antibacterial activity

Minimum inhibitory concentration (MIC) of the compound was tested on gram positive (Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, and Staphylococcus epidermidis), and gram negative (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, and Shigella flexneri) bacteria by broth microdilution method described by Andrews et al . The compound was dissolved in dimethyl sulfoxide (DMSO), and a two-fold serial dilution in 96-well plates was carried out. Bacterial suspensions were prepared in normal saline solution and adjusted to a turbidity of the 0.5 McFarland standard. Final concentration of the DMSO did not exceed 0.5% (v/v). The plates were incubated at 36 °C (±1) for 20 h, and the absorbance was measured at 600 nm to determine the MICs of the tested compounds. Chloramphenicol, tetracycline and vancomycine were used as the standard drugs. The MIC is defined as the lowest concentration of the compound that inhibits the growth of microorganism.

#### Antimycobacterial activity

Microplate alamar blue assay (MABA) was used to determine the antimycobacterial activity of compounds and Mycobacterium tuberculosis H37Ra (non-virulent strain) was used as the test organism. Compounds were individually dissolved in DMSO, and they were diluted two folds in 100 µL of Middlebrook 7H9GC medium in 96-well plates. A mycobacterial suspension was prepared in 0.04 % Tween 80 and diluted with sterile distilled water to a turbidity of the 1.0 McFarland standard. The mycobacterial suspension was subsequently diluted 1:50 with 7H9G medium, and 100 µL was added to the wells. The highest final concentration of DMSO was 0.156% (v/v). The plates were incubated at 37 °C (±1) for approximately 7 days. Then 12.5 uL of 20 % Tween 80 and 20 µL of alamar blue (SeroTec Ltd., Oxford, United Kingdom) were added to all wells. The growth of the mycobacteria was determined after reincubation at 37 °C for 16 to 24 h by visual determination of a color change from blue to pink. The MIC is defined as the lowest concentration of the compound which prevents the color change. Rifampicin was used as the reference drug.

#### Results

## Structure elucidation and characterization of compounds 1 and 2

Compound 1 was a yellow coloured oil. The high resolution electrospray ionization mass spectrometry (HRESI-MS) showed a pseudomolecular ion peak at m/z of 287.2309 (M+H)<sup>+</sup>, calcd. m/z 287.2312 for (C<sub>20</sub>H<sub>20</sub>O+H)<sup>+</sup>, suggesting the molecular formula of the compound 1 as C<sub>20</sub>H<sub>30</sub>O. The infra-red (IR) spectrum of the compound exhibited strong absorption peaks for C-H stretching at 2962 and 2929 cm<sup>-1</sup>, and a carbonyl peaks at 1713 cm<sup>-1</sup>. The ultraviolet (UV) spectrum of the compound showed absorption peaks at 368, 228.5 and 204.5 nm.

The proton nuclear magnetic resonance ( ${}^{1}H$  NMR spectrum) (300 MHz, acetone- $d_{o}$ ) (Figure 1) of compound 1 indicated the presence of four methyl protons at  $\delta$  1.05 (s, H<sub>3</sub>-16 and 17) and 1.72 (s, H<sub>3</sub>-18 and 19), six methylene protons which resonate between  $\delta$  2.16- 2.53, one  $sp^{3}$  methine protons that resonate at  $\delta$  2.42 (m, H-15), four  $sp^{2}$  methine protons that resonate at  $\delta$  5.16 (t, J = 6.4, H-7), 5.89 (d, J = 11.0, H-3), 5.97 (d, J = 11.0, H-2), and 6.43 (t, J = 7.7, H-11) ppm. Moreover, proton signal of an aldehyde was observed at  $\delta$  9.28 (s, H-20).

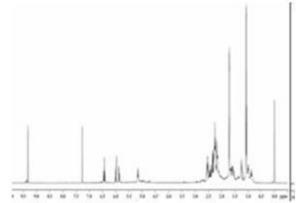


Figure 1 H NMR spectrum of compound 1

The carbon-13 NMR (13C NMR) spectrum (75 MHz) (Figure 2) of the compound displayed signals for 19 carbons. Distortionless enhancement by polarization transfer (DEPT) spectral data classified these carbons as six methine carbons, six methylene carbons, four methyl carbons, and four non-protonated carbons. Analysis of the chemical shifts of <sup>13</sup>C NMR spectrum indicated the presence of five sp2 methine carbons, one sp3 methine carbons, six sp3 methylene carbons, four methyl carbons, and four sp<sup>2</sup> non-protonated carbons. The carbons resonance at  $\delta$  118.4 (C-2), 119.9 (C-1), 129.0 (C-7), and 155.0 (C-1) were sp<sup>2</sup> methine carbons. Carbon resonances at  $\delta$  134.1 (C-8), 135.5 (C-4), 143.9 (C-12), and 146.3 (C-1) were sp2 nonprotonated carbons. Moreover, a carbons resonance at  $\delta$  196.0 (C-20) was a carbonyl carbon of an aldehyde. The heteronuclear single quantum coherence (HSQC) spectral data were used to assign the protons being attached to carbon (Table 1). The core structure of the compound 1 was established by analyzing the proton correlation spectroscopy ('H-'H COSY), heteronuclear multiple bond correlation (HMBC), and nuclear overhauser enhancement spectroscopy (NOESY) spectral data.

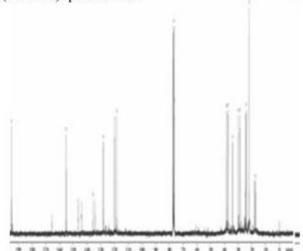


Figure 2 13C NMR of compound 1

Establishment of a core structure of compound 1

The <sup>1</sup>H-<sup>1</sup>H COSY spectrum of 1 established the fragments of H-2/H-3; H-5/H-6/H-7; H-9/H-10/H-11; H-13/H-14; and H-15/H-16/H-17. The cyclic core structure of compound 1 was established by analyzing the HMBC correlations including H-2 toC-1, C-4, and C-15; H-3 to C-5 and C-18; H<sub>2</sub>-6 to C-4and C-7; H-7 to C-5, C-9 and C-19; H<sub>2</sub>-10 to C-8, C-11, and C-12;H-11 to C-10,C-13 and C-20;and H<sub>2</sub>-14 to C-1 and C-15. The position of the aldehyde group was determined by the HMBC correlations of both H-11 and H<sub>2</sub>-13 to C-20 and an aldehyde proton H-20 to C-11, C-12, and C-13.

The <sup>1</sup>H NMR signal of H-2 was a doublet, coupling with H-3. The coupling constant 11.0 Hz indicated a *trans* double bond (*E* geometry) of the H-2/H-3 double bond. This was further confirmed by the NOESY correlations between H-2 andH<sub>3</sub>-16, H<sub>3</sub>-17, and H<sub>3</sub>-18. Moreover, the NOESY correlations H-3 and H<sub>2</sub>-5 and H-7 and H<sub>2</sub>-9 suggested an *E* geometry of C-3/ C-4 and C-7/C-8 double bonds, while a correlation between H-11 and H<sub>2</sub>-13 suggested a *Z* geometry of C-11/C-12 double bond. On the basis of these spectral data core structure of compound 1 was established.

Compound 2 was a white solid, and had a molecular formula of C<sub>20</sub>H<sub>30</sub>O<sub>2</sub> as determined by atmospheric pressure chemical ionization coupled time of flight mass spectrometry (APCI-TOF MS). Its IR spectrum showed absorption bands at 1713 cm<sup>-1</sup> and 3422 cm<sup>-1</sup> of carbonyl and hydroxyl functionalities, respectively. The <sup>1</sup>H NMR spectrum of compound 2 was similar to compound 1 except the missing signal for an aldehyde proton (Figure 3).

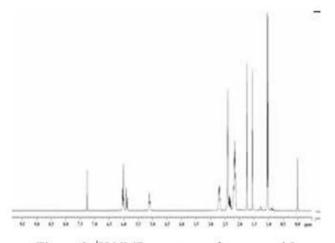


Figure 3 H NMR spectrum of compound 2

Moreover, <sup>13</sup>C NMR spectral data revealed that the carbonyl carbon ( $\delta$  194.0) of an aldehyde group in compound 1 has been replaced by a carboxylic acid group in compound 2 ( $\delta$  174.7) (Figure 4).

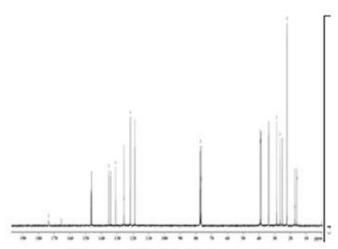


Figure 4 13C NMR of compound 1

Similar to compound 1, the core structure of compound 1 was established with the aids of <sup>1</sup>H-<sup>1</sup>H COSY, HSQC, HMBC and NOESY spectral data (Table 1).

Table <sup>1</sup>H and <sup>13</sup>C NMR data of compounds 1 and 2 in deuterated chloroform (CDCl<sub>3</sub>)

Position	25	1	2		
	δ <sub>C</sub> (ppm)	$\delta_{H}$ (ppm), multiplicity ( $J$ in Hz)	$\delta_{\mathbb{C}}$ (ppm)	$\delta_{11}$ (ppm), multiplicity ( $J$ in Hz)	
1	146.3, C		146.4, C	•	
2	118.4, CH	5.97 (d, J=11.0)	118.8, CH	6.02, d (10.5)	
3	119.9, CH	5.89 (d, J = 11.0)	121.6, CH	5.89, d (11.3)	
4	135.5, C		135.2, C	•	
5	38.5, CH <sub>2</sub>	2.28 (m)	39.2, CH <sub>2</sub>	2.15, m	
6	37.4, CH <sub>2</sub>	2.16 (m)	25.1, CH <sub>2</sub>	2.20, m	
7	129.0, CH	5.16 (t, J = 6.4)	125.7, CH	5.10, t (5.9)	
8	134.1, C	-	134.0, C	-	
9	24.8, CH <sub>2</sub>	2.25 (m)	38.6, CH <sub>2</sub>	2.15, m	
10	30.0, CH <sub>2</sub>	2.53 (m)	26.5, CH <sub>2</sub>	2.70, m	
11	155.0, CH	6.43 (t, J = 7.7)	146.7, CH	6.01, t (5.2)	
12	143.9, C	¥ 1000	130.9, C	-	
13	24.1, CH <sub>2</sub>	2.34 (m)	28.7, CH <sub>2</sub>	2.39, m	
14	28.7, CH <sub>2</sub>	2.23 (m)	33.6, CH <sub>2</sub>	2.40, m	
15	34.0, CH	2.42 (m)	33.8, CH	2.35, m	
16	22.1, CH <sub>3</sub>	1.05 (d, J = 3.2)	22.1, CH <sub>3</sub>	1.03, d (6.8)	
17	22.1, CH <sub>3</sub>	1.05 (d, J=3.2)	22.1, CH <sub>3</sub>	1.03, d (6.8)	
18	18.0, CH <sub>3</sub>	1.72 (s)	17.0, CH <sub>3</sub>	1.74, s	
19	17.3, CH <sub>3</sub>	1.73 (s)	15.8, CH <sub>3</sub>	1.54, s	
20	194.0, C=O	9.32 (s)	174.7, C=O	-	

300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C, chemical shift (δ) in ppm

Crotocembranal (1): Yellow oil (20.2 mg); UV (EtOH) λmax (log ε): 368.0 (1.85), 228.5 (4.16), 204.5 (4.19) nm; IR (UATR) vmax: 3422, 2962, 2929, 1713, 1452, 1377, 1266, 1163, 1047, 983. 735, 703 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) see Table 1; ESI-TOF MS: calcd. for C<sub>20</sub>H<sub>31</sub>O, m/z 287.2312 (M+H)+, found 287.2309.

Crotocembraneic acid (2): White crystals (77.7 mg); melting point 105-107 °C; UV (EtOH) λmax (log ε): 204.0 (3.39) nm; IR (UATR) vmax: 3422, 2957, 2927, 1712, 1454, 1377, 1180, 1059, 736, 703 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>1</sub>), <sup>13</sup>C NMR (75 MHz, CDCl<sub>1</sub>) see

Table 1; ESI-TOF MS: calcd. for  $C_{20}H_{31}O_2$ , m/z 303.2311 (M+H)+, found 303.2314.

#### Biological activities of the compounds 1 and 2

Compound 1 exhibited cytotoxic activity against MOLT-3, HepG2, HuCCA-1, and A549 cell lines with IC<sub>50</sub> values of 16.09, 29.01, 30.60 and 48.15 μg/mL, respectively. However, compound 2 exhibited cytotoxic activity against MOLT-3 cell line with IC<sub>50</sub> valueof 46.22 μg/mL, but it was not active against other cell lines.

Table 2 Cytotoxic activities of the compounds 1 and 2

Compound	IC <sub>50</sub> values for cancer cell lines (μg/mL)						
Compound	MOLT-3	HepG2	HuCCA-1	A549			
1	$16.09 \pm 1.38$	$29.01 \pm 2.58$	$30.60\pm0.08$	$48.15 \pm 0.04$			
2	$46.22 \pm 2.17$	I	I	I			
Etoposide	$0.04\pm0.01$	$24.32 \pm 1.88$	ND	ND			
Doxorubicin Hydrochloride	ND	$0.22\pm0.03$	$0.33 \pm 0.04$	$0.27 \pm 0.02$			

Both compounds were inactive for cancer chemopreventive tests. Crotocembraneic acid exhibited antimycobacterial activity with a MIC of 25 µg/mL and antibacterial activity against Staphylococcus epidermidis with a MIC of 100 µg/mL. Crotocembranal did not show any activity for antibacterial and antimycobacterial tests.

#### Conclusions

The plant Croton oblongifolius Roxb. is a rich source of ditepene compounds with diverse structures including cembrane type diterpenoids. Compounds that belong to this class are produced by terrestrial and marine organisms and the most remarkable property is cytotoxicity. Even though crotocembranal showed mild cytotoxicity towards MOLT-3, HuCCA-1 and A549 cancer cell lines, it exhibited IC<sub>50</sub> value of 29.01 for HepG2 which was in line with the standard drug etoposide. As an anti-infective agent, crotocembraneic acid is more effective compared to crotocembranal.

#### Acknowledgements

The author would like to acknowledge Dr. Prasat Kittakoop for his invaluable support and correct guidance, Chulabhorn Graduate Institute, Thailand for awarding a master's degree scholarship and Thailand International Cooperation Agency (TICA) for a student's grant. This research was financially supported by the Center of Excellence on Environmental Health and Toxicology, Science and Technology Postgraduate Education and Research Development Office (PERDO), Ministry of Education of Thailand.

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## Do surveillance cultures help in the management of neonatal infections? A study at a Neonatal Intensive Care Unit (NICU) in a tertiary care hospital in Southern Sri Lanka

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#### Abstract

#### Introduction

Timely and effective intervention in management can improve the outcome of neonatal infections. Aims were to find the incidence of culture proven episodes of infections, to identify bacterial pathogens isolated and their antibiotic sensitivity patterns, to decide the most appropriate empirical antibiotics for current use in the NICU, to identify the colonization with possible or potential pathogens on admission and to assess the usefulness of screening tests in predicting causative organism(s) of sepsis.

#### Methodology

This study was conducted prospectively from January to April 2011 at the NICU, Teaching Hospital, Karapitiya. Fifty neonates were screened on admission and weekly during their NICU stay for colonization with possible or potential pathogens. Relevant samples were cultured on clinical suspicion of sepsis. Sensitivity tests were done on all possible pathogens isolated.

#### Results

Culture positive rate was 340 per 1000 admissions (1/3 of the clinically suspected episodes of sepsis). Nosocomial infection rate was 13.61 episodes per 1000 patient days. Coliforms represented 76% of all culture isolates with more than 75% of them producing extended spectrum β lactamase (ESBL). More than 80% of the isolates causing LOS were sensitive to imipenem, meropenem and amikacin. Four babies were colonized with ESBL producers on admission. Of the culture positive episodes, 41% (7/17) had the same organism isolated in the surveillance samples.

#### Conclusions

Degree of clinical suspicion was high in this unit. Coliforms were the predominating pathogens and a high proportion was resistant to the commonly used antibiotics. Carbapenems and aminoglycosides were the recommended empirical therapy for LOS. Surveillance samples had some value in predicting the likely pathogen(s).

**Keywords:** empirical antibiotics, ESBL producers, neonatal sepsis, surveillance swabs

#### Introduction

Neonatal infections continue to be a major cause of morbidity & mortality in newborns throughout the world. The increasing populations of very low birth weight (VLBW) premature infants, who now survive due to improved neonatal care, represent the group at highest risk for infections. Neonatal infections are divided into two main categories, early onset sepsis (EOS); infections occurring during the first 48 hours of life and late onset sepsis (LOS); infections occurring thereafter respectively.

Neonatal sepsis causes approximately 1.6 million neonatal deaths annually in developing countries(1). A Malaysian study reports rates of neonatal sepsis of 5-10% with case fatality rates of 23-52% (2). Klein et al, 1995 have shown that neurological sequelae as a complication in 20-30% of the survivors of neonatal bacteraemia and 40% or more of those with meningitis(3). However, the increase in medical emergencies and following major surgeries needing advanced life support, the number of NICU admissions has gone up dramatically in the recent past.

NICU at the Teaching Hospital Karapitiya (THK) is one of the few NICUs with level 3 facilities in the country. It receives neonates with various surgical or medical problems from other institutions in Southern province. Data from the Microbiology & Infection Control Unit of THK showed that during the last two-year period there had been three clusters of neonatal sepsis caused by extended spectrum beta lactamase

(ESBL) producing coliforms in this NICU with increase in mortality. Inappropriate empirical antibiotic therapy delays recovery and increases the morbidity. However, prospective studies on neonatal infections have not been conducted in this unit previously, making it a timely necessity as it would help to identify the common pathogens and their current susceptibility pattern guiding early effective antibiotic therapy.

#### Materials and methods

Objectives were to find the incidence of culture proven episodes of infections among neonates admitted to the NICU, to identify the bacterial pathogens causing infections, to determine the most appropriate empirical antibiotic therapy for the current use in the NICU, to assess the usefulness of screening tests in predicting the causative organism(s) of sepsis.

A hospital based prospective study was conducted with a cohort of fifty neonates (aged < 28 days) admitted to the NICU at the THK from 1st January 2011 to 30th April 2011. They were followed until 48 hours of discharge from the NICU. Data regarding patients' demography, risk factors for sepsis, signs and symptoms, investigation results and antibiotic treatment were recorded. A peripheral blood culture, deep ear swab, nasal swab, umbilical swab and a rectal swab were collected from every neonate for culture on admission to the unit. During the stay, babies were screened weekly with a rectal swab and respiratory tract secretion cultures; pharyngeal aspirates and gastric aspirates from non-intubated babies and endotracheal (ET) secretions from intubated babies. Clinical suspicion of sepsis in babies was based on criteria from different studies (4) (1) as well as criteria for Integrated Management of Childhood Illness. Haematological and biochemical parameters were also used to support the diagnosis of sepsis. Appropriate clinical specimens were collected from babies for culture. Centre for Disease Control and Prevention guidelines were followed in collection and transport of specimens. Specimens were cultured and organisms were identified according to standard operating procedures given by the Sri Lanka College of Microbiologists. Antibiotic sensitivity tests (ABST) were performed on all significant isolates, according to the Clinical Laboratory Standards Institute methods. The culture and ABST results of surveillance samples and clinical samples were documented.

#### Results

On admission age ranged from 1 to 27 days. Male babies consisted 58% of the sample. Birth weights ranged from 0.700 kg to 3.600 kg with a mean of 2.200 kg. Twenty-six babies were preterm and mean gestational age of the study group was 34 weeks + 4 days. Identified risk factors which made our study participants more vulnerable for infections were sub optimal birth weight, prematurity, cardiac anomalies, congenital diaphragmatic hernia, ventriculoperitoneal shunt, abdominal surgery, mechanical ventilation and central lines for more than 48 hours and intra-partum problems like meconium aspiration with birth asphyxia, obstructed-prolonged labour and premature rupture of membranes.

Twelve babies had clinically suspected EOS (240 per 1000 admissions) and all were present on admission. LOS was noted in 43 occasions (860 per 1000 admissions) and 33 of this was thought to be acquired during 661 NICU patient days. Clinically suspected sepsis rate was 1100 per 1000 admissions. Culture proven rates for EOS and LOS were 2 (40 per 1000 admissions) and 15 episodes (300 per 1000 admissions) respectively. Culture proven sepsis rate was 340 per 1000 admissions. Nine episodes of LOS had been contracted during 661 patient days. Nosocomial infection rate (of culture proven sepsis) was 13.61 per 1000 patient days.

Pathogens isolated were mainly Gram negative organisms. (Table 1) In more than 90% of the occasions bacteraemia was due to coliforms. (Table 2) Klebsiella pneumoniae pneumoniae was the commonest pathogen in blood cultures (72.72%) and they were all extended spectrum beta lactamase (ESBL) producers. Antibiogram of the coliforms is shown in table 3. Coagulase negative Staphylococcus (CoNS) was resistant to oxacillin and ciprofloxacin. The Acinetobacter isolate was sensitive only to netilmicin and amikacin. It was only intermediately sensitive to cefoperazone + sulbactam. Streptococcus pneumoniae and Enterococcus isolates were penicillin sensitive.

Commonest surface colonizer on admission was CoNS. Coliforms and methicillin resistant *Staphylococcus aureus* (MRSA) were present in 40% and 10% of the neonates respectively. Relationship between clinical isolates and surveillance isolates is shown in table 4. Out of 7 screening positives, 4 (57.14%) were from rectal swabs.

Table 1 - Organisms isolated from cases of neonatal infections in the study

Organisms	Number of times isolated in clinical samples
EOS - Gram +ves - Enterococcus spp	1
Gram negatives Coliforms	1
LOS - Gram +ves - CoNS	1
Streptococcus pneumoniae	1
Gram -ves - Acinetobacter spp	1
Coliforms	12

Table 2 - Distribution of infections in this study as per organisms

Coliforms	Enterococcus	Acinetobacter	Strep.pneumoniae	CoNS
Septicaemia	congenital pneumonia	nosocomial pneumonia	meningitis	nosocomial pneumonia
EVD	CONTRACTOR CONTRACTOR CONTRACTOR	**************************************		Washington and a demonstration of
shunt infection				
NEC				
VAP				
Meningitis				

Table 3 - Antibiotic sensitivity pattern of the coliforms

	Sensitive	· · ·	Resistant		Intermedi	ate	Total	
Antibiotic	Number	%	Number	%	Number	%	Number	%
Ampicillin	1	7.70	12	92.30			13	100
Co-	1	7.70	12	92.30			13	100
amoxyclav								
Cefuroxime	0	0	13	100			13	100
Cefotaxime	1	7.70	12	92.30			13	100
Ceftriaxone	1	7.70	12	92.30			13	100
Cefepime	2	15.40	11	84.61			13	100
Timentin	1	7.70	12	92.30			13	100
Piperacillin-	2	15.40	11	84.61			13	100
Tazobactam								
Aztreonam	2	15.40	11	84.61			13	100
Ciprofloxacin	8	61.53	5	38.50			13	100
Gentamicin	7	53.84	6	46.15			13	100
Netilmicin	10	76.92	1	7.70	2	15.38	13	100
Amikacin	11	84.61	1	7.70	1	7.70	13	100
Imipenem	13	100	0	0			13	100
Meropenem	13	100	0	0			13	100

Table 4 - Relationship between clinical isolates and surveillance isolates

Clinical specimens	Screening /surveillance specimens		
	Screening positive	Screening negative	
Culture positive (17)	7 (41%)	10	
Culture negative (38)	29	9	

#### Discussion

Clinically over suspicion of infections and commencing antibiotic therapy prior to collecting cultures could be possible reasons for the discrepancy between clinically suspected infection rate and the culture proven rate seen in this study. Practical difficulties in repeated and adequate sample collection for cultures from neonates could have been another contributing factor for low culture proven rate.

The overall nosocomial infection rate of 13.61 episodes per 1000 patient days is higher than in studies from developed countries. Neonatal nosocomial infection rates across the Australian and New Zealand network 2009, had found an overall rate of 5.02 episodes of infections per 1000 patient days for infants of less than 1000 g birth weight. (5) Comparing the blood stream infection rate of 7.56 per 1000 patient days in this NICU, with the findings in Gastmeier et al 1998 which has recorded a rate of 6.4 per 1000 days in a birth weight less than 1000 g cohort from Berlin shows that our values are not very high. (6) Comparatively the small sample size and the characteristics of the study population, which is comprised of critically ill babies with complicated clinical problems, may have influenced the higher infection rates in our study. We included more highrisk neonates with nearly 20% of the total admissions being very low birth weight (VLBW) and 6% with extremely low birth weight (ELBW).

Gram negative organisms predominated as pathogens, which is similar to what was reported from neonatal units in other developing countries. (7) (8) Two clusters of *Klebsiella pneumoniae pneumoniae* bacteraemias occurred during this period which also has accounted for the high proportion of Gram negatives. Group B streptococcus was not isolated in any of the samples, probably due to effective screening and intra-partum antibiotics. Studies have

shown a shift from Gram positives to Gram negatives over the period of time. Gladstone I et al, 1990 have described group A streptococci and Staphylococcus aureus as the predominating pathogens in the preantibiotic era, while in 1940's and 1950's Gram negative organisms, particularly Escherichia coli has become the most common cause of neonatal sepsis. (9)(10)

ABST patterns of coliforms in culture proven LOS episodes were used to decide on the most effective empirical antibiotic therapy. Clinical response of patients were not considered in assessing the efficacy of antibiotics to avoid any personal bias. Numbers of other pathogens and the number in EOS cases were not adequate for analysis. Though we recommend carbapenems and aminoglycosides, another study has suggested combination of vancomycin plus amikacin. (11)

The value of routine surveillance of surface colonizers in neonates is a controversial issue. Though surface swabs are inefficient and not cost effective in guiding empirical therapy of neonatal sepsis, some value with surveillance ET cultures have been found by Jolley AE, 1993. (12) In 41% of the occasions of sepsis, we could have predicted the aetiological agent using the isolates in surveillance samples, which is comparable to the findings of Petra J, 2010, where they could predict the potential pathogen in 41% of the infections by surveillance cultures. (13) The most useful surveillance culture type was rectal swabs, which yielded the same organism as in clinical sample in 57% of the occasions. This is a useful finding, especially in order to prevent use of inappropriate antibiotics in empirical therapy.

#### Conclusions

Sepsis is diagnosed with a very low threshold among neonates in this NICU. Multi drug resistant Gram negatives predominated as pathogens indicating breach in infection control practices. Carbapenems and aminoglycosides are the first choice combination empirical antibiotic therapy for LOS in babies treated here. We conclude that surveillance cultures including nasal swabs, ET cultures and umbilical swabs are of limited value in predicting and identifying causative pathogens of neonatal infections. However, routine weekly surveillance with rectal swabs is useful in neonates to predict the likely pathogens of sepsis and guiding the empirical antibiotic therapy of sepsis.

#### Acknowledgements

We would like to thank Professor Ajith Nagahawatte for reviewing the manuscript. We appreciate the mothers and newborns who participated in this study, for their contribution to better understanding of the nature of neonatal sepsis and the value of screening swabs. We would like to extend our sincere gratitude towards NICU staff and the staff of the Microbiology Laboratory at THK for their co-operation in conducting this study and the Ministry of Health, Sri Lanka for the financial support.

#### Conflicts of interest

Authors declare that there are no conflicts of interest.

#### Ethical clearance

Ethical approval was obtained from Ethical Review Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka. Informed written consent was obtained from the mothers or the guardians of the babies.

#### **Authors' Contributions**

NPW designed and wrote the study plan and was involved in data collection, analysis and writing of the manuscript. DV also designed and wrote the study plan and was involved in analyzing and writing of the manuscript. BP helped in designing the study sample and analyzing the data.

This study was carried out as a partial requirement of MD Medical Microbiology programme and has been published in the journal of Sri Lanka College of Microbiologists.

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## A study on hospital admissions with wife battery referred to JMO's office, Teaching Hospital, Karapitiva

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#### Abstract

#### Introduction

There is convincing evidence that violence against women is a significant health and social problem affecting virtually all societies. In countries where reliable, large-scale studies carried out, it has been reported that 20% to 67% of females were assaulted by the men they live with.

#### Objective

Routine data pertaining to alleged wife battery victims were analyzed to document associated epidemiological and socio-economic factors and to describe the medico-legal aspects of injuries.

#### Materials and Methods

All the alleged wife battery cases examined by the author at the JMO's office, teaching hospital, Karapitiya, Galle form 1st of January 2013 to 31st of June 2017 were retrospectively analyzed.

#### Result

During the study period, 2683 assault cases were examined and out of that 183 (6.8%) were alleged wife battery cases. The age of the victims ranged from 16 years to 63 years. 106 (58%) victims were between 21-30 years. 117 (63%) alleged incidences had taken place between 6pm to 10pm. Out of the victims, only 26 (14%) had any independent income source. In 89% of times, victims were assaulted inside their own houses. Slapping (56%) was the commonest method of assault followed by punching with fists (51%). Sharp force injuries were present only in 7 (4%) of cases. Face (47%) was the commonest target site followed by lower limbs (41%). Genitals were spared in most of the cases. Suspicion of extra marital relationships (both husband and wife) was the commonest cause for alleged assaults (31%) and out of that, majority was due to suspicious usage of mobile phones and internet and social media.

#### Conclusions

Head and face were the commonest target sites which is compatible with reported literature. Contribution made by electronic devices for causing marital disharmonies was highlighted in this study.

#### Introduction

There is convincing evidence that violence against women is a significant health and social problem affecting all societies(1). One of the most common forms of violence against women is that performed by a husband or an intimate male partner. Intimate partner violence refers to any behaviour within an intimate relationship that causes physical, psychological or sexual harm to those in the relationship. When abuse occurs repeatedly in the same relationship, the phenomenon is often referred to as "battering" (2,3).

Wife battery occurs in all countries, irrespective of social, economic, religious or cultural back ground. Although women can be violent in relationships with men, and violence is also sometimes found in same-sex partnerships, the overwhelming burden of partner violence is borne by women at the hands of men (4,5). In every country where reliable, large-scale studies have been done, it has been reported that 20% to 67% of females have been assaulted by the men they live with (6).

Wife-battering in Sri Lanka has not been studied in detail from a medico-legal point of view. A study done by Saravanapavananthan mainly described the weapons and injuries associated with wife battering (7). A study by Samarasekera described associated factors and injury patterns of the survivors of wife-battering (8). Though the life time prevalence of wife battering was 20-60% in 2011 in Sri Lanka, only few victims seem to have reported to authorities (9).

It is difficult to ascertain the exact incidence of wife battery. Some statistics are available, but it is uncertain how accurate they are, as many cases go unreported. One estimate based on the work of several investigators has placed the annual number of cases of violence against women in the United States at 1.5 million; it has also been estimated to occur in up to 50% of all familial relationships (4). A study performed at Yale University reported that 3.8% of women who came to the surgical services and 3.4% of women who came to the psychiatric services of the emergency department had been victims of battering (10).

Lack of medico-legal data pertaining to wife battery in Sri Lanka lead to this study. This study was conducted to ascertain the nature and circumstances of violence against women. A battered wife was defined as a woman who had received deliberate severe and repeated demonstrable physical injury from her husband.

#### Materials and Methods

A retrospective cross-sectional descriptive study on victims of wife battery was conducted at the JMO's office, Teaching Hospital, Karapitya, Galle. All Medico-legal examination forms (MLEFs) examined by the author from 1<sup>st</sup> of January 2013 to 31<sup>st</sup> of June 2017 were screened and 183 wife battery victims were identified.

The forensic medical practitioner routinely records the demographic data and details of the incident obtained from victims on the MLEF. Further, the referrals to relevant specialists and their opinions are also documented. The bed head tickets were perused as a secondary source of data. The relevant information was collected on to a data collection form and the data were analyzed using Microsoft excel and SPSS packages.

#### Results

During the study period, 2683 victims were referred for medico-legal examination with an alleged history of assault and out of that 183 (6.8%)were alleged wife battery incidences.

Table 1 - Characteristics of the victim

Age of the victim (Years)	No (%)	Level of education	No (%)	Source of income	No (%)
21-25	17 (6.5%)	Not schooled	03 (2%)	Husband	159(87%)
26-30	177 (68%)	Grade 1-5	12 (7%)	Wife	26(14%)
31-35	30 (11.5%)	Up to O/L	131(71%)	Extended family	17(9%)
36-40	09 (3.4%)	Up to A/L	33 (18%)	Other sources	14(7.5%)
41-45	04 (1.5%)	Above A/L	04 (2%)		
46-50	03 (1.1%)				
51-55	06 (2.3%)				
56-60	02 (0.7%)				
61-65	03 (1.1%)				

The age of the victims ranged from 16 years to 63 years (table 1). Out of that, 117 (63%) victims were between 21 to 30 years of age. Out of the victims, 146 (79%) had not studied beyond grade 10 (table 1). Out of the victims, only 26 (14%) had any independent income source (table 1). Majority (66%) of the victims belonged to lower socio-economic classes where as 31% to middle classes and only 3% to higher classes.

Table 2 - Characteristics of the abuse

Place where the incident occur	No(%)	Time of the incident	No(%)
	163 (89%)	6.00 am - 10.00 am	03(1.5%)
Parents house	09 (6%)	10.00 am – 2.00 pm	17(9%)
Public place	06 (3%)	2.00 pm – 6.00 pm	34(19%)
Deserted place	2 (1%)	6.00 pm – 10.00 pm	117(63%)
Other	3 (1%)	10.00 pm – 2.00 am	09(5%)
		2.00 am - 6.00 am	03(1.5%)
Extent of abuse of the victim	No(%)	Type of physical abuse	No(%)
Physical aggression	183(100%)	Throw something at spouse	21(11%)
Psychological abuse	135(73%)	Pushed, grabbed, shoved spouse	89(49%)
Forced sexual intercourse and other forms of sexual coercion	13(7%)	Slapped spouse	103(56%)
Various controlling behaviours	43(23%)	Kicked, bit or hit with fist	94(51%)
		Hit or tried to hit with blunt object	34(18%)
		Threatened with a knife	21(11%)
		Used a knife	7(4%)
Reason for abuse	No (%)	Action taken after the abuse	No (%)
Issues related to drunkenness	54(29%)	Nothing	108(59%)
Monetary	43(23%)	Seek help from extended family	41(22%)
Suspicion of extramarita l affairs	31 27 (31%)	Seek help from other relatives	10(5%)
Dispute over mobile phones, social media and internet	48(26%)	Seek help from friends	13(7%)
Minor arguments	35(19%)	Seek help from others Seek legal assistance	05(2.5%) 06(3%)
Previous abuse	No (%)		
No abuse	45(24%)	7.	
1-3 times	81(44%)		

The alleged incident had taken place at their place of living in 163 (89%) cases (Table 2). Between 6 pm to 10 pm, 117 (63%) alleged incidences had taken place (Table 2). Majority of the victims were subjected to physical aggression followed by psychological abuse (Table 2). Slapping (56%) was the commonest method of assault followed by punching with fists (51%) (Table 2). Suspicion of extra marital relationships (either husband or wife) was the commonest cause for alleged assaults (31%). Issues related to drunkenness (29%) and disputes over electronic devices (26%) also remained significant contributors in this regard (Table 2). After the alleged incident, only 6 (3%) requested legal help directly (Table 2). Majority (74%) of the victims complained of previous abuse by their husbands (Table 2).

Table 3 – Characteristics of the injuries

Type of injury	No(%)	Site of injury	No(%)
No injuries	13(7%)	Head	31(16%)
Abrasion	67(36%)	Face	87(47%)
Contusion	87(47%)	Neck	04(2%)
Laceration	14(7%)	Chest	18(9%)
Fracture	06(3%)	Abdomen	06(3%)
Burn	02(1%)	Upper limbs	42(22%)
Cut	04(2%)	Lower limbs	76(42%)
Stab	02(1%)	Genitals	04(2%)
Firearm	00		

Category of hurt	No(%)
Non-grievous	156(85%)
Grievous	24(13%)
Endangering life	02(1%)
Fatal in the ordinary	01(0.5%)
cause of nature	

Contusions (47%) were the commonest type of injury found on the victims followed by abrasions (36%) (Table 3). Face (47%) was the commonest target site followed by lower limbs (41%). Genitals were spared in most of the cases (Table 3). Majority of the victims had non-grievous injuries (85%) (Table 3).

#### Discussion

Although the extent of wife battery is unknown, there is substantial evidence to suggest that wife battering is a major social problem that has escalated into significant proportions in recent years. It has attracted considerable attention, which has led to an increasing realization that the family far from providing a place of safety is one of the most dangerous institutions.

In this study, the prevalence of wife-battering among the reported medico-legal cases was 6.8%. In Sri Lanka, life time prevalence of wife battering is 20-60% (9). This indicates either the victims are reluctant to report to the authorities or the clinicians are unable to recognize the victims. Majority of the victims were younger (21-30 years) and similar findings were reported by Hofner et al (11) in a study done in Switzerland with victims of violence attending an

emergency department. Higher proportion of victims was unemployed and therefore, they were dependent on husbands. This is compatible with a retrospective study done by Chan et al in a regional hospital in the New Territories of Hong Kong(12). Similar to a study done by Koenig et al. (13), on individual- and community-level influences on domestic violence in Uttar Pradesh, North India, few of the victims were unmarried. In a study done by Linda and walker (14) in Wisconsin, they have observed majority of events to occur at night. Similar findings were observed in this study.

Majority of the injuries were non-grievous as the husband did not want to cause severe harm. This is compatible with the findings of Saravanapavananthan (7). The most common site of injury was the face and the most common injury type was contusion. This is similar to Saravanapavananthan's observations (7).

Similar to a study done by MacLeod (16) in Canada, majority faced more than one episode of assaults. The victims themselves contributed to the problem. Many of them do not seek redress or leave the batterer, because the battered woman seeking legal intervention or leaving the home carries a social stigma (14). Because of the need to maintain the social status and positive image attached to a successful marriage and to evade the social stigma, many women are battered secretly in their homes without coming out in the open (14).

Similar to study done by Kraanen and others (17) Amsterdam, Netherlands, majority of husbands consumed alcohol regularly. Suspicion of an extramarital affair remains as one of the most common triggering factors of wife battery. Mobile phones, social media and internet also found to be playing an important in this regard. In psychological abuse, similar to Koenig and others (11), the victims continued to suffer the effects of controlling behavior of the husband.

In the final analysis, it is best perhaps to conclude that violence against wives is a function of the patriarchal power structure within the family, where men are superior to women and the women are men's possession to be treated as they wish and consider appropriate.

This study has some limitations and strengths. The study is retrospective, and the choice of variables was limited. Data collection was dependent on the victim's report, and it is not clear how reliable it is to collect data within the context of a forensic examination shortly after an assault because of peri-traumatic dissociation. Some women did not recall or were unwilling to relate assault details. Data were collected from one hospital and that in most cases probably would reduce generalizability.

#### Conclusions

Wife-battering is not uncommon in Sri Lanka and many have faced repeated events. The pattern of injuries and their consequences are significant and the vigilance of clinicians is needed to identify them. According to literature, head and face were the commonest target sites and findings in this study (63%) were compatible with that. Contribution made by electronic devices for causing marital disharmonies was highlighted in this study.

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# **Abstracts** FAMS 2017

#### Colour Vision Deficiency Among 10-16 Year-old School Children In Galle District, Sri Lanka

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#### Introduction

Colour vision deficiency (CVD) is an X-linked recessive disease. Prevalence of CVD varies from race to race. This study was conducted to determine the prevalence of CVD among school children (ages 10-16) in Galle district, Sri Lanka.

#### Methods

A total of 566 students (260 males, 306 females) were examined with Ishihara pseudo-isochromatic colour plates. Parental questionnaire was used to obtain family history of CVD, the ability of the child to select coloured objects and past medical history.

#### Results

Overall prevalence of CVD was 1.4% (8 boys) with prevalence of 3.1% for males. There were no female patients. Six Sinhala, one Tamil and one Muslim boy had CVD. All affected children had negative histories of previous systemic and ocular disease or chronic use of medications. The visual acuity and the fundus were normal in all affected children. Seven (1.23%) had deuteranomaly while one (0.17%) had protanomaly.

Six parents (75%) of CVD children were unaware of their child's defect while 12.5% (01) reported a positive family history. Parental reporting of difficulties in selecting coloured objects in child's day-to-day activities were not significant (p>0.05 Chi-square test). CVD children had reported difficulties in identifying colour objects in Television/computer programmes and separation of cooked from uncooked food (p<0.05 Fisher's exact test). Four (50%) children were humiliated by their friends for their difficulties.

#### Conclusions

CVD is not uncommon. Affected children face difficulties. A large population-based study is needed to define the ethnicity-specific prevalence of CVD. CVD can be identified and counseling can be given at school medical inspection.

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## The Prevalence And Clinical Profile Of Abdominal Pain Predominant Functional Gastrointestinal Disorders (ap-fgids) In 5-12 Year Olds In Sri Lanka

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#### Introduction

Abdominal pain predominant functional gastrointestinal disorders (AP-FGIDs) are common among Sri Lankan adolescents and teenagers and it is an important healthcare problem. However, studies are rarely conducted to determine the prevalence and clinical profile of AP-FGIDs in 5-12-year age group. The main objective of the study was to determine the prevalence and the determine the clinical profile of AP-FGIDs among aged 5-12 years old, school children in Gampaha district, Sri Lanka.

#### Methods

A cross sectional survey was conducted in four randomly selected schools in Gampaha district of Sri Lanka. All children aged 5-12 years were recruited after obtaining parental consent. Data was collected using a translated and validated parental questionnaire. AP-FGIDs were diagnosed using Rome III criteria.

#### Results

A total of 1000 questionnaires were distributed and 709 (70.9%) returned. Properly filled 653 (65.3%) questionnaires were included in analysis [309 (47.3%) boys. mean age 8.8 years (SD 1.9 years)]. Eighty-two (12.6%) fulfilled criteria for the AP-FGIDs. Prevalence of AP-FGIDs was higher in females than male (7.9% vs. 4.5%, p=0.037, Chi-square test). Thirty-two (4.9%) had FAP, 26 (4.0%) had IBS, 21 (3.2%) had FD and 3 (0.4%) had AM. Prevalence in AP-FGIDs in girls has shown a positive correlation with age (r=0.718, p=0.045, Pearson correlation). The majority of the children had moderate to very severe pain (83.1%), lasting less than one hour (73.1%). In 24.4%, the duration of abdominal pain was more than one year, which was significantly higher in girls (9.27 vs. 6.03, p=0.021 independent T test). Epigastric (24.4%) and periumbilical (18.3%) were the commonest sites for abdominal pain. Extra-abdominal pain (56.2%), abdominal fullness (51.2%) and headache (46.3%) were the common associated symptoms.

#### Conclusion

The AP-GIDs is a common disorder with a prevalence of 12.6% in 5-12 year olds in Sri Lanka. It is more common in girls.

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## Psychological defense mechanisms and coping strategies of patients with lymphedema due to lymphatic filariasis

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#### Introduction

Psycho-Social Burden (PSB) in Chronic Filarial Lymphedema (CFL) is well recognized and psychological defense mechanisms and coping strategies develop within the patients to manage it. Therefore, exploring them will be helpful to develop interventional guidelines to address the burden.

#### Methods

Forty-six semi-structured, in-depth interviews were carried out on CFL patients Mathotagama in Matara and on patients who attend to filariasis clinics at Teaching hospital Karapitiya and Department of Parasitology Faculty of Medicine, until themes get saturated. Coding was done by extensive rending of transcribed scripts. Known Coping strategies and psychological defense mechanisms were identified while eliciting some new strategies of defenses used by the particular community.

#### Results

Acceptance, avoidance, conversion, denial, displacement, suppression, sublimation, rationalization, self-serving bias, intellectualization, humility, projection and denial, were the leading psychological defense mechanisms identified within the CFL patients. Emotional focused coping strategies were more prominent than other strategies and the disclaiming, accepting the blame, exercising self-control were some of them. Personality dependent resolution of problems, tempered within a known social group, media dependent expansion of psychosocial experiences seem to be another dimension of strategies seen within the patients.

#### Conclusion

Existence of the immature defenses instead of mature defenses and lack of appraisal focused or problem focused strategies are barriers for management. Non-existence of occupation focused strategies could be acceptable with the demographic background.

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# Evaluation of acute and sub chronic toxicity of selected medicinal plant extract mixture in healthy Wistar rats.

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#### Introduction

Poly herbal mixture composed of leaves of Murraya koenigii (L.), cloves of Allium sativum (L), fruits of Garcinia queasita Pierre and seeds of Piper nigrum(L) has been popular among Sri Lankan Ayurvedic medicine practitioners for the management of diabetes mellitus. However, its use in the management of diabetes is not scrutinized. The water: acetone plant extract mixture at a dose of 1.0 g/kg (therapeutic dose in diabetic rats) showed the most potent antihyperglycaemic effects in diabetic rats. The aim of this study was to evaluate the acute and sub-chronic toxicity ofwater: acetone extract of the above mixture in healthy Wistar rats in order to establish its safety in the long term management of diabetes.

# Materials and Methods

In the acute toxicity assessment, the plant extract mixture (1.0 g/kg b wt.) was administered to healthy Wistar rats (n=6/group) and the animals were observed for mortality and clinical signs for 14 days. In the sub-chronic toxicity study, the plant extract (1.0 g/kg) was administered orally for 28 days. The selected serum biochemical parameters (including liver enzymes, kidney function tests), hematological parameters (full blood count), relative organ weight, intake of water and consumption of food, were evaluated.

# Results

In the acute toxicity study, neither mortality nor any significant alterations in the behavior of rats were observed. In the sub chronic toxicity study, there was no significant difference (p > 0.05) in body weights of animals, consumption of food ,intake of water and relative weight of the organs between plant extract (at the therapeutic dose) treated group and the control group over 28 days. There was no statistical difference in tested biochemical parameters including alanine aminotransferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP), total protein (TP), creatinine and blood urea nitrogen between the test group and the control group. Further, no significant changes were observed in the hematological parameters in plant extract treated rats compared to the control rats (p>0.05).

# Conclusions

The results indicate that the oral administration of selected medicinal plant extract mixture did not produce any significant acute and sub chronic toxic effects at its therapeutic dose in healthy Wistar rats.

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# Frequency of problem drinking among the alcohol consuming males in a tertiary care setting in Southern Sri Lanka.

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# Background

Problem drinking, namely alcohol dependence (AD) and alcohol abuse (AA) are associated with major medical, social and economic adverse consequences. It is seen in those admitted to hospital due to alcohol related medical complaints as well as those with unrelated complaints. The aim is to determine the frequency of AD and AA among the alcohol consuming males in a tertiary care setting in Southern Sri Lanka.

#### Materials and Methods

Two-hundred male patients in medical and surgical wards in Teaching Hospital Karapitiya (THK) who reported alcohol consumption on history taking were assessed. The J12 questionnaire of the Mini International Neuropsychiatric Interview (MINI), which had been validated and translated to Sinhalese in a previous research was administered to determine the frequency of AA and AD.

#### Results

Problem drinking was noted in 63 (31.5%) of participants. Of them, 38 (19%) had AD and 25 (12.5%) had AA. Out of the problem drinkers 61.9% were within the age group of 51-60 years followed by 26.98% within the age group of 41-50. Highest percentage of problematic drinkers in this sample consisted of heavy vehicle drivers (33.3%) followed by military personnel (23.8%) and fishermen (19%).

### Discussion and Conclusion

Approximately one-third of patients who consumed alcohol had either AA or AD. It was high even among those admitted with complaints unrelated to alcohol consumption. We propose that alcohol consumption needs to be enquired in all medical consultations. If present, further evaluation for problem drinking is needed. The high frequency noted supports strengthening of alcohol cessation support services in the hospital setting.

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# Quantitative evaluation of urine protein in the lower range: Clinical utility and cost-effectiveness of an improved sulfosalicylic acid turbidimetric method

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#### Introduction

Sulfosalicylic acid method (SSA) is a widely used semi-quantitative test to detect proteinuria. This study was conducted to investigate the feasibility of SSA turbidimetric method for quantitative evaluation of proteinuria in the lower range (0-50 mg/dL) which could be useful in early diagnosis of CKDu.

#### Materials and methods

The optimum SSA concentration to detect urine protein in the lower range was detected by performing protein assays with SSA solutions of three different concentrations; 3%, 6% and 25% which are currently in-use for qualitative detection of urine protein. Initially, a series of albumin standards were prepared with varying concentrations (0-100 mg/dL), constant volumes of SSA were added to each solution and the turbidity was measured by spectrophotometry. Standard curves were generated from the protein assays performed using the three different concentrations of SSA. The optimum SSA concentration was determined by comparing the gradients of standard curves which were validated by the automated pyrogalol red (PGR) method. To assess the clinical validity, a comparative cross sectional study was performed using 61 patient samples. The cost-effectiveness was evaluated against the PGR method and the turbidimetric microalbumin assay.

#### Results

SSA of 25% gave the highest sensitivity, having the highest gradient for detection of protein in the lower range. The linearity range was 3-75 mg/dL. An intra-assay precision of 7.3% and 4.5% were observed for urine protein concentrations at 20.0 mg/dL and 40.6 mg/dL, respectively. The sensitivity was 94% and the specificity was 88% when compared with the PGR method at a cut-off of 20 mg/dL for a positive test. The cost per test for 25% SSA method is Rs 0.27 whereas, for PGR method and for microalbumin it is Rs. 25.00 and Rs.168.00, respectively.

# Conclusion

Improved 25% SSA method is suitable and cost-effective for quantitative measurement of total protein in urine at lower levels facilitating early detection of proteinuria.

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# Physical and emotional violence exposure of adolescents in Bope-Poddala MOH area

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# Background

Exposure to violence is common all around the world. Present study therefore examined different types of home and community violence exposures among adolescents in Bope-Poddala MOH area.

#### Methods

A school based violence exposure screening was conducted in 5 schools selected conveniently in Bope-Poddala MOH area. All students present on the day of data collection in the late adolescent age; 17-19 years were selected for the study. Sample consisted of 300 students with 50% boys. Culturally modified version of child exposure to domestic violence check list (CEDV) was used as a self-reported questionnaire to measure violence exposure.

# Results

There were 58.3% of students exposed to at least one type of violence. Among them, 81.7% was exposed to domestic violence and 90.9% was exposed to community violence. Physical violence exposure was high at home (86.0%) and in the community (80.2%). There was a statistically significant difference in violence exposure between girls and boys. (t = 5.0077, p = <.001). However, in our sample, violence exposure of children did not associate with alcohol consumption of father  $x^2$  (300,1) = 0.35, p > 0.05, family income  $x^2$  (300,2) = 1.619, p > 0.05, and time duration of mother spent at home  $x^2$  (300,1) = 2.473, p > 0.05.

### Conclusion

Domestic and community violence exposure was common among our sample of adolescents in Bope-Poddala MOH area. It is therefore recommended to conduct further research to implement evidence based interventions to minimize this common problem in the society.

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# Factors affecting delay in fibrinolytic therapy among patients with Acute Myocardial Infarction admitted to Base Hospital, Elpitiya

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#### Introduction

Early fibrinolysis is the mainstay of treatment in Acute Myocardial Infarction (AMI) in Sri Lankan health care settings. Delaying fibrinolytic therapy can considerably increase morbidity and mortality. Many factors affect the time delay between onset of symptoms and fibrinolytic therapy. This study assessed the patient and pre-hospital factors that delay fibrinolysis in patients admitted to a secondary care setting.

#### Materials and methods

This study included 94 patients with AMI admitted to Base Hospital, Elpitiya. Data on study variables were collected using an interviewer-administered questionnaire and from patient records. Data analysis was done using SPSS.

#### Results

Mean age ( $\pm$ SD) of the sample was 60.4 ( $\pm$ 11.5) years. The majority (84%) were males. Median total ischemic time was 180 (range: 40-2170) minutes and much of that was due to pre-hospital delay (median=130 minutes; range: 10-1868 minutes). Pre-hospital delay was significantly greater in patients with lower educational status (p<0.05) and who sought medical advice prior to admission (p<0.05). Patients who recognized the pain to be of cardiac origin and who knew they had risk factors for AMI reported significantly less delay in reaching hospital (p<0.01 for both). Age, gender and occupation of patient, a positive family history of AMI or having prior advice to seek medical attention in case of chest pain did not show significant associations with pre-hospital delay.

# Conclusions

Delay in fibrinolytic therapy in AMI is significantly affected by patients' educational level, ischemic pain and risk factors recognition. Patient education with emphasis on recognizing ischemic pain and risk factor education can minimize pre-hospital delay and improve chances of recovery.

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# Cervical cytology screening: Knowledge, Attitudes and Behaviour among Public Health Midwives in Galle district in Sri Lanka

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#### Introduction

Cervical cancer is the second commonest cancer among females in Sri Lanka. The main aim of screening for cervical carcinoma is to reduce the incidence of cervical carcinoma by detecting and treating pre-cancerous lesions. Public Health Midwives (PHMM) play a major role in women's health.

#### Method

Descriptive cross-sectional study was carried out among PHMM attached to the Medical Officer of Health (MOH) divisions in Galle district using self-administered questionnaire. Assessment was done under categories of their knowledge, attitude and behavior on cervical cytology screening.

#### Results

Total number of PHMM participated in the study was 274. Percentage of women who has had an above average knowledge on cervical cytology screening and identified cervical cytology as a screening method for cervical carcinoma are 90% and 94% respectively. Awareness on Human Papilloma Virus (HPV) and DNA testing was low (26%). Correct identification of the target group, recommended interval of screening and benefits of screening by the PHMM were 90%, 98% and 83% respectively. Ninety four (94%) of PHMM had a favourable attitude towards their role and capacity in cervical cytology screening. Only 64% of PHMM had undergone cervical cytology screening themselves. There was a statistically significant increase in overall knowledge with higher levels of education (p=0.004).

### Conclusion

A vast majority of the PHMM had better understanding and positive attitude towards cervical cytology screening and identified it as a screening method. Their awareness regarding HPV DNA as a screening tool for cervical carcinoma was not satisfactory. Considerable number of PHMM had not undergone cervical cytology screening themselves.

Key words: Cervical carcinoma screening, cervical cytology, Public Health Midwife.

# Knowledge and practices regarding management of fever among mothers of the febrile children under 5 years of age: Teaching Hospital Karapitiya(THK).

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# Background

Childhood fever can be effectively controlled if mothers have appropriate knowledge on the subject. Understanding mothers' current knowledge and practice will assist to develop a proper education programme. The objective of this study was to evaluate the knowledge and practices regarding management of fever.

#### Materials and methods

A descriptive cross sectional study was carried out on 150 mothers of children, under 5 years admitted to THK. Interviewer administered questionnaire was used to collect data. Descriptive statistics and chi square were used to analyze the results. Ethical approval was obtained from the Faculty of Medicine, University of Ruhuna.

#### Results

Majority (84.7%) were educated beyond GCE O/L. Around two third had sufficient knowledge (67%) about fever management and practices which was recommended by WHO (69%). Forty four percent (44%) knew what the 'normal body temperature' was while 62.7% knew that fever could be associated with convulsions. 88.7% had an adequate knowledge on non-pharmacological methods of fever management. 52% had the knowledge on using the thermometer accurately. 82% knew that paracetamol dose varies with weight. Undressing (99.3%) and giving paracetamol (95.3%) were the common practices. 57.3% used non-scientific methods to check the fever. Both knowledge (P=0.000) and practices (P=0.002) were significantly correlated with the level of education. Adequacy of the knowledge significantly correlated with good practice (P=0.000).

#### Conclusion

Knowledge and correct practice on management of 'fever' was average. Knowledge on 'normal body temperature' and the use of a thermometer was poor. Majority of mothers knew that paracetamol dose depends on weight. Knowledge on non-pharmacological methods was adequate. A Health education session is recommended for all new mothers.

Key words - Knowledge, practices, mothers, fever management

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# Influence of environmental factors associated with the etiology of cleft palate

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#### Introduction

Cleft palate is one of the commonest craniofacial malformations that involves many environmental factors in the process of morphogenesis of the palates. Teratogenic agents, maternal nutrition and folic acid deficiency were identified as the risk factors in animal and population studies.

# Objective

Objective of this study is to identify the influence of environmental factors associated with the etiology of cleft palate.

#### Methods and materials

Subjects with isolated cleft palate registered from January 2001 to December 2016 at the Regional Cleft Centre & Maxillo-Facial Department, Teaching Hospital, Karapitiya, Galle, were recruited for the study. Interview based standard questionnaire was used to collect the data.

#### Results

Two hundred twenty subjects participated in the study and a statistically significant female predominance (p< 0.05) was identified (n=128; 58.2%). Majority were in the low social economy group. Only few mothers of cleft palate subjects (n=12; 5.45%) have taken the periconceptional folic acid supplementation. There was a statistically significant (p<0.05) higher frequency of 1<sup>st</sup> rank births (n=114; 51.85%) found in this cohort of cleft palate subjects. Significantly higher number (n=26; 11.82%) of cleft palate subjects were born in the month of March and fewer (n=6; 2.73%) subjects in the month of October (p<0.05).

#### Discussion and conclusion

Folic acid is the one of key vitamins associated with the etiology of the cleft palate. Literature reported that various adverse environmental factors are associated with the occurrence of the cleft palate. According to the results of this study, majority of mothers have not taken periconceptional folic acid supplementation. Interestingly there were statistically significant association of cleft palate with first rank birth and the birth month. These findings are important in identifying the exact adverse environmental factors associated with cleft palate.

Key words: Cleft palate, etiology, Birth rank, Birth month, Folic acid supplementation

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# Prevalence, management and outcome of anemia in patients undergoing cardiopulmonary bypass surgery in Teaching Hospital, Karapitiya

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#### Introduction

Anemia places patients undergoing cardiac surgery at risk of postoperative mortality and morbidity. Alternatively, a number of reports suggest that blood transfusion itself confer similar risk and adverse sequels, especially long-term. Thus, clinicians face uncertainty regarding the optimal strategy for peri-operative management of anemia. As a part of a survey on nutritional status, we analyzed prevalence, management and outcome of anemia in patients who underwent cardiopulmonary bypass surgery in cardiothoracic unit, Teaching Hospital, Karapitiya (CTU/THK).

#### Materials and methods

Clinical records of 397 patients who underwent cardiopulmonary bypass surgery between 2015-2016 in CTU/THK, with details on haemoglabin levels were retrospectively analyzed. Data on relevant demographic, haematological and clinical parameters were collected using a data extraction sheet and analyzed using SPSS software.

#### Results

Majority of the sample was Sinhalese (92.4%) and males (75.8%) from rural areas (71.5%) with a mean age(±SD) of 59.4(±7.7) years. Mean haemoglobin (Hb) concentration (±SD) was 12.5(±1.5)g/dl (range:7.9-16.2g.dl). Overall prevalence of anaemia was 48.1%, prevalence in females and males being 45.8% and 48.8% respectively. Anaemia was significantly more prevalent among older and undernourished patients (p<0.001 and p<0.01 respectively). Approximately 68% received blood transfusions, 18.9% receiving more than two pints. Neither presence of anaemia nor receipt of blood transfusions was associated with increased post-operative mortality/morbidity in this sample.

#### Conclusion

Although pre-operative anaemia was prevalent in patients who underwent cardiopulmonary bypass surgery, it has not adversely affected their clinical outcomes. Receiving blood transfusions did not pose any additional risk for these patients, however, its long-term sequel should be further studied.

# Evaluation of Risk Factors associated with Breast Cancer in women attending oncology clinic, Teaching Hospital Karapitiya.

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# Background

Breast cancer is the most common cancer in women globally and the leading cause of cancer deaths among Sri Lankan women. An age matched case control study was conducted to study the associated risk factors among breast cancer patients followed up at oncology unit, Teaching Hospital Karapitiya (THK).

# Methodology

Study involved 100 breast cancer patients attending oncology clinic within 5 years of diagnosis as cases and 200 patients from dermatology clinic at THK as controls. An interviewer –administrated questionnaire was used to gather data on sociodemographics, reproduction and menstruation, exposure to smoking, dietary pattern and nutrition of the participants. Data analysis was done manually using odds ratio and chi-square tests. Odds ratio (OR)  $\geq 1.5$  was taken as a significant risk and  $\leq 0.5$  was taken as a protective factor.

### Results

Study revealed that nulliparity(OR=1.605), high maternal age during first child birth(OR=1.765), duration of exposure to smoking>10 years(OR=4.129), increased frequency of red meat consumption(OR=1.607), family history of breast cancer(OR=2.59) were risk factors for breast cancers.

Breast feeding at any time of life (OR=0.438), consumption of vegetables and fruits grown at home garden (OR=.399) were protective factors against developing breast cancers.

According to the study, social class, age at menarche, age at menopause, use of OCP, use of HRT, duration of use of HRT, duration of pregnancy, vegetable and fruit consumption had no direct association with breast cancer development.

#### Conclusion

Increased duration of exposure to smoking was identified as a leading cause for developing breast cancer. To minimize the breast cancers, identified risk factors should be controlled.

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# Survey on road traffic accidents (RTA) admitted to Teaching Hospital Karapitiya (THK)

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### Background

RTA constitute a major public health problem in Sri Lanka, contributing to significantly high morbidity & mortality. WHO data published in 2014, shows RTA Deaths in Sri Lanka reached 2773 or 2.19% of total deaths, ranking 106<sup>th</sup> in the world. Therefore this study was carried out with the objective of identify the incidence and the major contributory factors of RTA causing admissions to THK.

# Methodology

Data was collected using interviewer based questionnaire, among simple randomized sample of the patients admitted to wards 58, 59, 60 of THK following RTA from 21/05/2015 to 19/06/2015.

#### Results

During the study period, out of 3334 admissions 315 were RTA rating 9.4%. Out of them 240 participated in the study.80.9% were males & 19.1% were females. In both genders the highest incidence was in age group 20-29.

Majority were motorbike accidents (65%) followed by three wheelers (22.9%), light vehicles (5.8%), push bikes (4.16%) and heavy vehicles (2.08%). Majority were drivers (46%) followed by passengers (36%) and pedestrians (18%).

From all victims, 18% has consumed alcohol, 10% of drivers were tired or sleepy. Considering morbidities, 6.53% had vision problems, 1.3% had hearing problems. Fifty four (54%) of drivers had driving license and 7% hadn't. In 39% of RTAs, the victim didn't know the availability of license of the driver. Only 5% of drivers exceeded the legal speed limits while 11% of drivers have not used seat belts and 21% of bike riders have not used helmets. High RTA incidence was observed among people, educated more than O/L and in crowded and bended roads.

Majority were superficial injuries, followed by fractures, head &internal organ injuries.

#### Conclusion

To minimize RTAs, awareness should be improved specially in motorbike riders.

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# Breast feeding practices and factors affecting breast-feeding of the primi mothers delivered in two Teaching Hospitals in Sri Lanka.

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### Background

Breast feeding (BF) is one of the most effective interventions to improve nutritional status of children. Despite various interventions the coverage of breast feeding practices in Sri Lanka is still not in satisfactory levels. The current study aimed to describe the BF practices and factors associated with exclusive breast feeding among the primi mothers from two Teaching Hospitals in Sri Lanka.

#### Materials and methods

Primi mothers who delivered live babies from February to March 2017 at Teaching Hospital Anuradhapura and Peradeniya were included in the study following ethical approval to the study. A structured pre-tested, interviewer administered questionnaire was used to collect the data by trained data collectors following informed written consent by the participants.

#### Results

There were 189 mothers recruited to the study. The mean (SD) age was 28 (5.3) years. Majority (88.4%) of them have received satisfactory lactation management training (LMT) and most common method of LMT was by lectures (78.3%). Live demonstration was significantly associated with mothers' satisfaction (p=<0.000). Most of the mothers (90.5%) were able to start BF within 1st hour. Special Care Baby Unit admission (44.4%) was the main reason for the failure to establish BF within 1st hour. Among the mothers, several nonscientific beliefs observed, such as working mothers can start formula feeds earlier (26.5%), formula feeds enhance healthy growth (15.9%), formula feeds should be given within five months of age (11.6%) and feeding on time schedule (45%).

#### Conclusions

Though the coverage of lactation management system was satisfactory, significant proportion of mothers still believe on non-scientific myths. New modes of knowledge dissemination methods with high quality must be applied as considerable proportion of mothers received information from via non-medical persons so the quality is questionable.

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# An audit of major surgeries performed in the gastrointestinal surgical unit of Teaching Hospital Karapitiya.

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#### Introduction

Surgical conditions of the gastrointestinal system are managed mainly in the gastrointestinal surgical units of Tertiary Care centers. The aim of the study was to assess the number and types of major surgeries carried out in the gastrointestinal surgical unit of Teaching Hospital Karapitiya (GISU of THK) which may help in prioritizing the facilities needed for the unit.

#### Material and Method

This was a retrospective study which was performed in the GISU of THK. Demographic data of those patients who underwent major surgeries, part of the gastrointestinal system involved, the nature of the illness and the type of surgery was recorded from patients treated in the GISU of THK during the two year period.

#### Results

Total of 96 patients had undergone major surgical interventions during this period. There were more males [57 (59%)] than females [39(41%)]. The age of the patients ranged from 21 to 79 years. Most of the surgeries done in the unit were colorectal [56 (58.3 %)] and the other surgeries included oesophageal [12 (12.5%)], stomach [11 (11.5%)] small bowel [7 (7.3%)], pancreas [6 (6.3%)], liver [2 (2.1%)], urogenital [1 (1%)] and other [1 (1%)]. Most of the surgeries were performed for malignancies [75(78%)]. Out of the 78% malignancies 65% were colorectal cancer.

### Conclusion

Most of the surgeries carried out in the unit during this period were colorectal surgeries with a considerable amount of esophageal and stomach surgeries. Majority of those surgeries were performed for malignancies.

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